

5-Aminolevulinic Acid Induces Regulation in Growth, Yield and Physio-Biochemical Characteristics of Wheat under Water Stress

(Asid 5-aminolevulinik Mengaruh Pengawalatan terhadap Pertumbuhan, Hasil dan Ciri Fisis-Biokimia Gandum pada Tekanan Air)

NUDRAT AISHA AKRAM*, SHAMIM KAUSAR, NAILA FARID, MUHAMMAD ASHRAF & FAHAD AL-QURAINY

ABSTRACT

The production of wheat crop is below average in many regions of the world which is ascribed to adverse environmental conditions including drought stress. The present study was conducted to appraise the beneficial role of exogenously-applied 5-aminolevulinic acid (ALA) on growth, yield and some key physio-biochemical characteristics of two commercially important wheat cultivars (Shafaq-06 and Uqab-2000) under well watered [100% field capacity (FC)] and water-deficit (60 and 80% FC) conditions. Imposition of varying water regimes significantly decreased fresh and dry weights of shoots, photosynthetic pigments (a and b), non-photochemical quenching of chlorophyll fluorescence (NPQ), quenching coefficient for non-photochemical (N) of chlorophyll fluorescence (qN), K⁺ (potassium ion), Ca²⁺ (calcium ion) and P (phosphorus) accumulation in shoot and root and yield-related attributes. In contrast, water deficit regimes caused improvement in F_v/F_m (chlorophyll fluorescence measurement), coefficient of photochemical quenching (qP), proline, glycinebetaine (GB) and hydrogen peroxide (H₂O₂) contents. Foliar spray of ALA at the rate of 50, 100 and 150 mg/L along with control (no spray (NS) and/or water spray (WS)) significantly enhanced chlorophyll a and b pigments, qN, NPQ, qP, K⁺, Ca²⁺ and P accumulation in both roots and shoots, proline, GB, total phenolics and malondialdehyde (MDA) contents and yield. The wheat Shafaq-06 was better in shoot dry weight, qN, NPQ and F_v/F_m, shoot and root K⁺, root Ca²⁺, proline, GB accumulation and yield attributes, while Uqab-2000 was better in chlorophyll a contents, root P and MDA contents. Overall, better growth and yield of Shafaq-06 than Uqab-2000 under water deficit regimes was found to be associated with ALA improved leaf fluorescence (qN, NPQ and F_v/F_m), shoot and root K⁺, root Ca²⁺, proline and GB accumulation.

Keywords: Chlorophyll contents; drought stress; wheat; yield; 5-Aminolevulinic acid

ABSTRAK

Penghasilan tanaman gandum adalah di bawah purata di kebanyakan kawasan di dunia dianggap sebagai keadaan alam sekitar yang buruk termasuk tekanan kemarau. Kajian ini dijalankan untuk menilai kelebihan eksogen-aplikasi 5-aminolevulinik asid (ALA) terhadap pertumbuhan, hasil dan ciri-ciri fisis-biokimia kedua-dua kultivar gandum yang komersial utama (Shafaq-06 dan Uqab-2000) dalam keadaan kecukupan air [100% keupayaan lading (FC)] dan defisit air (60 dan 80% FC). Pengenaan rejim air yang berbeza telah mengurangkan berat segar dan kering tunas secara berkesan, pigmen fotosintetik (a dan b), pelindapan tanpa fotokimia fluoresens klorofil (NPQ), pekali pelindapan untuk tanpa fotokimia (N) fluoresens klorofil (qN), K⁺ (ion kalium), Ca²⁺ (ion kalsium) dan P (fosforus) pengumpulan pada tunas, akar serta sifat berkaitan hasil. Sebaliknya, rejim defisit air menyebabkan penambahbaikan F_v/F_m (pengukuran fluoresens klorofil), pekali pelindapan fotokimia (qP), kandungan prolina, glycinebetaine (GB) dan hidrogen peroksida (H₂O₂). Semburan daun ALA pada kadar 50, 100 dan 150 mg/L selari dengan kawalan (tanpa semburan (NS) dan semburan air (WS)) meningkat dengan ketara pengumpulan pigmen klorofil a dan b, qN, NPQ, qP, K⁺, Ca²⁺ dan P pada akar dan tunas, serta kandungan dan hasil prolina, GB, jumlah fenolik dan malondialdehid (MDA). Gandum Shafaq-06 lebih baik dalam berat kering tunas, qN, NPQ dan F_v/F_m, tunas dan akar K⁺, tunas dan akar Ca²⁺, prolina, pengumpulan GB dan sifat hasil, manakala Uqab-2000 lebih baik dalam kandungan klorofil a, tunas P dan kandungan MDA. Keseluruhannya, pertumbuhan dan hasil Shafaq-06 lebih baik daripada Uqab-2000 dalam regim defisit air adalah berkaitan dengan ALA yang dapat perambahbaik fluoresens daun (qN, NPQ, dan F_v/F_m), tunas dan akar K⁺, akar Ca²⁺, prolina dan pengumpulan GB.

Kata kunci: Asid 5-aminolevulinik; gandum; hasil; kandungan klorofil; tekanan kemarau

INTRODUCTION

Water shortage is a devastating environmental stress which adversely affects growth, production and development

of crop plants (Ashraf et al. 2011). At all stages of plant growth, drought stress acts as a limiting factor. Water stress is believed to alter many biochemical and physiological

processes such as nutrient uptake and metabolism, water relations, osmotic adjustment, photosynthesis, respiration, fluorescence and energy metabolism (Ashraf et al. 2011). Plants have developed a wide range of mechanisms to maintain survival and productivity under water deficit conditions (Akram et al. 2007; Ashraf et al. 2011). Many defensive mechanisms help the plant to stay alive and develop properly before the reproductive stage i.e. up-regulation of antioxidants, osmo-regulation, ionic homeostasis and hormonal system (Akram & Ashraf 2013). Drought stress promotes the rate of synthesis of reactive oxygen species (ROS) (Navarri-Izzo et al. 1994), however, plants produce different antioxidants and osmolytes to neutralize ROS under drought stress (Ashraf 2009; Mittler 2002). According to a prediction, 1.8 billion people i.e. 65% of the total world's population will face water deficient conditions up to 2025 (Nezhadahmadi et al. 2013).

Aminolevulinic acid (ALA), a five carbon plant growth regulator (Akram & Ashraf 2013; Naeem et al. 2010) is involved in the biosynthesis of phycobilins, chlorophyll, vitamin B₁₂ and heme in plants (Akram & Ashraf 2013). Generally, optimal production of ALA is necessary in order to synthesize optimal levels of chlorophyll in plants. If the natural production of this biomolecule is very low in a plant, then this deficiency can be overcome by its exogenous application. It is strongly believed that exogenous application of ALA can effectively stimulate a number of physiological processes including biosynthesis of chlorophyll and photosynthates under stress and non-stress conditions (Akram & Ashraf 2013, 2011b). For example, ALA improved photosynthesis in strawberry (Liu et al. 2006), pakchoi (Wang et al. 2004), spinach (Nishihara et al. 2003) and radish (Wang et al. 2005). In view of some reports ALA can enhance agricultural productivity under drought stress due to increase in photosynthetic rate (Wang et al. 2003), antioxidants, nitrogen and carbon fixing processes (Maruyama-Nakashita et al. 2010). It is now widely known that ALA containing fertilizers improves photosynthetic assimilation (Youssef & Awad 2008) and provides protection against insects and herbs (Duke & Rebeiz 1994).

Production of wheat is below from average in many regions of the world mainly due to unfavorable climatic factors. Drought is one of the main environmental cues affecting quantity and quality of wheat all-over the world. Generally, different physio-biochemical processes, growth and yield of wheat plants are affected due to insufficient irrigation (Metwaly 2012). Therefore, an experiment was carried-out to examine whether exogenous application of ALA could enhance plant growth and/or yield by altering different physiological attributes of wheat under water deficit regimes.

MATERIALS AND METHODS

A completely randomized design (CRD) experiment with four replicates in a three factor factorial arrangement was conducted from November 2012 to April 2013. Two wheat cultivars, Shafaq-06 and Uqab-2000, were grown

under different (control, 80% and 60% field capacity; FC) water deficit regimes. Plastic pots filled with 6000 g sandy-loam soil were used. During growing season, RH (relative humidity) was 33.0-63.0%, day/night temperature was $31 \pm 3.5^\circ\text{C}/26.5 \pm 2^\circ\text{C}$ and day-length was 11 to 12 h. Six seeds were planted in each plastic pot and normal watering was done before seed germination. After germination, thinning of plants was done and four seedlings were maintained per pot. After 20 days of seed germination, water stress treatments were maintained up to the yield on the basis of FC. After 30 days of initiation of drought stress treatments when the wheat plants were at the vegetative stage, different levels (NS, WS, 50, 100 and 150 mg/L) of ALA were applied foliarly. After 14 days of ALA spray, leaf samples for key variables were collected and stored at freezing temperature (-20°C). Then, two plants per treatment were harvested and rinsed with tap water. After separating roots and shoots, fresh weights were recorded and then placed in an oven for 3 days at 70°C . The dry weights were measured. The remaining two plants in each pot were allowed to grow up till yield formation. The following attributes were determined during the study:

CHLOROPHYLL FLUORESCENCE

Using a chlorophyll fluorometer (Model, Multi-Mode-OS5P, USA), chlorophyll fluorescence attributes were recorded of intact leaves following Strasser et al. (1995).

CHLOROPHYLL CONTENTS

Photosynthetic pigments were analyzed pursuing the method of Arnon (1949). 500 mg Fresh leaf was collected in an ice containing container. Then, it was extracted in 10 mL (v/v) acetone (80%). The O.D (optical density) of the filtrate was read using a UV-visible spectrophotometer (IRMECO, U2020, Germany) at 645 and 663 nm.

MINERAL NUTRIENTS (K⁺, CA²⁺ AND P)

The method of Wolf (1982) was used to digest plant material for analyzing different inorganic nutrients. The values of K⁺ and Ca²⁺ in the aliquots were recorded using a flame photometer (Jenway, PFP-7). Phosphorus was determined according to the method of Jackson (1962).

FREE PROLINE

Fresh leaf material (500 mg) was extracted with 3% sulfosalicylic acid following the method of Bates et al. (1973).

GLYCINEBETAINE (GB)

A total of 250 mg dry leaf was mixed with 5 mL 0.5% toluene and kept overnight at 4°C . 1.0 mL 2 N H₂SO₄ (sulfuric acid) was added to 1 mL filtrate and mixed. Then, 0.2 mL KI₃ (potassium tri-iodide) solution was added to 0.5 mL mixture. This mixture was mixed well and cooled in an ice bath. The absorbance of the lower organic layer

was recorded at 365 nm using a spectrophotometer (Grieve & Grattan 1983).

HYDROGEN PEROXIDE (H₂O₂)

In a pre-chilled mortar, 500 mg fresh leaf material was ground in 5 mL 0.1% TCA (trichloroacetic acid solution) and centrifuged for 15 min at 12,000 × g following Velikova et al. (2000).

MALONDIALDEHYDE (MDA)

A total of 250 mg fresh leaf was extracted in 3 mL 1% TCA under chilling conditions. 1 mL of the supernatant was mixed with 4 mL 0.5% TBA (thiobarbituric acid) and the mixture was incubated at 95°C for 1 h. After cooling, the absorbance of the supernatant was read at 532 and 600 nm (Carmak & Horst 1991).

TOTAL PHENOLICS

Leaf total phenolics were examined following Julkenen-Titto (1985).

STATISTICAL ANALYSIS

A three-factor (cultivars, drought stress and ALA) factorial randomized design with four replicates was employed. Mean square values were obtained through analysis of variance (ANOVA) technique using Costat version 3.0 (MSTAT Development Team 1989).

RESULTS AND DISCUSSION

Under water-deficit conditions (60% and 80% FC), shoot fresh and dry weights of both wheat cultivars decreased ($p \leq 0.001$) considerably as compared to the plants grown under well watered conditions. Different levels (50, 100 and 150 mg/L) of ALA slightly improved shoot fresh and dry weights in both wheat genotypes under all water regimes. Both wheat cultivars were similar in shoot fresh weight, while wheat Shafaq-06 was slightly superior to the other wheat cultivar in shoot dry weight under water stress (Table 1 & Figure 1). Water is a crucial entity for all organisms including plants because functioning of all metabolic processes depends on it (Ashraf et al. 2011). Plant growth and yield reduction due to water deficiency appearing at any plant growth stage has been well studied (Pervez et al. 2009). However, this study was carried out to observe the effectiveness of ALA on growth, yield and some key physio-biochemical characteristics of wheat plants under well watered (100% FC) and water-deficit (60% and 80% FC) conditions. Foliar-applied with 100 and 150 mg/L 5-aminolevulinic acid (ALA) reduced the drought-induced inhibition in plant growth (shoot fresh and dry weights) of both wheat cultivars. This is comparable to what has already been observed in cucumber plants when Li et al. (2011) applied 3 μ M ALA as a seed pretreatment. They found a significant improvement in plant growth under drought stress. In addition, they attributed this

improvement to ALA-induced increase in antioxidant enzyme activities and reduction in ROS generation. In another study, Liu et al. (2013) observed that foliar-applied ALA at the rate of 30 mg/L considerably maintained water stress-affected chlorophyll contents, water status in terms of RWC (relative water content), photosynthetic capacity, F_v/F_m , qP , NPQ and electron transport rates of rapeseed plants. Furthermore, foliar-applied ALA also suppressed the drought-induced accumulation of H₂O₂ and MDA coupled with improved activities of different (CAT (catalase) & POD (peroxidase)) antioxidant enzymes (Liu et al. 2013). Similarly, in our study, foliar-applied ALA also decreased drought-induced increase in H₂O₂ contents.

Chlorophyll contents (*a* and *b*) reduced considerably ($P \leq 0.001$ and $P \leq 0.05$, respectively) under different water regimes (Figure 1). Higher reduction in chlorophyll pigments was observed at 60% FC as compared to that of control (100% FC) and mild (80% FC) water stress conditions. ALA improved photosynthetic pigments and maximum improvement in chlorophyll *a* and *b* contents was found at 100 and 150 mg/L levels of ALA particularly under water stress conditions. Cultivar Uqab had higher chlorophyll *a* contents than the other cultivar under stress conditions in particular at 60% FC. While both wheat cultivars showed a similar trend in chlorophyll *b* contents under different water regimes and exogenously applied ALA.

5-Aminolevulinic acid-induced modifications in multiple physiological processes of different crops have been observed under different environmental conditions including drought stress (Akram & Ashraf 2013, 2011a, 2011b; Liu et al. 2013). In this study, ALA improved chlorophyll of wheat plants under all water regimes. ALA-induced improvement in chlorophyll contents under water-deficit conditions has already been observed in maize (Dolatabadian et al. 2009), oilseed rape (Liu et al. 2013, 2011) and *Phoenix dactylefera* (Youssef & Awad 2008). ALA is known as one of the major precursors of chlorophyll biosynthesis, so increase in chlorophyll content under stress and non-stress conditions could be attributed to increased endogenous level of ALA following its external application. Of different leaf fluorescence attributes, qN and NPQ of all the plants decreased under water stress. However, photochemical quenching (qP) and efficiency of PS-II (measured as F_v/F_m) increased significantly under water-deficit conditions as compared to those in 100% FC. Exogenously-applied varying levels of ALA considerably improved all the above-mentioned leaf fluorescence attributes except F_v/F_m . Overall, cultivar Shafaq-06 was better in qN , NPQ and F_v/F_m as compared to cultivar Uqab-2000 under both water regimes (Figure 1).

Accumulation of K⁺, Ca²⁺ and P in shoot and root decreased significantly in both wheat cultivars due to imposition of different water regimes (Table 1; Figure 2). Varying levels of foliar-applied ALA improved accumulation of K⁺, Ca²⁺ and P in both roots and shoots of both wheat cultivars under 60, 80 and 100% FC (Figure 2). Occurrence of nutritional imbalance in plant cells/tissues is one of the major physiological disorders caused

TABLE 1. Mean square (ANOVA) data for growth, yield and some key physio-biochemical attributes of wheat plants subjected to foliar-applied varying levels of 5-aminolevulinic acid (ALA) under water-stressed and non-stressed conditions

Source of variation	df	Shoot FW	Shoot DW	Chl. <i>a</i>	Chl. <i>b</i>	<i>qN</i>
Cultivars (Cvs)	1	1.397**	0.0003ns	3.111***	1.133ns	0.384***
Drought stress (D)	2	2.100***	0.487***	2.048***	1.267*	0.293***
ALA	4	0.224ns	0.027ns	0.669***	0.929*	0.093***
Cvs × D	2	0.021ns	0.059*	0.372*	0.270ns	0.034***
Cvs × ALA	4	0.036ns	0.005ns	0.124ns	0.073ns	0.012*
D × ALA	8	0.014ns	0.008ns	0.165ns	0.231ns	0.016***
Cvs × D × ALA	8	0.044ns	0.013ns	0.120ns	0.307ns	0.009*
	df	F _v /F _m	NPQ	<i>qP</i>	Shoot K ⁺	Root K ⁺
Cultivars (Cvs)	1	0.207***	2.624***	0.0855ns	1162.5***	1928.0***
Drought stress (D)	2	0.025***	1.057***	0.433***	259.6*	265.0***
ALA	4	0.019***	0.480***	0.296***	495.1***	233.9***
Cvs × D	2	0.021***	0.123**	0.077*	154.5ns	43.72ns
Cvs × ALA	4	0.004ns	0.071*	0.037ns	19.98ns	56.81ns
D × ALA	8	0.020***	0.05*	0.014ns	141.3ns	32.15ns
Cvs × D × ALA	8	0.008***	0.084***	0.025ns	188.9*	11.41ns
	df	Shoot Ca ²⁺	Root Ca ²⁺	Shoot P	Root P	Proline
Cultivars (Cvs)	1	7.252ns	902.5***	0.0035ns	0.0003***	59.17***
Drought stress (D)	2	242.5***	116.4***	0.0007**	0.00047***	9.155*
ALA	4	79.89***	67.3***	0.0008***	0.00002***	18.015***
Cvs × D	2	22.15ns	34.53*	0.0001ns	0.00003***	0.0089ns
Cvs × ALA	4	2.793ns	71.88***	0.00007ns	0.000019***	2.486ns
D × 5-ALA	8	6.211ns	14.05ns	0.00007ns	0.0002ns	1.375ns
Cvs × D × ALA	8	42.73**	9.082ns	0.00001ns	0.0004ns	2.525ns
	df	GB	Total Phenolics	MDA	H ₂ O ₂	100-grain weight
Cultivars (Cvs)	1	9278.2***	7.413ns	8600446.0***	131920.4ns	6.31***
Drought stress (D)	2	15484.3***	55.98ns	1539561.4ns	890373.2***	5.32***
ALA	4	1959.5**	326.8***	2865627.1***	116812.6*	4.35***
Cvs × D	2	9763.4***	124.9ns	2928279.5**	343163.9***	0.100ns
Cvs × ALA	4	244.2ns	51.49ns	1358985.5*	29430.5ns	0.159ns
D × ALA	8	305.7ns	96.97*	414547.5ns	31664.5ns	0.128ns
Cvs × D × ALA	8	327.8ns	108.5*	365203.1ns	25213.7ns	0.271ns
	df	No of grains/ plant	Grain yield/ plant			
Cultivars (Cvs)	1	9.847ns	1.281ns			
Drought stress (D)	2	291.5***	26.44***			
ALA	4	36.61*	2.347*			
Cvs × D	2	8.88ns	1.007ns			
Cvs × ALA	4	7.57ns	0.877ns			
D × ALA	8	5.851ns	0.326ns			
Cvs × D × ALA	8	5.315ns	0.620ns			

ns = non-significant; *, ** and *** = significant at 0.05, 0.01 and 0.001 levels, respectively; FW, Fresh weight; DW, Dry weight; MDA, Malondialdehyde; H₂O₂, Hydrogen oxide; GB, Glycinebetaine

by water shortage (Ashraf et al. 2011). We observed that K⁺, Ca²⁺ and P accumulation in shoot and root was suppressed under water deficit conditions similar to that

in wheat under saline stress (Ashraf & Khanum 1997) and exogenously applied ALA levels were effective in improving accumulation of these ions in both wheat

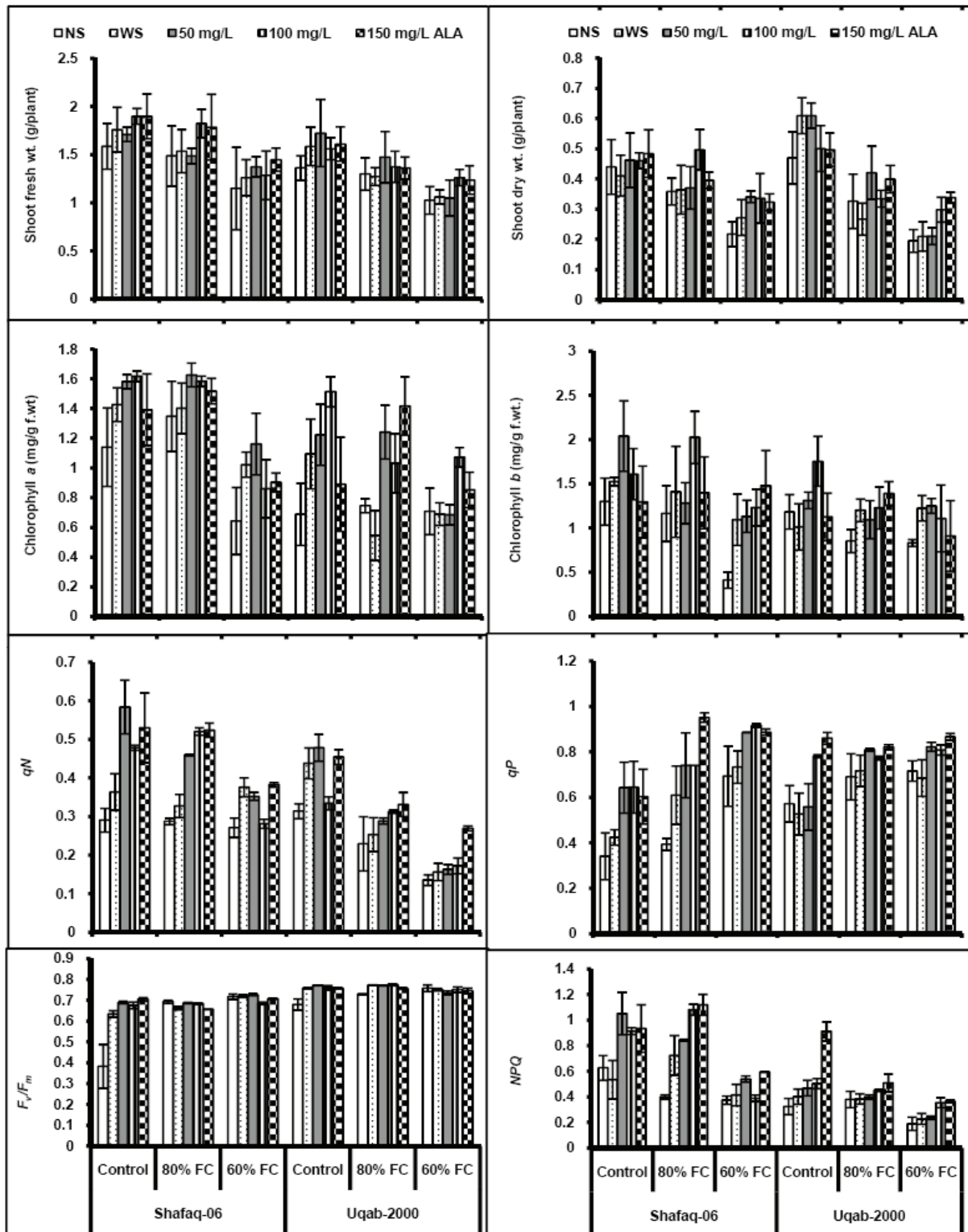


FIGURE 1. Shoot fresh and dry weights, chlorophyll *a* and *b* contents and different leaf fluorescence attributes of wheat plants treated with aminolevulinic acid (ALA) foliarly at vegetative stage under control and drought stress (60% & 80% field capacity) conditions (Mean \pm S.E)

cultivars at all water regimes. Earlier to the present study, not a single report is available in the literature on ALA induced changes in mineral nutrient accumulation in plants subjected to drought stress conditions. However, under saline conditions, Akram and Ashraf (2011a) observed that exogenously-applied ALA altered the uptake of Na^+ and K^+ in the roots of sunflower plants. In addition, Na^+

accumulation declined significantly in salt-stressed plants of cotton (Watanabe et al. 2000), *P. dactylifera* (Youssef & Awad 2008) and oilseed rape (Naeem et al. 2010) by exogenously applied ALA. Therefore, further research needs to find out ALA-induced effectiveness in pattern of uptake and accumulation of different nutrients in plants of different species under drought stress.

High free proline contents were observed in both wheat cultivars due to water stress particularly at 80% FC (Figure 2). ALA improved proline accumulation in all wheat plants subjected to all water stress levels. Cultivar Shafaq-06 being better than cultivar Uqab-2000 in this variable. Drought stress-induced increase ($P \leq 0.001$) in GB contents was observed only in wheat Shafaq-06, while GB accumulation in wheat Uqab-2000 remained almost unchanged under well-watered and water-deficit conditions (Table 1; Figure 2). ALA-induced considerable improvement in GB accumulation was observed. Of all the ALA levels, 100 and 150 mg/L were found to be the most effective. Osmoregulation/osmotic adjustment caused by accumulation of osmotica in high concentration is one of the promising strategies of plants for maintaining plant growth as well as yield under low water environment

(Ashraf et al. 2011). High accumulation of proline and GB in response to drought stress has been observed in many plants such as rice (Cha-um et al. 2013), okra (Sankar et al. 2007) and sunflower (Iqbal et al. 2005), which induced drought tolerance in these plant species. All these reports are analogous to what has been observed in the present study as increased accumulation of GB and proline also occurred in wheat plants grown under drought conditions. In the present study, exogenously-applied ALA enhanced GB and proline accumulation in drought-stressed wheat plants, but not a single report is available on the effect of ALA on proline or GB stimulation in growth under water deficit with which our findings could be compared. However, there are some other studies on the effect of ALA on the accumulation of these osmoprotectants under stress conditions other than drought. For example, ALA caused

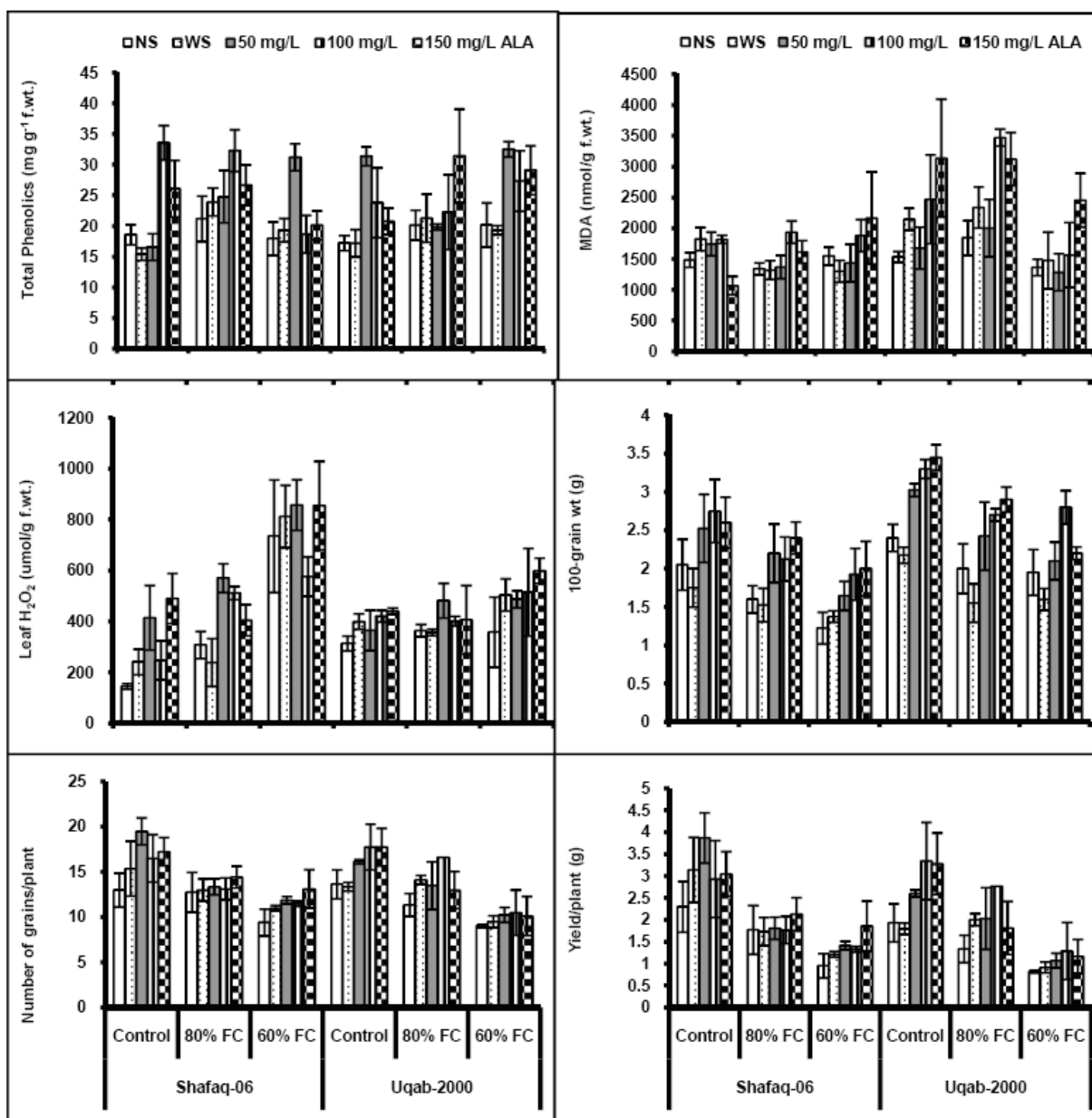


FIGURE 2. Shoot and root K⁺, Ca²⁺ and P and leaf glycinebetaine (GB) and free proline contents of wheat plants treated with aminolevulinic acid (ALA) foliarly at vegetative stage under control and drought stress (60% & 80% field capacity) conditions (Mean ± S.E)

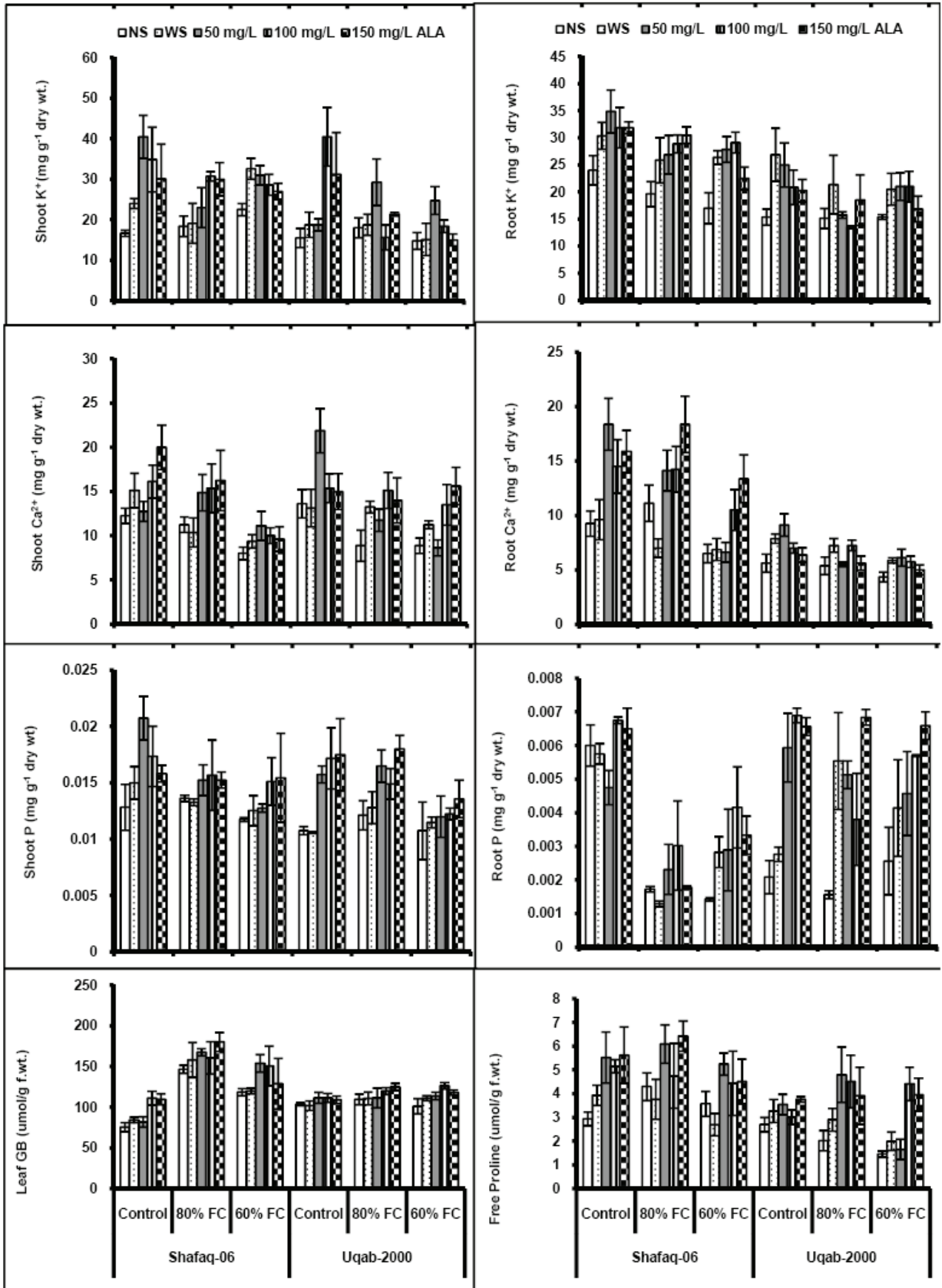


FIGURE 3. Leaf phenolics, malodialdehyde (MDA), hydrogen peroxide (H₂O₂) contents and different yield [100-grain wt. (weight), number of grains per plant and yield per plant] attributes of wheat plants treated with aminolevulinic acid (ALA) foliarly at vegetative stage under control and drought stress (60% & 80% field capacity) conditions (Mean ± S.E)

high accumulation of proline in pepper plants under low temperature stress (Korkmaz et al. 2010), cucumber under heat stress (Zhang et al. 2012) and oilseed rape under NaCl stress (Naeem et al. 2011). Therefore, ALA-induced change in biosynthesis of osmoprotectants/osmolytes particularly under arid environment still needs to be explored.

No significant change in leaf total phenolic and MDA contents observed in both the wheat cultivars under drought stress conditions (Table 1). Overall, cultivar Uqab-2000 was significantly higher in MDA contents than cultivar Shafaq-06 under all the water regimes, and the response of both cultivars to water deficit conditions or ALA in terms of leaf total phenolics was almost same (Figure 3). Exogenously-applied ALA markedly increased both attributes in wheat plants subjected to water stress. These results are analogous to the observations of Liu et al. (2011) who found that exogenously-applied ALA (0.1-1.0 ppm) improved plant growth and chlorophyll, while inhibited MDA and ROS in *Brassicca rapa* under water-deficit conditions. In addition, they also suggested that ALA could improve drought tolerance by maintaining growth due to improved antioxidants, while reduced ROS and MDA levels.

Yield in terms of yield per plant, grains per plant along with 100-grain weight decreased due to water stress markedly ($P \leq 0.001$) in both wheat cultivars. However, ALA foliar spray significantly improved all yield related attributes under varying water stress conditions. Cultivar Uqab-2000 was considerably better in 100-grain weight and cultivar Shafaq-06 in number of grains and yield per plant under stress and/or non-stress conditions (Table 1; Figure 3). A considerable ALA-induced modulation in different physio-biochemical processes under stress conditions has been observed, but little information is available on the role of ALA in crop yield improvement under drought stress (Akram & Ashraf 2013). In the present study, yield and yield related attributes of wheat plants decreased due to water stress in all the wheat plants, however foliar spray of ALA significantly improved all the yield related attributes of all the wheat plants under varying water regimes. ALA-induced improvement in grain yield has earlier been observed in wheat plants by Al-Thabet (2006). He ascribed this yield improvement to ALA-induced increase in water-use-efficiency and plant growth under drought stress. In another study, Al-Khateeb (2006) also observed improvement in drought-induced suppressed grain yield, spike length and number of grains which were positively correlated with ALA-induced improvement in rate of photosynthesis and stomatal conductance in barley plants. It is now widely known that ALA in low quantity augments chlorophyll synthesis resulting in enhanced photosynthesis, which in turn regulates growth and finally the yield of different crops (Akram & Ashraf 2013).

CONCLUSION

Overall, ALA application considerably enhanced leaf chlorophyll pigments, qN , NPQ, qP , root and shoot K^+ ,

Ca^{2+} and P accumulation, leaf proline, GB, total phenolics, MDA contents and grain yield. Better growth and yield of cultivar Shafaq-06 than those of cultivar Uqab-2000 under water deficit regimes was found to be associated with ALA-induced improvement in leaf fluorescence (qN , NPQ and F_v/F_m), shoot and root K^+ , root Ca^{2+} , proline and GB accumulation.

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REFERENCES

- Akram, N.A. & Ashraf, M. 2011a. Pattern of accumulation of inorganic elements in sunflower (*Helianthus annuus* L.) plants subjected to salt stress and exogenous application of 5-aminolevulinic acid. *Pakistan Journal of Botany* 43: 521-530.
- Akram, N.A. & Ashraf, M. 2011b. Improvement in growth, chlorophyll pigments and photosynthetic performance in salt-stressed plants of sunflower (*Helianthus annuus* L.) by foliar application of 5-aminolevulinic acid. *Agrochimica* 55: 94-104.
- Akram, N.A. & Ashraf, M. 2013. Regulation in plant stress tolerance by a potential plant growth regulator, 5-aminolevulinic acid (ALA). *Journal of Plant Growth Regulation* 32: 663-679.
- Akram, N.A., Shahbaz, M. & Ashraf, M. 2007. Relationship of photosynthetic capacity and proline accumulation with the growth of differently adapted populations of two potential grasses (*Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L.) to drought stress. *Pakistan Journal of Botany* 39: 777-786.
- Al-Khateeb, S.A. 2006. Promotive effect of 5-aminolevulinic acid on growth, yield and gas exchange capacity of barley (*Hordeum vulgare* L.) grown under different irrigation regimes. *Journal of the Saudi Society of Agricultural Sciences* 18: 103-111.
- Al-Thabet, S.S. 2006. Promotive effect of 5-aminolevulinic acid on growth and yield of wheat grown under dry conditions. *Journal of Agronomy* 5: 45-49.
- Arnon, D.T. 1949. Copper enzyme in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* 24: 1-15.
- Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology Advances* 27: 84-93.
- Ashraf, M., Akram, N.A., Al-Qurainy, F. & Foolad, M.R. 2011. Drought tolerance: Roles of organic osmolytes, growth regulators, and mineral nutrients. *Advances in Agronomy* 111: 249-296.
- Ashraf, M. & Khanum, A. 1997. Relationship between ion accumulation and growth in two spring wheat lines differing in salt tolerance at different growth stages. *Journal of Agronomy and Crop Science* 178: 39-51.
- Bates, L.S., Waldren, R.P. & Teare, I.D. 1973. Rapid determination of free proline for water stress studies. *Plant Science* 39: 205-207.
- Carmak, I. & Horst, J.H. 1991. Effects of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase

- activities in root tips of soybean (*Glycine max*). *Physiologia Plantarum* 83: 463-468.
- Cha-um, S., Samphumphuang, T. & Kirdmanee, C. 2013. Glycinebetaine alleviates water deficit stress in indica rice using proline accumulation, photosynthetic efficiencies, growth performances and yield attributes. *Australian Journal of Crop Science* 7: 213-218.
- Dolatabadian, A., Sanavi, A.M. & Sharifi, M. 2009. Alleviation of water deficit stress effects by foliar application of ascorbic acid on *Zea mays* L. *Journal of Agronomy and Crop Science* 195: 347-355.
- Duke, S.O. & Rebeiz, C.A. 1994. Porphyrin biosynthesis as a tool in pest management: An overview. In *Porphyrin Pesticides: Chemistry, Toxicology and Pharmaceutical Applications*, edited by Duke, S.O. & Rebeiz, C.A. Washington: American Chemical Society. pp. 1-17.
- Grieve, C.M. & Grattan, S.R. 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant and Soil* 70: 303-307.
- Iqbal, N., Ashraf, M.Y. & Ashraf, M. 2005. Influence of water stress and exogenous glycinebetaine on sunflower achene weight and oil percentage. *International Journal of Environmental Science and Technology* 2: 155-160.
- Jackson, M.L. 1962. *Soil Chemical Analysis*. London: Contable Co. Ltd.
- Julkenen-Titto, R. 1985. Phenolic constituents in the leaves of northern willows: Methods for the analysis of certain phenolics. *Journal of Agricultural and Food Chemistry* 33: 213-217.
- Korkmaz, A., Korkmaz, Y. & Demirkiran, A.R. 2010. Enhancing chilling stress tolerance of pepper seedlings by exogenous application of 5-aminolevulinic acid. *Environmental and Experimental Botany* 67: 495-501.
- Li, D., Zhang, J., Sun, W., Li, Q., Dai, A. & Bai, J. 2011. 5-aminolevulinic acid pretreatment mitigates drought stress of cucumber leaves through altering antioxidant enzyme activity. *Scientia Horticulturae* 130: 820-828.
- Liu, D., Wu, L., Naeem, M.S., Liu, H., Deng, X., Xu, L., Zhang, F. & Zhou, W. 2013. 5-Aminolevulinic acid enhances photosynthetic gas exchange, chlorophyll fluorescence and antioxidant system in oilseed rape under drought stress. *Acta Physiologiae Plantarum* 9: 2747-2759.
- Liu, D., Pei, Z.F., Naeem, M.S., Ming, D.F., Liu, H.B., Khan, F. & Zhou, W.J. 2011. 5-Aminolevulinic acid activates antioxidative defense system and seedling growth in *Brassica napus* L. under water deficit stress. *Journal of Agronomy and Crop Science* 197: 284-295.
- Liu, W.Q., Kang, L. & Wang, L.J. 2006. Effect of 5-aminolevulinic acid (ALA) on photosynthesis and its relationship with antioxidant enzymes of strawberry leaves. *Acta Botany Boreal-Occident Sinica* 26: 57-62.
- Maruyama-Nakashita, A., Hira, M.Y., Funada, S. & Fuek, S. 2010. Exogenous application of 5-aminolevulinic acid increases the transcript levels of sulfur transport and assimilatory genes, sulfate uptake, and cysteine and glutathione contents in *Arabidopsis thaliana*. *Soil Science and Plant Nutrition* 56: 281-288.
- Metwaly, M.M. 2012. Ecophysiological and anatomical responses of drought stressed wheat plants (*Triticum aestivum* L.) treating with some bacterial endophytes. Ph.D. Thesis, Faculty of Agriculture, Kafre El-Sheikh University, Kafre El-Sheikh, Egypt (Unpublished).
- Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Sciences* 7: 405-410.
- MSTAT Development Team. 1989. MSTAT User's Guide: A Microcomputer Program for the Design Management and Analysis of Agronomic Research Experiments. Michigan State University, East Lansing.
- Naeem, M.S., Rasheed, M., Liu, D., Jin, Z.L., Ming, D.F., Yoneyama, K., Takeuchi, Y. & Zhou, W.J. 2011. 5-Aminolevulinic acid ameliorates salinity-induced metabolic, water-related and biochemical changes in *Brassica napus* L. *Acta Physiologiae Plantarum* 33: 517-528.
- Naeem, M.S., Jin, Z.L., Wan, Z.L., Liu, D., Liu, H.B., Yoneyama, K. & Zhou, W.J. 2010. 5-aminolevulinic acid improves photosynthetic gas exchange capacity and ion uptake under salinity stress in oilseed rape (*Brassica napus* L.). *Plant and Soil* 332: 405-415.
- Navari-Izzo, F., Pinzino, C., Quartacci, M.F. & Sgherri, C.L.M. 1994. Intracellular membranes: Kinetics of superoxide production and changes in thylakoids of resurrection plants upon dehydration and rehydration. *Proceedings of the Royal Society of Edinburgh* 102B: 187-191.
- Nezhadahmadi, A., Prodhan, Z.H. & Faruq, G. 2013. Drought tolerance in wheat. *Scientific World Journal* 2013: 610721.
- Nishihara, E., Kondo, K., Parvez, M.M., Takahashi, K., Watanabe, K. & Tanaka, K. 2003. Role of 5-aminolevulinic acid (ALA) on active oxygen-scavenging system in NaCl-treated spinach (*Spinacia oleracea*). *Journal of Plant Physiology* 60: 1085-1091.
- Pervez, M.A., Ayub, C.M., Khan, H.A., Shahid, M.A. & Ashraf, I. 2009. Effect of drought stress on growth, yield and seed quality of tomato (*Lycopersicon esculentum* L.). *Pakistan Journal of Agricultural Sciences* 46: 174-178.
- Sankar, B., Jaleel, C.A., Manivannan, P., Kishorekumar, A., Somasundaram, R. & Panneerselvam, R. 2007. Drought-induced biochemical modifications and proline metabolism in *Abelmoschus esculentus* (L.) Moench. *Acta Botanica Croatica* 66: 43-56.
- Strasser, R.J., Srivastava, A. & Govindjee. 1995. Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. *Photochemistry and Photobiology* 61(1): 32-42.
- Velikova, V., Yordanov, I. & Edreva, A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines. *Plant Science* 151: 59-66.
- Wang, L.J., Jiang, W.B., Liu, H., Liu, W.Q., Kang, L. & Hou, X.L. 2005. Promotion by 5-aminolevulinic acid of germination of pakchoi (*Brassica campestris* ssp. *Chinensis* var. *communis* Tsen et Lee) seeds under salt stress. *Journal of Integrative Plant Biology* 47: 1084-1091.
- Wang, L.J., Shi, W., Liu, H., Liu, W.Q., Jiangm, W.B. & Hou, X.L. 2004. Effects of exogenous 5-aminolevulinic acid treatment on leaf photosynthesis of pakchoi. *Journal of Nanjing Agricultural University* 47: 1084-1091.
- Wang, L.J., Jiang, W.B., Zhang, Z., Yao, Q.H., Matsui, H. & Ohara, H. 2003. Biosynthesis and physiological activities of 5-aminolevulinic acid (ALA) and its potential application in agriculture. *Plant Physiology Communications* 39: 185-192.
- Watanabe, K., Tanaka, T., Hotta, Y., Kuramochi, H. & Takeuchi, Y. 2000. Improving salt tolerance of cotton seedlings with 5-aminolevulinic acid. *Plant Growth Regulation* 32: 99-103.
- Wolf, B. 1982. A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Communications in Soil Science and Plant Analysis* 13: 1035-1059.

Youssef, T. & Awad, M.A. 2008. Mechanisms of enhancing photosynthetic gas exchange in date palm seedlings (*Phoenix dactylifera* L.) under salinity stress by a 5-aminolevulinic acid-based fertilizer. *Journal of Plant Growth Regulation* 27: 1-9.

Zhang, J., Gao, Y., Yu, B., Xia, C.X. & Bai, J.G. 2012. Pretreatment with 5-aminolevulinic acid mitigates heat stress of cucumber leaves. *Biologia Plantarum* 56: 780-784.

Nudrat Aisha Akram*, Shamim Kausar & Naila Farid
Department of Botany
Government College University Faisalabad
Pakistan

Muhammad Ashraf
Pakistan Science Foundation
Islamabad
Pakistan

Muhammad Ashraf & Fahad Al-Qurainy
Department of Botany and Microbiology
King Saud University, Riyadh
Saudi Arabia

*Corresponding author; email: nudrataauaf@yahoo.com

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