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Promotive Effect of 5-aminolevulinic Acid on Growth, Yield and Gas Exchange Capacity of Barley (*Hordeum Vulgare* L.) Grown under Different Irrigation Regimes

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Abstract. The effect of ALA on growth, yield and gas exchange capacity of barley (*Hordiun vulagare*) cv. Gesto grown under three water regimes was evaluated. Barley was irrigated every 7, 14 and 21 days. ALA was applied as foliar spray at 25, 50 and 100 ppm as well as control. Irrigation interval of 21 days significantly reduced grain and straw yields/ha and this reduction was attributed to the reduction in plant height, spike length, number of grains/spike and weight of grains/spike. ALA spray significantly increased grain yield, particularly under 50 and 100 ppm. This increase was accompanying with an increase in plant height, spike length, number of grains/spike and weight of grains/spike. The high grain yield was noticed under 7 days interval and spraying with 50 or 100 ppm ALA. Net photosynthesis (NP), stomatal conductance (gs), transpiration rate (T), mesophyll conductance and intercellular CO_2 concentration were significantly reduced with increasing irrigation intervals, while the opposite was noticed in chlorophyll content. ALA did not significantly affect NP, T, Ci and chlorophyll content, while gm and gs was significantly increased with increasing ALA concentration. However, NP was increased by 24.3% under 100 ppm ALA compared with the control. The highest NP and gs appeared under 7 days irrigation intervals and 50 and 100 ppm ALA. Promotive effects on barley yield were clearly appeared under 7 and 14 days irrigation intervals.

***Keywords*:** 5-ALA, Drought, Water stress, Grain and straw yields, Net photosynthesis, Stomatal conductance, Transpiration.

Introduction

Barley (*Hordiun vulagare* L.) is grown as dual purpose crop, human food and for feeding animals. In most dry areas, barley is used for grazing by sheep in the field or harvested for animal feed [1]. It is highly adapted to a water stress environment and can be produced even in land with very marginal rainfall. Drought negatively affects cereal yield in many regions of the world and it was more severe in arid and semiarid regions. Reduction in growth and yield of barley grown under drought conditions is extensively reported [1-4]. Improving drought tolerance of cereal species is a major goal of most breeding programs through evaluating yield performance under drought conditions [5]. This approach is costly and time consuming which emphasizes improving growth and productivity of cereal by the benefit of the physiological behavior of these plants.

5-aminolevulinic acid (ALA) is a key precursor in the biosynthesis of porphyrins such as chlorophyll and heme. ALA application on plant has been often reported in relation to chlorophyll biosynthesis and plant greening [6]. However, the physiological properties of ALA have not been sufficiently reported. ALA application at low concentrations has been reported to promote growth and yield of crops and vegetables by 10-60% over the control as observed in barley [7, 8], radish, potatoes, garlic and kidney bean [8], wheat [6] and rice [7, 9].

Recently, ALA has been used to mitigate negative effects on plants grown under saline stress [10]. Salinity affects growth of crops due to toxicity and/or osmotic stress [11, 12]. Therefore, there is a possibility of positive promotion of ALA spraying on crops grown under drought stress. In this study, the effect of foliar spraying of ALA on growth, yield and gas exchange capacity of barley plants grown under different irrigation regimes were studied.

Material and Methods

This investigation was conducted on the Agricultural and Veterinary Training and Research Station, King Faisal University during the winter seasons of 2001/2002 and 2002/2003. A split plot design with four replicates was used in this study. The main plots were devoted to three irrigation regimes, i.e. irrigation every 7, 14 and 21 days with the volumes of 500, 650 and 800 m^3/ha/irrigation, receiving 20, 10 and 6 irrigations/season. The sub plots were devoted to three 5-ALA concentrations, 25, 50 and 100 ppm in addition to the control, i.e. water. The dimension of the experimental unit was 3 x 5 m, occupying an area of 15 m^2. Barley seeds (cv. Gesto) were hand drilled in rows, 15 cm apart with the rate of 150 kg/ha. Thereafter, the field area was watered with a volume of 800 m^3/ha for all units. This was done on the first week of November in both seasons. Barley plants were fertilized with nitrogen in the form of urea (46.6% N) at the rate of 200 kg N/ha, which was added into three equal portions, the first was added prior planting during land preparation. The second portion was applied at the first tillering stage and the rest was added at the panicle initiation stage. The plots were weeded using Brominal 2.5 L/ha at 30 days after sowing. Other recommended cultural practices for barley production were followed, except the studied treatments. Foliar spraying of 5-ALA (2000 L/ha) was applied biweekly starting from tillering stage to milk-ripe stage.

At maturity, 160 days after sowing, i.e. when barley plants turned into straw color and grains became solid, 10 guarded plants were randomly collected from each examined treatment to estimate the following characters: Plant height (cm), spike length (cm), number of grains/spikes, weight of grains/spike and 1000-grain weight (g). The plants in the two central square meters in each plot were harvested, left to dry, tied and threshed. Thereafter, grains and straw were separated and estimated in g/m^2, which converted to grain and straw yields (t/ha). Net photosynthesis (NP), stomatal conductance (gs), transpiration rate (T), mesophyll conductance (g), and intercellular CO_2 concentrations (Ci) were measured using Infra Red Gas Analyzer (IRGA) Ci-301 PS (CID, INC, USA) portable photosynthesis apparatus, open system. Measurement periods were in the morning from 8:00 am to 11:00 am where leaves of barley were exposed to a saturation photon irradiance exceeding 1200 µmol/m^2/s. Leaf temperature was 25 ± 5.0 °C. Air flow into the cuvette was 350 ml/min. The boundary layer conductance to water vapor was measured [13] and found to be 0.26 mmol /m^2/s over the measurement period. Calculation of NP, gs, T and Ci were done [14]. Mesophyll (residual) conductance, which is a composite measure of all liquid phase conductance to CO_2 (Cell wall, plasmalemma, cytoplasm, chloroplast membranes) as well as conductance associated with carboxylation [15] was calculated as gm= NP/Ci mol/m^2/s [16]. Chlorophyll content was measured in intact flag leaf using chlorophyll meter (SPAD 502-Japan) which provides a rapid and accurate non-destructive estimate of leaf chlorophyll content. During the grain filling stage, the measurement of photosynthesis gas exchange capacity was done one week after ALA spraying on flag leaves in six plants per treatment.

The obtained data in the two seasons were subjected to the proper combined (over seasons) analysis of variance of the split plot design [17]. Baysian Least significant difference (BLSD) at 0.05% level of significant was used to compare the treatment means [18]. Regression models were fitted to the data to describe the relationship between net photosynthesis (NP) and each of stomatal conductance (gs) and mesophyll conductance (gm). Statistical analysis and computations were done using SAS [19].

Results and Discussion

Results presented in Table 1 show that irrigation intervals significantly affected all estimated characters. Increasing irrigation intervals up to 21 days was associated with significant decreases in plant height, spike length, number of grains/spike, 1000-grain weight as well as grain and straw yields/ha. The reduction in grain and straw yields/ha with increasing irrigation intervals from 7 to 21 days was 37.2 and 18.4%, respectively. The reduction in number of grains/spike was 9.1%, compared with 15.5% in weight of grains/spike when irrigation interval increased from 7 to 21 days. It seems that the reduction of grain yield was much associated with reduction in weight than the number of grains which show that 21 days irrigation regime used in the present study did not affect severely anthers or pollination processes and the main effect of this regime could be attributed to the lack of assimilate required for grain filling. The reduction of growth and yield of barley grown under drought conditions was extensively reported in the literature [2-4, 20]. This reduction is expected to be much pronounced under arid and semiarid regions like Saudi Arabia. Net photosynthesis was significantly reduced with increasing irrigation intervals which confirm the suggested explanation reported earlier of lack of assimilate required for grain filling under drought conditions. Similar findings were obtained by others [2, 21]. Since photosynthesis is the source of organic carbon and energy for plants, it considers the source of the growth and production of biomass and yield formation. Reduction of straw and grain yields reported in the present study could be partially attributed to the reduction of net photosynthesis. Chlorophyll content was significantly increased with prolonged irrigation interval. Stomatal conductance, mesophyll conductance, intercellular CO_2 concentration and transpiration rate were significantly reduced with increasing irrigation intervals (Table 2). The lower rate of stomatal conductance with increasing irrigation intervals from 7 to 21 days could partially explain the lower net photosynthesis and transpiration rates reported under this condition. The changes in transpiration rate (18%) relatively corresponded with changes in stomatal conductance. It seems that stomatal conductance did restrict entry of CO_2 into leaf which contribute partially to the reduction of net photosynthesis with $r^2 = 0.78\%$ (Fig. 1). Reduction of net photosynthesis (28%) under 21 days irrigation intervals did not correspond with the reduction on stomatal conductance (12%). This suggests that in addition to any stomatal responses, the biochemical properties of the photosynthesis apparatus in the mesophyll cells may also play a significant role in decreasing the rate of net photosynthesis with $r^2 = 0.84\%$ (Fig. 2). The considerable changes in mesophyll conductance reported in the present study were consistent with the changes in net photosynthesis. With a significant increase in chlorophyll under 21 days irrigation regime, it could not be possible to explain underlaying mesophyll conductance effects. This suggests that the inhibition of net photosynthesis reported in the present study on barley plant grown under 21 days irrigation regime could be due to inhibition of chloroplast enzymes [21]. Foliar application of 5-ALA significantly affected all estimated characters. Application of 5-ALA at 100 ppm showed the highest values of all parameters. Also, there was a significant increase with each increase in 5-ALA concentration. The difference between 50 and 100 ppm was insignificant, particularly in number of grains /spike and weight of grains/spike and grain yield/ha. The increase in barley yield with the application of 25, 50 and 100 ppm 5-ALA reached 19.57, 41.01 and 50.0[illegible]% in grain yield and 19.10, 30.63 and 35.87% in straw yield, compared with the control, respectively (Table 1). Promotive effect of 5-ALA application on barley yield was also reported by many investigators [7, 8]. A significant increase in number of grains/spike and spike weight with the application of ALA on barley plants was reported [8].

5-ALA did not significantly affect net photosynthesis, transpiration, intercellular CO_2 and chlorophyll content. However, mesophyll conductance and stomatal conductance were significantly higher under foliar spraying of 100 ppm 5-ALA (Table 2). Foliar application of 5-ALA with low concentration increased CO_2 fixation in raddish [8], spinach [22]. However, such increase was not reported in potatoes [9]. Chlorophyll content in potatoes was significantly increased with 5-ALA application [9]. It seems that the increase in photosynthesis activity was much related to the foliar spraying with 50 and 100 ppm 5-ALA. Hotta *et al.* [9] reported a significant increase in radish photosynthesis activity two days after 5-ALA spraying, but after five days a reduction of about 11% in photosynthesis activity was reported. This trend may explain the absence of significant increase in net photosynthesis in the present study, since the measurement of NP on barley was done one week after 5-ALA spraying.

Table 1. Barley plant height, spike length, number of grains/spike, weight of grains/spike, 1000-grain weight and straw and grain yields as affected by irrigation intervals, 5-ALA concentrations and their interaction

Treatments		Plant height (cm)	Spike length (cm)	Grains/spike (No)	Grains weight/ spike (g)	1000-Grain wt. (g)	Straw yield (t/ha)	Grain yield (t/ha)
A: Irrigation period								
7 days		67.4	5.8	49.8	2.9	57.8	12.924	7.149
14 days		69.1	5.7	50.8	2.9	56.0	12.767	6.605
21 days		61.7	5.1	42.1	2.0	45.8	10.547	4.488
BLSD 5%		3.0	0.5	7.0	0.3	1.3	0.625	0.512
B: 5-ALA conc.								
0 ppm		60.1	4.9	41.7	2.1	49.4	9.950	4.757
25 ppm		65.5	5.5	44.6	2.4	52.8	11.850	5.688
50 ppm		69.0	5.7	51.4	2.8	53.5	12.998	6.708
100 ppm		69.5	5.9	52.6	3.0	57.1	13.519	7.167
BLSD 5%		2.9	0.4	6.0	0.3	0.9	0.518	0.488
Interaction (A*B)								
7 days	0 ppm	63.8	5.3	42.1	2.3	55.5	10.975	5.712
7 days	25 ppm	65.8	5.6	46.1	2.6	56.5	13.020	6.685
7 days	50 ppm	69.1	6.1	55.4	3.2	57.5	13.721	7.936
7 days	100 ppm	70.8	6	55.7	3.4	61.8	13.980	8.262
14 days	0 ppm	63.5	5	44.6	2.2	48.8	10.522	4.840
14 days	25 ppm	69.0	5.6	46.5	2.6	56.8	12.105	5.980
14 days	50 ppm	72.6	5.7	55.2	3.1	57.0	13.714	7.409
14 days	100 ppm	71.1	6.3	56.8	3.5	61.5	14.753	8.190
21 days	0 ppm	53.1	4.3	38.3	1.7	44.0	8.374	3.720
21 days	25 ppm	61.8	5.3	41.3	1.9	45.0	10.425	4.400
21 days	50 ppm	65.2	5.4	43.5	2.1	46.1	11.563	4.780
21 days	100 ppm	66.5	5.5	45.3	2.2	48.0	11.825	5.050
BLSD 5%		N.S	0.7	10.2	0.5	1.7	1.070	0.845

Table 2. Net photosynthesis, NP (μmol/m²/s), transpiration rate, T(mmol/m²/s), intercellular CO_2 concentration, Ci (μmol/mol), stomatal conductance, gs (mmol/m²/s), mesophyll conductance, gm (mmol/m²/s) and chlorophyll content (SAPAD reading) as affected by irrigation intervals, 5-ALA concentrations and their interaction

Treatments		NP	gs	T	gm	Ci	Chl.
A: Irrigation period							
	7 days	8.28	153.1	2.20	70	76.53	40.6
	14 days	6.85	140.3	1.67	50	54.40	45.4
	21 days	5.93	134.7	1.79	50	56.00	45
BLSD 5%		1.31	10.1	0.24	10	7.3	3.8
B: 5-ALA conc.							
	0 ppm	6.03	127.0	2.13	40	49.67	39.33
	25 ppm	6.70	134.6	2.00	40	56.17	43.10
	50 ppm	7.37	144.5	1.77	60	64.40	44.00
	100 ppm	7.97	164.7	1.64	70	79.00	48.20
BLSD 5%		**N.S**	**8.65**	**N.S**	**10**	**N.S**	**N.S**
Interaction (A*B)							
7 days	0 ppm	7.51	139.8	2.53	50	63.6	33.3
7 days	25 ppm	7.79	146.7	2.3	50	70.4	40.6
7 days	50 ppm	8.73	156.5	2.08	80	75.6	41.0
7 days	100 ppm	9.08	169.5	1.89	90	96.5	47.4
14 days	0 ppm	5.59	120.5	1.89	40	40.2	40.8
14 days	25 ppm	6.81	132.3	1.81	40	43.2	44.8
14 days	50 ppm	7.2	139.9	1.56	60	61.6	46.2
14 days	100 ppm	7.79	168.5	1.41	60	72.6	50.0
21 days	0 ppm	4.98	120.7	1.98	30	45.2	43.9
21 days	25 ppm	5.51	124.9	1.88	40	54.9	43.9
21 days	50 ppm	6.18	137.0	1.68	50	56.0	44.8
21 days	100 ppm	7.03	156.1	1.63	60	67.9	47.2
BLSD 5%		**2.03**	**15.9**	**0.38**	**N.S**	**14.2**	**4.6**

Results of the statistical analysis revealed a significant interaction between irrigation treatments and 5-ALA concentrations on spike length, number of grains/spike, 1000-grain weight, weight of grains/spike, grain and straw yields/ha. The highest grain weight of spike and grain and straw yields/ha were obtained with irrigation barley plants every 7 and 14 days and foliar sprays with 50 and 100 ppm ALA (Table 1). It seems that foliar application of ALA mitigate the negative effects of prolonged irrigation regime on yield and its components of barley grown under field conditions. Yields of barley under

21 days irrigation intervals and 50 – 100 ppm ALA were less than the yield reported under 7 days irrigation intervals and control of ALA application. Net photosynthesis and stomatal conductance were significantly higher under 7 days irrigation interval with 100 ppm ALA (Table 2) which emphasis the promotive role of ALA in barley plant grown under the normal condition.

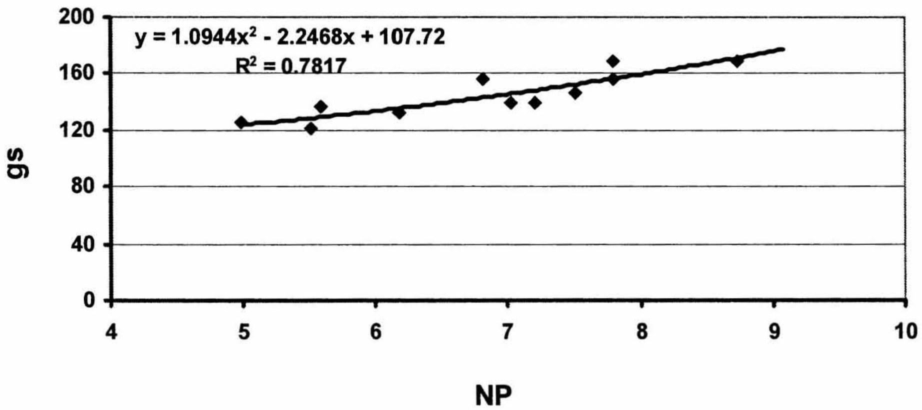

Fig. 1. Relationship between Photosynthesis rate (NP) and stomatal conductance (gs).

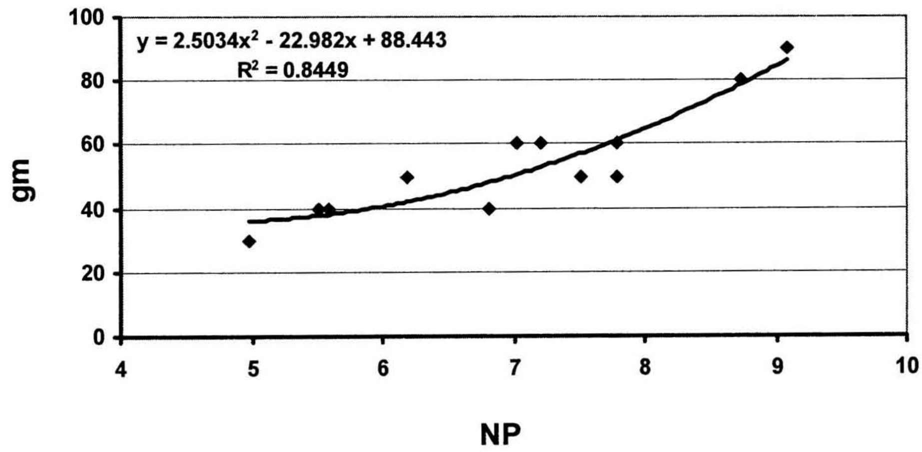

Fig. 2. Relationship between Photosynthesis rate (NP) and mesophyll conductance (gm).

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التأثير المنشط لمنظم النمو 5-ALA في النمو والإنتاج والتبادل الغازي للشعير تحت الظروف الجفافية

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(قدم للنشر في ١٤٢٦/١/١٧هـ ؛ قبل للنشر في ١٤٢٦/٩/١٣هـ)

ملخص البحث. استهدف هذا البحث دراسة تأثير منظم النمو 5-ALA في نمو ومحصول الشعير "صنف جستو" تحت ثلاث مستويات من الرى ، حيث تم ري الشعير كل ٧، ١٤ و ٢١ يوما كما تم رش منظم النمو 5-ALA على المجموع الخضري للنبات بتركيزات ٢٥، ٥٠ و ١٠٠ جزء في المليون بالإضافة إلى معاملة الشاهد. وأفادت نتائج الدراسة أن ري الشعير كل ٢١ يوما أحدث نقصا معنويا في محصول الحبوب والقش/هكتار، حيث عزي هذا النقص لانخفاض طول النبات وطول السنبلة وعدد ووزن الحبوب بالسنبلة. أدى رش منظم النمو 5-ALA إلى زيادة محصول الحبوب وخاصة عند التركيزات ٥٠ و ١٠٠ جزء في المليون. هذه الزيادة كانت مصحوبة بزيادة في طول النبات وطول السنبلة وعدد ووزن الحبوب بالسنبلة. أدى الري كل ٧ أيام مع الرش بمنظم النمو 5-ALA بمعدل ٥٠ و ١٠٠ جزء في المليون إلى أعلى محصول من الحبوب. وانخفض معنويا كل من معدل التمثيل الضوئي والتوصيل الثغري ومعدل النتح وتوصيل خلايا الميزوفيل وتركيز CO_2 في الثغور بزيادة الإجهاد المائي والعكس صحيح بالنسبة لتركيز الكلوروفيل. لم يكن لمنظم النمو 5-ALA تأثيرا معنويا على معدل التمثيل الضوئي ومعدل النتح وتركيز CO_2 في الثغور ومحتوى النبات من الكلوروفيل، إلا أن التوصيل الثغري وتوصيل خلايا الميزوفيل قد زاد معنويا بزيادة تركيز منظم النمو 5-ALA. ارتفع معدل التمثيل الضوئي بنسبة ٢٤,٣٪ عند رش منظم النمو 5-ALA بتركيز ١٠٠ جزء في المليون، مقارنة مع معاملة الشاهد. وقد لوحظ أن أعلى معدل للتمثيل الضوئي والتوصيل الثغري نتج مع معاملة الري كل ٧ أيام ورش منظم النمو 5-ALA بتركيز ٥٠ و ١٠٠ جزء في المليون. وظهر التأثير المنشط لمنظم النمو 5-ALA بوضوح عند الري كل ٧ و ١٤ يوما.