

Response of barley quality traits, yield and antioxidant enzymes to water-stress and chemical inducers

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Abstract

Two field experiments were carried out in order to investigate the effect of chemical inducers [benzothiadiazole 0.9 mM l⁻¹, oxalic acid 1.0 mM l⁻¹, salicylic acid 0.2 mM l⁻¹] on physiological and technological traits as well as on yields and antioxidant enzyme activities of barley grown under abiotic stress (i.e. water surplus and deficit conditions). Results showed that relative water content, leaf area, chlorophyll and yield as well as technological properties of barley were improved with chemical inducers application under water surplus and water-stress conditions. Antioxidant enzymes activity (i.e. catalase and peroxidase) were significantly increased in barley grown under water-stress and treated with chemical inducers. Yield and related parameters of barley presented also significant decrease under water-stress treatment, while chemical inducers application enhanced the yield-related traits. Starch and protein contents were higher in plants treated with salicylic acid than in untreated plants when water-stress was applied. In conclusion, results show that chemical inducers application have a positive interaction and synergetic influence and should be suggested to improve plant growth, yield and technological properties of water stressed barley. Salicylic acid application was better than oxalic acid and benzothiadiazole in terms of plant growth and yield improvement.

Keywords: *Hordeum vulgare* L.; Drought stress; Yield; Quality; Antioxidant enzymes.

Introduction

Barley (*Hordeum vulgare* L.) is considered the fourth crop among the most produced cereal grains worldwide after wheat, rice and maize. It is usually considered as one of the main sources for feeding animal (i.e. adult monogastrics and ruminants); however there is an interest in using it for human food (Biel and Jacyno, 2013). Barley can grow well in North Coastal Region as well as in newly reclaimed soils, in particular when it grows under water-stress as attributable to its tolerance to abiotic stress (El-Seidy et al., 2013). Therefore, barley cultivation is suited in Egypt owing to the shortage of water supply. Barley is a moderate salt tolerance crop and has the ability for growing in a varied environmental stresses conditions such as arid, poor or saline soils (Abd El-Hady, 2007).

Abiotic stress such as drought impedes steady global food disposal with growing population of the world. Abiotic stresses as a result of water-stress can cause a reduction in growth parameters such as shoot length and weight, leaf area and biomass of barley

plants (Fayez and Bazaid, 2014; Hafez et al., 2015). Furthermore, it lessens plant metabolic processes comprising relative water content (RWC), nutrient absorption and chlorophyll content (Cossania et al., 2012; Ahmed et al., 2013; Hafez et al., 2014b). It is stated that high RWC is a significant indicator for drought stress tolerance and such high RWC can cause high osmotic regulation or reduce elasticity of tissue plant cell walls (Ritchie et al., 1990). Additionally, abiotic stress can cause an oxidative stress in plant cells, consequently can result in a higher leakage of electrons towards O^2 through the two processes of photosynthetic and respiratory which resulting in an enhancement of reactive oxygen species generation (Asada, 2006). A lot of the damage on plants grown with abiotic stress is associated with oxidative damage at the cellular level and this can cause death of plant cells (Mittler, 2002).

Plants which contain high activity of antioxidant enzymes have shown a significant tolerance to the oxidative damage initiated by reactive oxygen species (Gapinska et al., 2008). Antioxidant advantages in cereals have been believed to be one of the main physiological mechanisms and can be pivotal in relation to water-stress (Cossania et al., 2012). Exogenous applications through chemical inducers have shown a vital role in scavenging free radicals and decrease the negative impacts of increased reactive oxygen species as a result of water-stress (Anjum et al., 2011).

Salicylic acid is known as a hormone-like substance, which can play a vital role in photosynthesis process and stomatal conductance (Khan et al., 2003; Arfan et al., 2007), increasing antioxidative protection (Xu et al., 2008). Benzothiadiazole is a chemical inducer of tolerance and a functional analogue to salicylic acid (Görlach et al., 1996). The synthetic chemical benzothiadiazole was also revealed to be an effective systemic acquired resistance (SAR) activator (Görlach et al., 1996; Lawton et al., 1996) that supplies protection in the field against some biotic and abiotic stresses of different plant species. Thus, benzothiadiazole seems to be appropriated application for applied agronomic use (Hafez et al., 2012; Hafez et al., 2014a).

Also, exogenous application of oxalic acid and salicylic acid were effective in the regulation of biotic and abiotic stresses as well as physiological processes (Gunes et al., 2007). Application of those chemical inducers induced the resistance of plants to drought stress as a result of antioxidant capability (Zheng et al., 1999; Malencic et al., 2004).

Although, various investigations have been carried out to investigate growth of barley grown with water-stress, still a little data are available regarding the role of exogenous application (i.e. benzothiadiazole, salicylic acid and oxalic acid) on growth, yield and technological properties of barley as well as the activity of antioxidant enzymes when grown under water-stress conditions.

Therefore, the objectives of the current investigation were to evaluate whether benzothiadiazole, salicylic acid or oxalic acid can improve the negative effect of water-stress on barley via monitoring yield and its components, physiological and quality traits in addition to the changes of antioxidant enzymes activity.

Materials and Methods

Plant materials and experimental design

The present investigation was conducted at the Experimental Farm, Faculty of Agriculture, Kafrelsheikh University, Egypt (31°05'N and 30°57'E) during the two

successive growing seasons of 2014/2015 and 2015/2016 to study the effect chemical inducers application on growth, yield and quality traits of barley [*Hordium vulgare* L., Cv. Giza 132, (six rows)] grown under surplus- and water-stress. The experiment was arranged in a Split-Plot design with three replicates. It included two factors (i.e. water regime and chemical inducers). Two water treatments (i.e. water-stress and well-watered) were arranged in main plots and plots were separated well to avoid the infiltration when irrigation was applied. Four treatments of chemical inducers [i.e. control, benzothiadiazole, oxalic acid and salicylic acid] were placed in sub-plots. Water-stress treatment was applied after two successive irrigations (i.e. at sowing day and tillering stage) by withholding the water supply from the end of tillering stage until physiological maturity stage. However, well-watered treatment was applied through adding water for three irrigations (i.e. at sowing day, tillering stage and booting stage). The three chemical inducers: benzothiadiazole, oxalic acid and salicylic acid were applied at concentrate of 0.9 mM l⁻¹, 1.0 mM l⁻¹ and 0.2 mM l⁻¹, respectively and were added as foliar spray on two equal doses at 45 and 60 days after sowing (DAS) for each plot during the vegetative growth stage. The amount of each chemical inducer was estimated based on 500 L ha⁻¹ for each dose. The size of experimental sub-plot was 20.0 m² (4.0 m width × 5.0 m length). Barley (*Hordium vulgare* L., Cv. Giza 132) is originated in barley department, Field Crops Research Institute, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Grains were sown on 2th December 2014 in first season, while were sown on 5th December 2015 in second season. The space between rows was 12.5 cm and the seeding rate was 120 kg grains ha⁻¹. The preceding crop in the cultivated farm was rice (*Oryza sativa* L.) during the two growing seasons. Calcium superphosphate (15.5% P₂O₅) was added during seedbed preparation prior to sowing at level of 125 kg ha⁻¹. Nitrogen fertilizer was applied at level of 110 kg N ha⁻¹ as Urea (46% N) at two doses during the growing of barley. First dose accounted for 40% of the total dose and was applied before first irrigation, while the second dose accounted for 60% and was applied before second irrigation. Weeds were controlled by using Topik 15% WP herbicide (Syngenta, Basel, Switzerland) as well as mechanical control was used. The harvest dates during two seasons were 2nd May 2015 and 10th May 2016.

The weather conditions (Figure 1) were obtained from an agro-meteorological Sakha Station, Kafrelsheikh Governorate, Egypt located at 2 km from the Experimental Farm. Soil samples were collected from 0-20 cm depth prior to sowing date in each season using a soil Auger, then were stored at -20 °C for further analysis. The soil was clayey and the bulk densities were 1.21 and 1.26 g cm⁻³ for first and second seasons, respectively. The soil contained; total organic matter: 1.40 and 1.38%, available nitrogen (N): 24 and 26 mg kg⁻¹, available phosphorus (P): 36.0 and 38.4 mg kg⁻¹, exchangeable potassium (K): 0.26 and 0.27 g kg⁻¹, EC: 1.03 and 1.10 ds m⁻¹ (1:5), pH 8.0 and 8.1 (1:2.5), annual precipitation: 1.40 and 1.43 cm in seasons of 2014–2015 and 2015–2016, respectively.

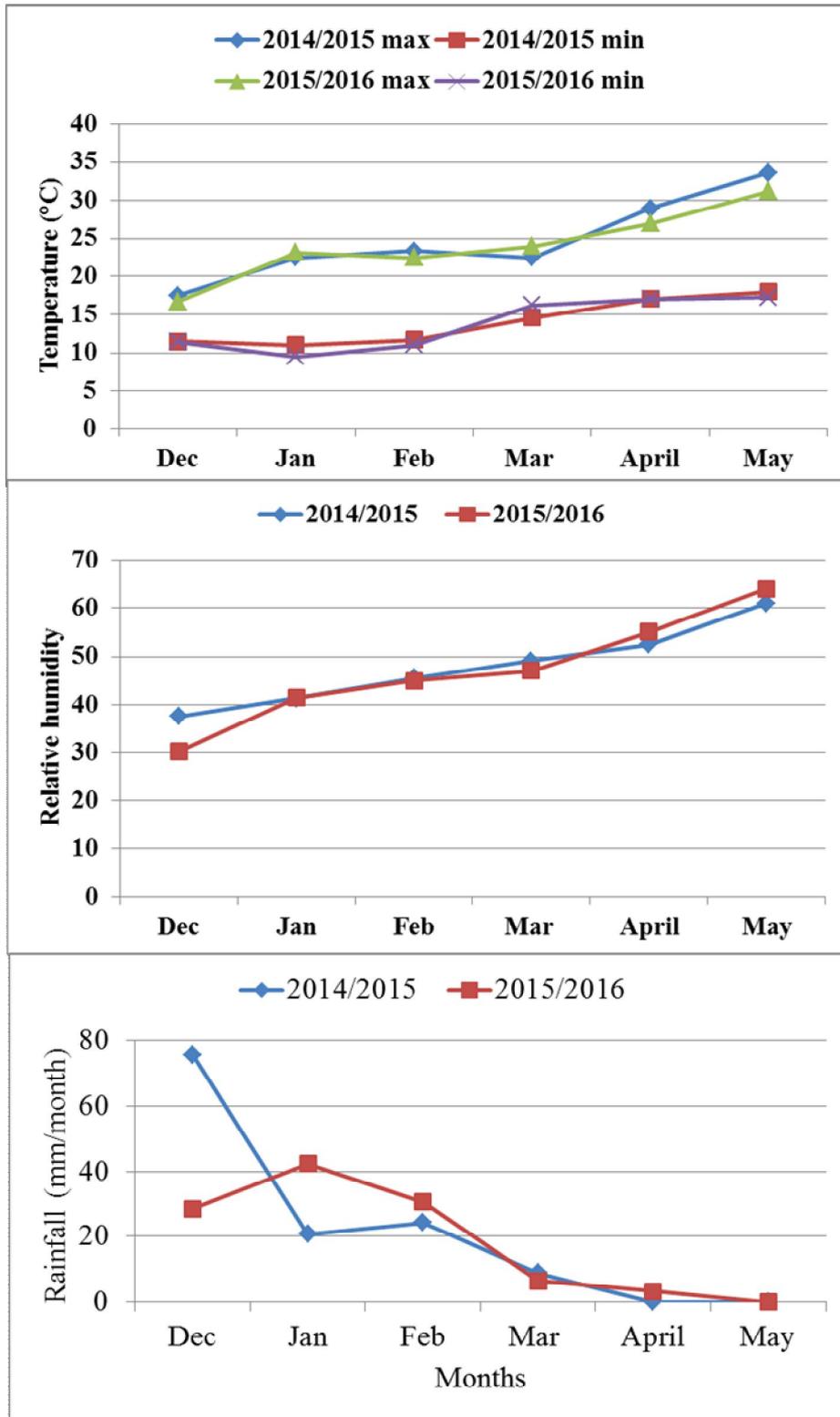


Figure 1. Monthly relative humidity (%), minimum and maximum air temperatures during the two winter growing seasons. *max* = maximum, *min* = minimum.

Measurements

Morphological and Physiological measurements

Leaf area index (LAI): From each plot, ten plants were collected at heading stage (BBCH stage 51, Meier 2001) to measure leaf area using Leaf Area Meter (Model: Li-Cor 3100, Lambda Instruments Co., USA). Leaf area of those plants was then divided by the specific ground area for ten plants to get LAI.

Chlorophyll content (SPAD): Hand-held chlorophyll meter (Model: SPAD-502, Minolta Sensing Ltd, Japan) was used to record SPAD readings from the topmost fully expanded leaves on each main stem at heading stage (BBCH stage 51, Meier 2001). SPAD values were measured at three different points along the flag leaf blade and then the readings were averaged to have a single value for a plant as described by Markwell et al. (1995).

Leaf relative water content: Relative water content was measured at heading stage (BBCH stage 51, Meier 2001) by using fully expanded leaves, weighted for fresh weight (FW). Turgid weight (TW) was measured by rehydrated the collected leaves in purified water into a closed container at +10 °C in the dark for 24 hour and weighted again. Dry weight (DW) was measured for the same leaves after oven-drying for 72 hour at +65 °C. Relative water content (RWC) was estimated as follows (Jeon et al., 2006):

$$RWC (\%) = \frac{FW - DW}{TW - DW} \times 100$$

Antioxidant enzymes (peroxidase and catalase activities): The studied antioxidant enzyme activities were measured in plant leaves. About 0.5 g of leaves was homogenized at 0-4 °C in 3 mL of 50 mM TRIS buffer (pH 7.8), containing 1.0 mM Ethylene Diamine Tetraacetic Acid DiSodium (EDTA-Na₂) and 7.5% polyvinylpyrrolidone. The homogenized samples were centrifuged with 12000 rpm for 20 min at 4.0 °C and then the activities of total soluble enzyme were measured spectrophotometrically in the supernatant (Hafez et al., 2012). All samples were poured into semi-micro-cuvettes and the absorbance was detected at +25 °C by using spectrophotometer at 430 nm (UV-160A, Shimadzu, Japan).

Catalase activity (CAT) was determined spectrophotometrically according to Aebi (1983). Changes in the absorbance at 240 nm were detected every 30 sec intervals for 3 min. Enzyme activity was expressed as the increase in absorbance min⁻¹ g⁻¹ fresh weight. Activity of guaiacol peroxidase activity was directly determined of the crude enzyme extract as described by Hammerschmidt et al. (1982). Changes in absorbance at 470 nm were recorded every 30 sec intervals for 3 min. Enzyme activity was expressed as the increase in absorbance min⁻¹ g⁻¹ fresh weight.

Yield and its components: Barley grain yield (14% moisture) was obtained by harvesting 4.0 m² (2 m × 2 m) from the middle of each plot, while yield components were determined by collecting 10 plants from two outer rows within each plot. After

harvest, biological and grain yield as well as yield components (i.e. number of spikes m^{-2} , number of grains spike $^{-1}$ and 1000-grain weight) were measured. Plant samples collected at harvest were separated into grains and straw and then dried in electric oven at +65 °C for 72 hour until constant dry weight. Dried samples were grounded and fine powder for each sample was passed through 0.5 mm sieve to be used for the analysis of quality traits.

Quality traits

Protein content: Nitrogen was analyzed by using the standard procedure of micro-Kjeldahl digestion with Sulfuric acid. N content was then multiplied by 5.83 to obtain the content of grain protein (A.O.A.C., 1990).

Ash content was determined from about 1.0 g ground grain samples. Samples were further dried in an oven at +105 °C overnight. The dry weight was determined (W_1) and the sample was put into in a muffle furnace at 580 °C for 8 h. Then, samples were cooled in a desiccator and weighed again (W_2). The ash content was calculated as follows: W_2/ W_1 and the net value multiplied by 100 to get ash %.

Starch: About 100 mg milled sample (0.5 mm) was weighted to analyze the starch content with an assay kit (K-TSTA-50A/K-TSTA-100A, Megazyme, Wicklow, Ireland) and protocol supplied by the manufacturer using the UV-spectrophotometer (Model UV-1800 240V IVDD, Shimadzu Inc., Kyoto, Japan).

Statistical analysis

All Data were subjected to an analysis of variance (ANOVA) procedures according to (Gomez and Gomez, 1984) using the MSTAT-C Statistical Software package. Different Means were compared using (Duncan, 1955) when the differences were significant ($P \leq 0.05$).

Results

Effects of water-stress and chemical inducers on barley growth at heading stage:

Water-stress treatment resulted in a negative effect on growth of barley in terms of leaf area index, chlorophyll and relative water content during both of growing seasons in comparison to well-watered treatment at heading stage (BBCH 51, Meier 2001), (Figure 2). Nevertheless, foliar application of chemical inducers resulted in improvement leaf area index, chlorophyll and relative water content in comparison to control treatment, practically when plants grown under water-stress conditions (Figure 2).

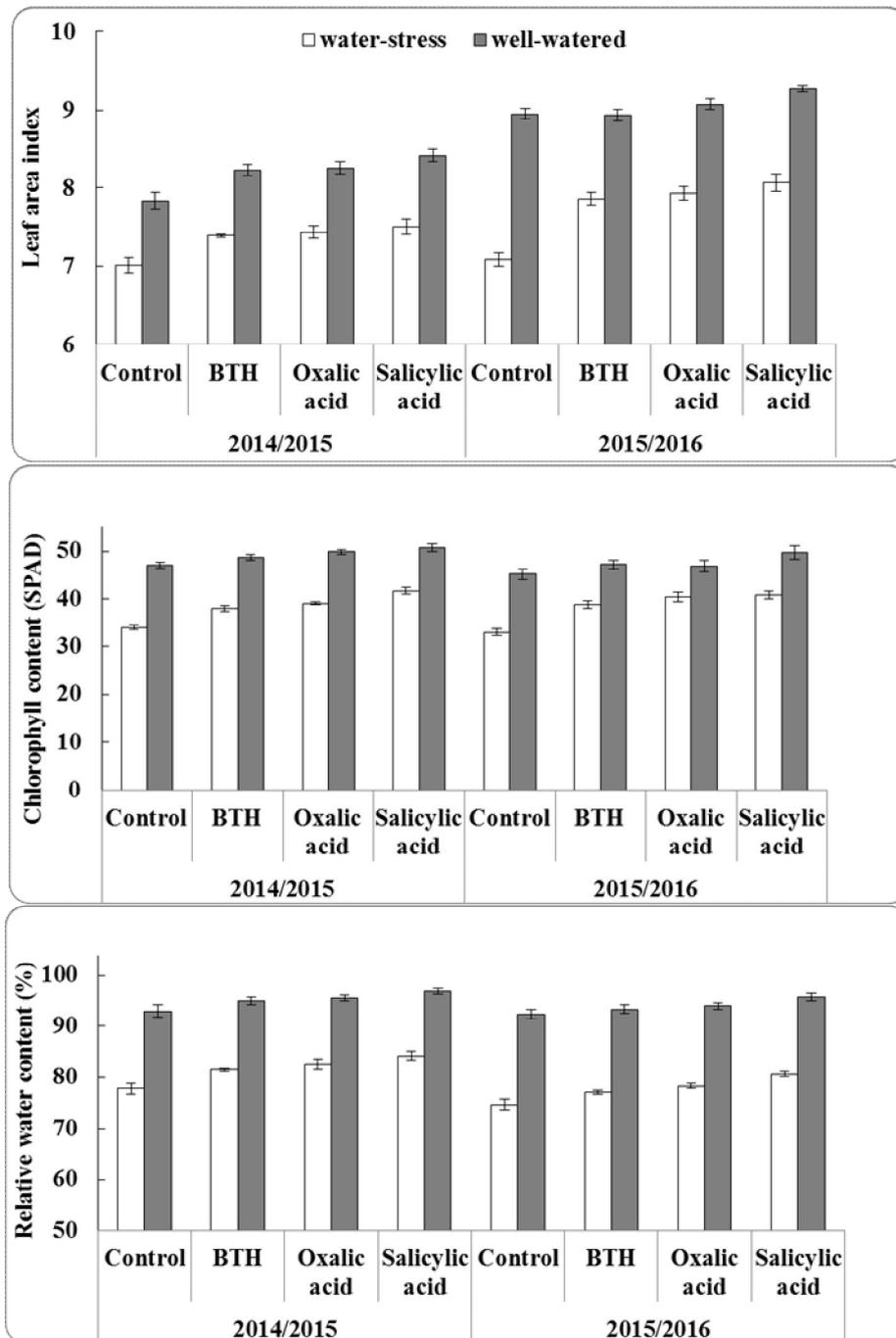


Figure 2. Effects of water-stress and chemical inducers on barley growth traits at heading stage. BTH= benzothiadiazole. Bars are standard error of means.

Effects of water-stress and chemical inducers on antioxidant enzymes activity

Peroxidase and catalase activities in leaves extracts of barley plants grown under water-stress or well-watered conditions were significantly increased during the two growing seasons when different chemical inducers were applied in comparison to the control treatment (Figure 3).

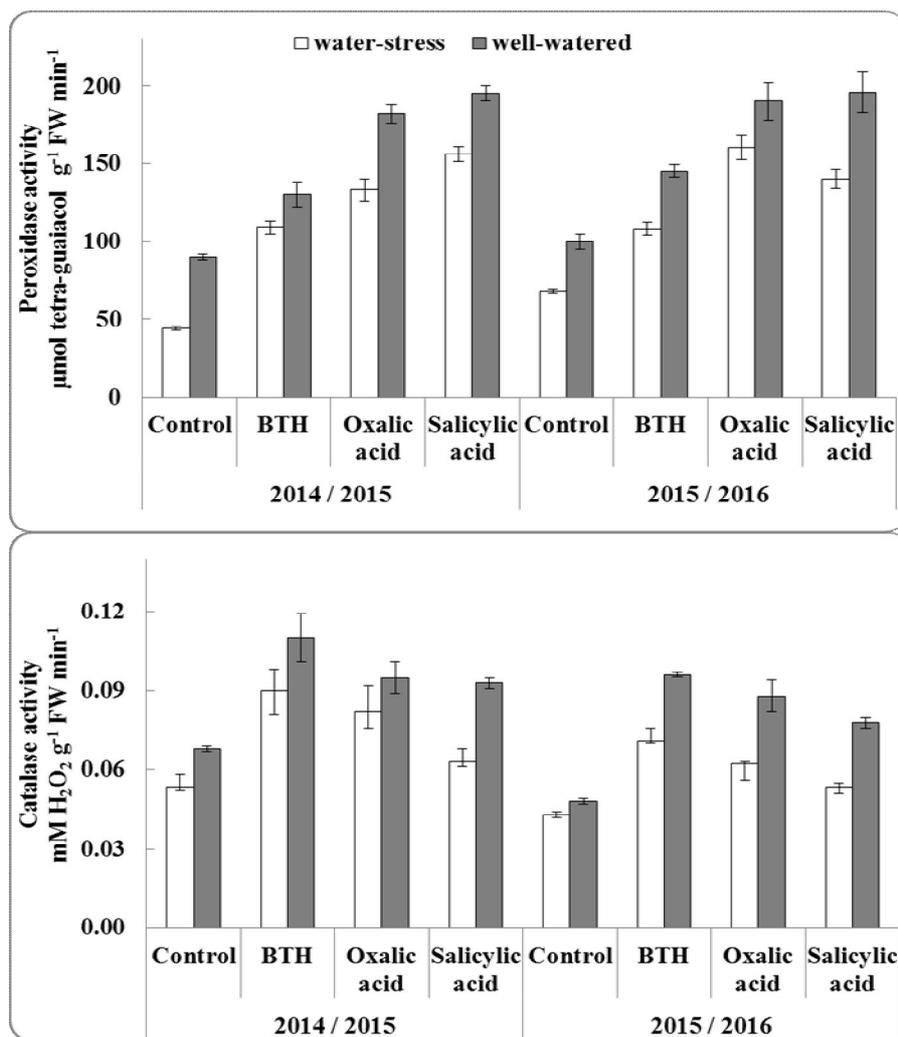


Figure 3. Effects of water-stress and chemical inducers on antioxidant enzymes activity in barley leaves. BTH= benzothiadiazole. Bars are standard error of means.

Salicylic and oxalic acids application resulted in higher activity of peroxidase in plant leaves grown under water-stress than in plants treated with benzothiadiazole, while the lowest activity was obtained from plants grown under control treatments. Foliar spray of benzothiadiazole, oxalic and salicylic acids increased the activity of catalase in leaves of barley by 67.4, 49.2 and 21.5% in comparison to the activity of catalase in leaves of untreated-plants grown under water-stress condition as average during both of the growing seasons.

Effects of water-stress and chemical inducers on yield and related traits

Data showed that water-stress treatment significantly reduced the yields (i.e. grain and straw yields) and its related parameters (i.e. 1000-grain weight, number of grains spike⁻¹, number of spikes m⁻²) in comparison to barley plants grown with well-watered treatment (Tables 1, 2). However, exogenous spraying of the chemical inducers on barley grown under water-stress or well-watered conditions improved the 1000-grain weight and increased number of grains spike⁻¹, number of spikes m⁻² and consequently increased grain and straw yield and harvest index (Tables 1, 2). Salicylic acid is

considered the best chemical inducer among the applied chemical inducers in the current study, because its applications resulted in the highest grain and straw yields when barley grown under water-stress conditions. Salicylic acid application increased 1000-grain weight by 7.5%, number of grains spike⁻¹ by 16.4%, number of spikes m⁻² by 4.5%, grain yield by 28.4% and straw yield by 9.4% as average during both of seasons in comparison to control treatment.

Table 1. Effects of water-stress and chemical inducers on 1000-grain weight, number of grains spike⁻¹ and number of spikes m⁻² during the two growing seasons.

Treatments	1000-grain weight (g)		No. grains spike ⁻¹		No. spikes m ⁻²	
	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂
Water treatments (W)						
Water-stress	40.03 ^b	41.45 ^b	26.45 ^b	26.35 ^b	300.93 ^b	302.66 ^b
Well-watered	43.52 ^a	44.28 ^a	29.23 ^a	29.72 ^a	318.34 ^a	319.55 ^a
Chemical inducers (C)						
Control	40.23 ^c	43.38 ^d	25.00 ^b	26.61 ^c	304.18 ^b	305.93 ^c
Benzothiadiazole	42.08 ^b	43.29 ^c	28.33 ^{ab}	27.27 ^b	316.59 ^a	315.49 ^b
Oxalic acid	42.02 ^b	44.26 ^b	30.50 ^a	29.03 ^{ab}	317.06 ^a	317.36 ^a
Salicylic acid	43.21 ^a	46.68 ^a	30.33 ^a	29.68 ^a	319.00 ^a	317.95 ^a
W	*	*	*	*	*	*
C	*	*	*	*	*	*
W × C	ns	ns	ns	ns	ns	ns

Data within columns followed by different letters are significantly different at * = $P \leq 0.05$; ns, no significant difference. S₁= 2014/2015 season, S₂= 2015/2016 season.

Effects of water-stress and chemical inducers on grain quality traits

Generally, the highest grain protein content was obtained from plants grown under water-stress conditions, while the highest ash and starch contents were obtained from well-watered plants (Figure 4). The highest protein content was obtained from barley when salicylic acid was applied under water-stress conditions followed by barley plants treated with oxalic acid application, while the lost protein content was obtained from barley treated with benzothiadiazole or untreated plants (Figure 4).

Table 2. Effects of water-stress and chemical inducers on yields and harvest index during the two growing seasons.

Treatments	Grain yield (ton ha. ⁻¹)		Straw yield (ton ha. ⁻¹)		Harvest index (%)	
	S ₁	S ₂	S ₁	S ₂	S ₁	S ₂
Water treatments (W)						
Water-stress	4.78 ^b	5.02 ^b	10.59 ^b	11.06 ^b	31.11 ^b	31.21 ^b
Well-watered	6.33 ^a	6.02 ^a	12.66 ^a	13.35 ^a	33.33 ^a	31.08 ^b
Chemical inducers (C)						
Control	5.02 ^c	5.33 ^c	11.44 ^c	12.39 ^c	30.49 ^c	30.07 ^b
Benzothiadiazole	5.99 ^b	5.78 ^b	12.18 ^b	12.99 ^b	32.98 ^b	30.80 ^b
Oxalic acid	6.04 ^b	6.06 ^b	12.09 ^b	12.92 ^b	33.33 ^b	31.95 ^{ab}
Salicylic acid	6.59 ^a	6.68 ^a	12.87 ^a	13.18 ^a	33.86 ^a	33.65 ^a
W	*	*	*	*	*	*
C	*	*	*	*	*	*
W × C	ns	ns	ns	ns	ns	ns

Data within columns followed by different letters are significantly different at * = $P \leq 0.05$; ns, no significant difference. S₁= 2014/2015 season, S₂= 2015/2016 season.

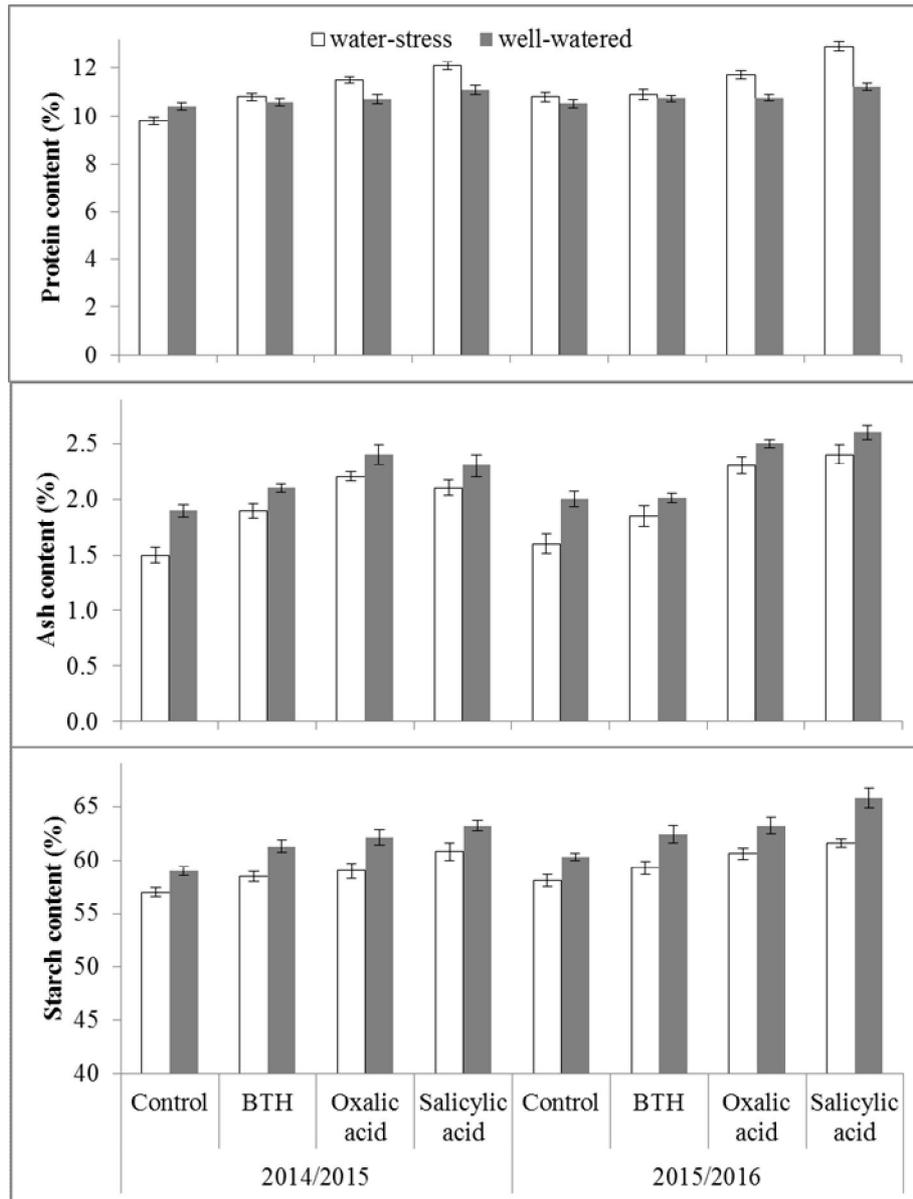


Figure 4. Effects of water-stress and chemical inducers on grain quality traits. BTH= benzothiadiazole . Bars are standard error of means.

Discussions

Generally, chemical inducers used in the current study improved growth, yield and technological properties of barley grown under water-stress or well-watered conditions.

Water-stress led to loss of turgor pressure in the leaves and consequently resulted in closeness of the pores and decreased of photo-assimilates (Wu et al., 2008; Hafez and Kobata, 2012). Low relative water content could be due to a reduction of water supply to the leaves (Ghotbi-Ravandi et al., 2014). Some authors demonstrated that negative impacts of water-stress on stomata closure and photosynthesis rate led to a decline in leaf area and chlorophyll content (Ghotbi-Ravandi et al., 2014) and this could be a main reason for decreasing leaf area index.

Exogenous application of salicylic acid resulted in a higher relative water content, leaf area index and chlorophyll content than the application of other used chemical inducers or even untreated-plants (Figure 2). This positive improvement could alleviate the negative effects of water-stress treatment through increasing the activity of antioxidant enzymes such as catalase and peroxidase as reported by (Hafez et al., 2012; Hafez et al., 2014a). Furthermore, it could be attributed to the lessen of osmotic potential, which could be a benefit to preserve the metabolic process and consequently improve plant growth via preserving cell turgor additionally regulating growth division of plant cells (Pirasteh-Anosheh et al., 2015).

Salicylic acid and its close analogues improved the leaf area and dry mass of maize and soybean production (Khan et al., 2003). Also, foliar application of salicylic acid on barley plants improved growth (Pancheva et al., 1996). In addition, a significant increase in maize growth in terms of photosynthetic rate was obtained when salicylic acid was sprayed (Khodary, 2004). The foliar application of salicylic acid also increased the content of carbohydrate in grain of maize (Khodary, 2004).

Enzymes activity (i.e. catalase and peroxidase) in barley leaves were significantly increased with the application of exogenous application through chemical inducers when plants were grown under water-stress or well-watered conditions. The increase in the activity of antioxidant enzymes could be attributed to the high defense capability, in addition to their characteristics which improve the plant's resistance against the oxidative damage, the powdery mildew and the severity of diseases (Hafez et al., 2012). The increase in the antioxidant enzymes activity could benefit the plants grown under water-stress to conserve their growth which could be a vital indicator of water-stress resistance (Noctor et al., 2014). The high activity of antioxidants enzymes can neutralize the harmful effects of reactive oxygen species in plants. Mostly, reactive oxygen species seems to have an important role through eliciting localized death of plant and pathogen cells and as a diffusible signal for the induction of antioxidant and pathogenesis-related genes in adjacent plant tissues (Hafez et al., 2012).

The reduction in yield and related parameters of barley grown under water-stress conditions could be due to the reduction of photosynthesis as a result for the low water supply (Samarah, 2005). Additionally, it might be attributed to less uptake of needed nutrients from soil and consequently affected cell division and differentiation with related decrease in and number of spikes m^{-2} , number of grains spike $^{-1}$ and 1000-grain weight (Pecio and Wach, 2015). Chemical inducers application was valuable in alleviating the negative effect of water-stress on barley plants. Our results are in agreement with earlier investigations which stated that application of salicylic acid (Bandurska and Stroinski, 2005) and oxalic acid in addition to benzothiadiazole (Hafez et al., 2014a) improved plant tolerance to water-stress through utilizing the soil water more efficiently in comparison to control treatment through increasing the level of abscisic acid and proline which can control the close of stomata.

Chemical inducers significantly improved grain protein content compared to control under water treatments in both seasons. Salicylic acid application resulted in the highest protein, ash and starch contents in grain of barely followed by oxalic acid or benzothiadiazole applications when barley was grown under well-watered or water-stress treatments. Gunes et al. (2007) found that salicylic acid application can play a vital role as endogenous signal molecule responsible for inducing abiotic stress

tolerance in plants. Also, they reported that chemical inducer of salicylic acid significantly improved content of protein when plants grown under water-stress or non-water-stress conditions.

Conclusion

It was obvious that the negative impact of water-stress on barley plants was significantly improved through application of different chemical inducers. Chemical inducers application improved yields and related traits as well as quality traits of barley in particular when plants grown under water-stress conditions. Salicylic acid is considered the most appreciated chemical inducers in the current study followed by oxalic acid and finally benzothiadiazole. Also, chemical inducers significantly enhanced the activity of antioxidant enzymes (i.e. catalase and peroxidase). Further research can be given to barley plants grown under different stressed-factors such as heat; heavy metal stress and etc. when foliar chemical inducers are applied.

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