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Alleviation The Adverse Effects Of Drought Stress On Maize Growth And Yield By Application Of Salicylic Acid And Abscisic Acid

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Abstract

This experiment was conducted to evaluate the effect of salicylic acid and abscisic acid on the growth and biochemical characteristics of maize under different humidity regimes. The study used split plot design in which main plot includes three levels of humidity regimes: 1- whole irrigation (100 % used water based on the plant demand at various growing stages), 2 and 3- two levels of deficit irrigations (75 and 50% used water). Secondary plot includes: 1- application of ABA, 2- application of SA, 3- control. Water stress decreased plant height and maize yield, but soluble protein, soluble carbohydrate, proline and malondialdehyde (MDA) significantly increased. Foliar spraying of abscisic acid and salicylic acid significantly increased plant height, yield and proline content. Concentration of antioxidant enzyme markedly enhanced in treatments combination of 75 and 50 % water availability and ABA and SA applications compared to the other treatments. In this study, irrigation based on 75 and 50 % of available water caused a significant decline in growth and yield of the maize. The exogenous application of ABA and SA compounds increased the plant's resistance to dehydration by significantly enhancing the amount of soluble cabohydrate, proline and dehydrin proteins, so that the yield of maize increased and the amount of the stress biomarker, MDA, significantly decreased.

Keywords: *Water stress, ABA, SA, yield, antioxidant enzymes, dehydrin protein, carbohydrate.*

Introduction

Maize (*Zea mays* L.) is one of the major cereal corn grown widely throughout the World as a food for both human and livestock, biofuel and crude material in industry. In Iran, the area used for the cultivation of corn was 166000 ha, which contributed to 1.46 % of the total crop products and 2.03 % of the total grain yield in 2014-2015/1. In these areas, maize is generally cultivated after wheat. It appears that maize is the second most important plant cultivated in arid and semi-arid regions of Iran. The scarce precipitation and water sources in this regions have created challenges for cultivation of maize which is reported to be very sensitive to drought.

Drought stress is a major threat to maize yield occurring in arid, semiarid, and sub-humid dryland environments3. Drought and oxidative stress are often interconnected, and may impairs numerous metabolic and physiological process such as oxidative stress, disruption of hemostasis and ion distribution in cells, changes in fluorescence which they lead to growth reduction and yield quality4,5. Oxidative stress may also cause denaturation of functional and structural proteins6. As a consequence, the water stresses often activate cellular responses, such as the generation of reactive oxygen species (ROS), the production of stress proteins, up-regulation of anti-oxidants enzymes and accumulation of compatible solutes. To mitigate ROS accumulation under drought stress, a protective enzymatic system is triggered in plant. The enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), which can eliminate the excess free radicals and prevent accumulation in plants, so as to improve the tolerance of plants to drought. The plant abscisic acid (ABA) is a key hormone involved in regulatory response to abiotic stresses, such as drought, salt and low temperatures, which coordinates an array of functions, enabling the plant to cope with the stresses. Increased level of endogenous ABA enhances activities of antioxidant enzymes to balance between ROS and anti-oxidant defense system. By activating antioxidant defense mechanism in maize seedlings under water deficit, ABA reduces oxidative damage. External application of ABA has been revealed to increase drought tolerance in some plant species. ABA treatments could decrease ROS accumulation and enhance abiotic tolerance of wheat by inducing activities or expression levels of many antioxidative enzymes such as glutathione (GSH) and ascorbate (ASA).

Salicylic acid (SA) is a phenolic phytohormones potentially generates a wide array of metabolic responses in plants and also affects photosynthetic parameters and plant water relations, especially in in stress management. Sharma et al. suggested that SA markedly increased drought-tolerance and could be used for increasing and stabilizing crop production under stress situations. SA acts as signaling molecule and activates enzymatic functions to trigger abiotic stressors. The oxidative damage occurred to the plant under stressful environment due to the increased generation of ROS was mitigated by the external application of SA. In *Cicer arietinum* L., exogenous SA not only increased biomass and photosynthesis, but also induces ROS activities of SO, APX and CAT.

Importance of ABA and SA in stress response has been extensively studied and reviewed. The present study was planned to explore role of SA and ABA treatments in ameliorating drought-tolerance in maize under different water stresses based on changes in growth, yield, and physiological and biochemical parameters. It was hypothesized that exogenous application of SA and ABA separately improve drought-tolerance of maize by enhancing photosynthesis efficiency and triggering anti-oxidant defense system of the maize.

Materials and methods

Experimental design and plant growth conditions and treatments. In order to evaluate the effect of ABA and SA on the agronomic and biochemical characteristics of fodder corn, single cross hybrid S.C647, under different humidity regimes, an experiment was carried out in Fars province, an agricultural farm in the Takht-e-Jamshid region, 60 km from Shiraz with the longitude 51 degrees and 39 min east and latitude 29 degrees and 55 min north and an altitude of 1770 meters above sea level in the crop year 2022-23. The study used split plot design in which main plot includes three levels of humidity regimes: 1- Whole irrigation (100 % used water based on the plant demand at various growing stages), 2 and 3- two levels of deficit irrigations (75 and 50 % used water based on the plant demand at various growing stages). Secondary plot includes: 1- application of ABA, 2- application of SA, 3- control (without ABA and SA application).

After leveling the land, plots were made with the dimensions of 3.5 x 75 m, each plot had 5 rows of crops and the distance between the rows was 75 cm and the distance on the rows was 18 cm. The seeds were manually sown in depth of 3-5 cm. Immediately, after planting the field was irrigated to saturate the soil moisture profiles for all treatments and also the seeds germinate easily and quickly.

The treatment of humidity regimes started from the 5-leaf stage of the maize and continued until the end of the growth period. To determine the amount of moisture at the time of irrigation (R), the amount of soil moisture in the state of field capacity (FC), permanent wilting point moisture (PWP) and available water are calculated, the coefficient of accessible water (a) for corn is 0.5 is considered. Available water (AW) is the difference between field capacity moisture and permanent wilting point.

$$AW = FC - PWP$$

$$R = FC - (a \times AW)$$

The foliar spraying was done twice, 7 and 14 days after applying the drought stress Shemi et al. SA was sprayed at the rate of 140 mg L-1 and ABA at the rate of 50 µM. In order to increase the solubility of SA in water, ethanol was used at a ratio of 1 g per 10 mg Hayat et al..

Plant morphological measurements and relative water content. The crop was harvested manually in the milky stage of the seeds. From each plot, 20 plants were selected completely randomly and morphological traits including plant height (PH) and maize yield were measured. In order to measure the relative water content of the leaf, the samples taken after measuring the weight (FW) were placed in distilled water in the dark for 24 h at a temperature of 4° C to swell. After removing the leaves from distilled water and removing excess moisture, their saturated weight (SW) was measured. Then the samples were placed at 105° C for 24 h to measure their dry weight (DW). Finally, the relative leaf water content (RWC) was calculated through the following equation (Sánchez et al., 2002):

$$\text{RWC (\%)} = (\text{FW-DW})/ (\text{SW-DW}) \times 100\%$$

Chlorophyll contents. To measure the concentration of chlorophyll (a+b) during growth before irrigation, a number of leaves were separated from each control and stress plots and 1g of fresh sample was prepared from the middle part of the leaves. In order to extract chlorophyll, each fresh sample was ground with 80 % acetone in a Chinese mortar. The obtained solution was passed through a filter paper several times until the remains of the leaf sample were completely colorless. The volume of the obtained solution was increased to 100 mL using 80 % acetone, and then the optical density of the leaf extract was read at 645 and 663 wavelengths using a spectrophotometer. The following relationships were used to calculate the concentration of chlorophyll (a+b): chlorophyll a content (mg/g tissue) = $(12.7D663-2.69D645) \times V/ (1000 \times W)$, chlorophyll b content (mg/g tissue) = $(22.9\ D645-4.69D663) \times V/ (1000 \times W)$. V is the volume of extracting liquid (cm3) and W is the weight of fresh leaf (mg).

Proline content. The proline content of drought stressed and irrigated (control) plants was determined using the method of Bates et al.. Proline was extracted from leaf samples (50 mg fW). The absorbance of the sample extract was spectrophotometrically determined at 520 nm. The proline concentration was determined as (μmol g/DW) using a standard curve.

Malondialdehyde content. Samples of equal weight (0.5 g) were homogenized in liquid nitrogen for physiological measurement. The malondialdehyde (MDA) level was measured using the thiobarbituric acid (TBA) method as described by Calmak and Horst (1991) with a slight modification. Briefly, 50 mg leaf tissue was homogenized with 10 mL phosphate buffer (pH 7.8) and extracted in 2 mL 0.6 % TBA made in 10 % trichloroacetic acid (TCA). The extract was incubated at 100° C for 15 min and then quickly cooled on ice. After centrifugation at 5000×g for 20 min, absorbance of the supernatant was measured at 450, 532 and 600 nm. The MDA-TBA complex was quantified using the extinction coefficient as 155 mM-1 cm-1.

Enzyme activities assays. For the estimation of antioxidant enzymes, the 0.5 g fresh leaves were fully ground with liquid N2, added with 5 mL pre-cooled 50 mM phosphate buffer (PBS) (pH 7.8) containing 1 % polyvinyl pyrrolidone to grind into homogenate. The homogenate was fully shaken and extracted at 4° C for 1 h. The supernatant was taken and sub packed after centrifugation at 12000 g 4° C for 20 min, and stored at -20° C for determination of hydrogen peroxide and enzyme assays.

Peroxidase (POD) activity was assayed as described by Maehly & Chance slightly modified. Briefly, enzyme was assayed with Guaiacol as the substrate in a total volume of 3 mL. The reaction mixture consisted of 200 mM phosphate buffer (pH 6.0), 1 % Guaiacol, 30 % H2O2, and enzyme extract. The increase in the absorbance due to the oxidation of Guaiacol was measured at 470 nm. One unit of POD activity was defined as the OD 470 nm value reduced by 0.01 in 1 min.

CAT activity was measured at 25° C according to the method of Ekinci et al. (2020). The reaction mixture contained 100 μL enzyme extract, 30 % H2O2 in 150 mM phosphate buffer (pH 7.0). CAT activity was estimated by following the decrease in absorbance of H2O2 at 240 nm and was expressed as mM H2O2 decomposed min-1 mg-1 protein using the extinction coefficient 39.4 mM-1 cm-1.

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) following the method of Beauchamp and Fridovich (1971). The reaction mixture consisted of 50 mM phosphate buffer (pH 7.8), 20 µM riboflavin, and 75 mM NBT, 13 mM methionine, and 0.1 mM EDTA. The mixture was illuminated with 2 fluorescent light tubes for 10 min. The absorbance was measured at 560 nm using a UV-visible spectrophotometer.

GR activity was determined by following the oxidation of NADPH at 340 nm (extinction coefficient 6.2 mM±1 cm±1) for 3 min in 1 mL of an assay mixture containing 50 mM potassium phosphate buffer (pH 7.8), 2 mM Na2EDTA, 0.15 mM NADPH, 0.5 mM GSSG, and 100 mL of enzyme extract. The reaction was initiated by adding NADPH. Corrections were made for the background absorbance at 340 nm, without NADPH (Schaedle and Bassham, 1977).

Soluble protein content. The level of soluble protein was measured using the Coomassie brilliant blue method (Bradford, 1976), 0.5 g leaf tissue was homogenized with 10 mL phosphate buffer (pH 7.8) and extracted in 2.9 mL react mixture contained 0.1 g Coomassie brilliant blue G-250, 50 mL 95 % ethanol and 100 mL 85 % (w/v) phosphoric acid. The absorbance of the supernatant was measured at 595 nm after 2 min reaction, and then figure out the protein content in the sample according to the standard curve with bovine serum albumin.

Soluble carbohydrates. The 0.1 mL of alcoholic extract was mixed with 3 mL of freshly prepared anthrone (150 mg of anthrone + 100 ml of 72 % sulfuric acid). This solution was placed in a boiling water bath for 10 min to make the reaction and color. Then, its absorbance was read with a spectrophotometer (UV-Vis Lamdba 25) at a wavelength of 625 nm and the amount of dissolved sugars was calculated.

Statistical analyses. All statistical analyses were performed using SPSS 17.0 program by tow-way analysis of variance (ANOVA). Significant differences among different treatments were presented at $p<0.05$. Before ANOVA analyses, normality and homogeneity of variance were determined using Kolmogorov-Smirnov and Bartlett tests, respectively.

Results

PH, corn yield, RWC. The independent effect of irrigation regimes (I) and application of SA and ABA (F) on PH, corn yield and RWC were significant (PH: I: $F_{2,8}= 16.4$, $P < 0.05$; F: $F_{2,8}= 8.7$, $P < 0.05$; corn yield: I: $F_{2,8}= 22$, $P < 0.05$; F: $F_{2,8}= 8.7$, $P < 0.05$; RWC: I: $F_{2,8}= 11.78$, $P < 0.05$; F: $F_{2,8}= 8.31$, $P < 0.05$; however, significant their interactive effects (I*F) weren’t observed (PH: I*F: $F_{4,8}= 0.1$, $P > 0.05$; corn yield: I*F: $F_{4,8}= 0.01$, $P > 0.05$, RWC: I*F: $F_{4,8}= 1.5$, $P > 0.05$). Irrigation based on 50 % of available water (I3) led to a significant decrease in the height of the plant, corn yield and RWC compared to non-stressed conditions (100 %) (Table 1). The application of SA and ABA markedly increased these parameters (Table 2).

Chlorophyll content. Chlorophyll content (a+b) of maize leaves was significantly influenced by interactive effects of irrigation regimes (I) and application of SA and ABA (F) (I*F: $F_{4,8}= 4.3$, $P < 0.05$) and irrigation regimes of 50 and 75 % available water, spraying SA and ABA significantly increased chlorophyll (a+b) (Table 3).

Proline and malondialdehyde content. The prolin and malondialdehyde content was significantly influenced by The independent effect of irrigation regimes and application of SA and ABA (prolin: I: $F_{2,8}= 17.53$, $P < 0.05$; F: $F_{2,8}= 8.7$, $P<0.05$; malondialdehyde: I: $F_{2,8}= 3.29$, $P < 0.05$; F: $F_{2,8}= 8.7$, $P < 0.05$), while their interactive effects didn’t have notable effect on these two stress indices (prolin: I*F: $F_{4,8}= 0.02$, $P > 0.05$; malondialdehyde: I*F: $F_{4,8}= 0.015$, $P > 0.05$). Under ABA and SA treatments, the maximum prolin content in maize leaves in comparison to control was observed (Table

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5). Water stress (50 and 75 % water availability) significantly enhanced malondialdehyde content in maize leaves in comparison to 100 % water available (Table 4).

Enzyme activities. All four antioxidant enzymes studied were significantly influenced by the interactive effects of irrigation regimes (I) and application of SA and ABA (F) (POD: I*F: F4,8= 5.28, $P < 0.05$, CAT: I*F: F4,8= 31.6, $P < 0.05$; SOD: I*F: F4,8= 35.4, $P < 0.05$; GR: I*F: F4,8= 22.2, $P < 0.05$). In 50 % available water and application of ABA and SA situation amount of POD, CAT, SOD and GR in maize leaves significantly increased in comparison to the other treatment combinations (Figure 1).

Total protein and carbohydrate. The irrigation regimes (I) and application of SA and ABA (F) had significant interactive effects on total soluble protein and carbohydrate (Pro: I*F: F4, 8= 4.77, $P < 0.05$, Carb: I*F: F4, 8= 3.56, $P < 0.05$). The total protein and carbohydrate significantly increased in 50 and 70 % available water and application of SA situation compared to the other treatment combinations (Figure 2).

Discussion

The environmental stresses like drought lead to decreased water availability and consequently affects many physiological and metabolic processes including changes in the metabolism of carbohydrates and nitrogen and the structure of proteins and the activity of enzymes. Here, the growth and yield of the maize was repressed under drought stress, however exogenous application of SA and ABA somewhat compensate loss of water recommended availability and thus improved growth parameters usingy biochemical changes in maize plants.

In the present study, morphological parameters such as PH, corn yield and RWC significantly increased under 75 and 50 % water availability. Many investigations have shown that drought stress decreased morphological growth indices of the maize[2,4,22]. Here, total chlorophyll content of maize leaves was reduced under water stress compared to maize grown in 100 % water availability condition. Drought leads to reduction of chlorophyll pigments which could restrict photosynthesis process[16]. Similarly, Yavas & Unay[23] revealed that water deficit considerably decreased chlorophyll content in wheat.

Lack of irrigation water, depending on the type of variety, growth stage, intensity and duration of stress, reduces the yield of maize. The negative effect of water deficiency on cell division[24], reduced leaf emergence speed, premature senescence due to the transfer of water from old leaves to young leaves, less absorption of radiation due to the loss of leaves[24], reduced efficiency of radiation use are the possible reasons for yield reduction under water deficit conditions. In biochemistry view, the decreases in maize growth parameters and yield under drought stress might be attributed to the overproduction of ROS which caused oxidative damage to the membranes, lipids and elevated the content of proline and MDA, which is a highly reactive three-carbon dialdehyde and one of biological marker for oxidative stress[2]. Several previous studies have documented that drought stress leads to an increase in the ROS production that destroys the cell membrane, causes damage to lipids, proteins, and chlorophylls, and finally decreases plant biomass accumulation[25]. Antioxidant enzymes protect the cell structure against ROS formation under stress conditions. In the present study, application of ABA and SA increased the growth and yield of the maize and ameliorated the adverse effects of drought stress using improve defense system using empowering antioxidant enzyme activities (SOD, POD, CAT and GR) and leaf proline content, and declining MDA content. Hayat et al.[14] indicated that SA foliar application enhanced activities of antioxidant enzymes and proline content in chickpea plant. Similar results were obtained in wheat[16]. Wei et al.[10] reported that exogenous ABA application increased tolerance of treated wheat under drought stress. Shemi et al.[2], reported that application of SA decreased MDA content in maize grown under water stress.

In addition to strengthen defense system, ABA and SA-enhanced drought tolerance might be related to its protective roles such as closing stomata, decreasing evaporation and solute uptake[26]. Here, ABA and SA applications in maize grown under 50 and 75 % water availability lead to increasing proline and soluble carbohydrate. Proline, acts as an osmolyte, as well as soluble carbohydrate are important components of osmoregulation in plant under stress[2]. The soluble sugars play a role in maintaining the osmotic balance of cells, maintaining the enzyme structure and protecting against free radicals, and by increasing the proteins stress keeps the osmotic power of the plant[27]. The increasing soluble protein in maize leaves treated by ABA and SA under drought was similar to findings of Riccardi et al.[28], who reported that a set of dehydrin proteins, is known stress protein, increased from 1.3- to 5.0-fold upon water stress has been characterized. Similar results were reported by Ge et al.[29].

Based on all the above results, resistance of the investigated corn variety, single cross hybrid S.C647, to water stress is low because its yield and growth parameters showed a significant decrease. So, it is not recommended to grow drought-sensitive maize cultivars in dry areas due to very low yield, but it is possible to increase maize resistance to drought, caused growth and yield improvement, by applying a reduced level of recommended irrigation along with SA and ABA foliar spraying, so that maize growing in semi-arid areas is cost-effective.

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Table1 Mean of maize height, yield and relative leaf water content (RWC) under different humidity regimes

Humidity regimes	Plant height (cm)	Plant yield (kg/ha)	RWC (%)
100 % water available	193.4 a	8889.9 a	80.6 a
75 % water available	190.5 a	7575.5 b	77.1 b
50 % water available	176.5 b	6172.9 c	74.2 c

Table 2 Mean of maize height, yield and relative leaf water content (RWC) under application of abscisic acid and salicylic acid

Hormone application	Plant height (cm)	Plant yield (kg/ha)	RWC (%)
Abscisic acid	188.3 a	7233.4 a	80.4 a
Salicylic acid	187.8 a	7044.3 b	78.1 b
Control	184.3 b	6911.4 c	73.5 c

Table 3 Mean of chlorophyll a+b application of abscisic acid and salicylic acid

Humidity regimes	Abscisic acid	Salicylic acid	Control
100 % water available	188.3 a	7233.4 a	80.4 a
75 % water available	187.8 a	7044.3 b	78.1 b
50 % water available	184.3 b	6911.4 c	73.5 c

Table 4 Mean of prolin and malondialdehyd under different humidity regimes

Humidity regimes	Prolin (µgl/g)	Malondialdehyd (µmol/g)
100 % water available	4.25 c	1.2 c
75 % water available	5.68 b	1.49 b
50 % water available	6.99 a	1.71 a

Table 5 Mean of prolin and malondialdehyd under application of abscisic acid and salicylic acid

Hormone application	Prolin (µgl/g)	Malondialdehyd (µmol/g)
Abscisic acid	6.1 a	1.84 a
Salicylic acid	5.9 a	1.68 b
Control	5.48 b	1.63 b

Figure 1 Mean (+SE) concentrations of POD, SOD, CAT and GR in maize leaves under combinations of three humidity regimes and three hormone applications situations

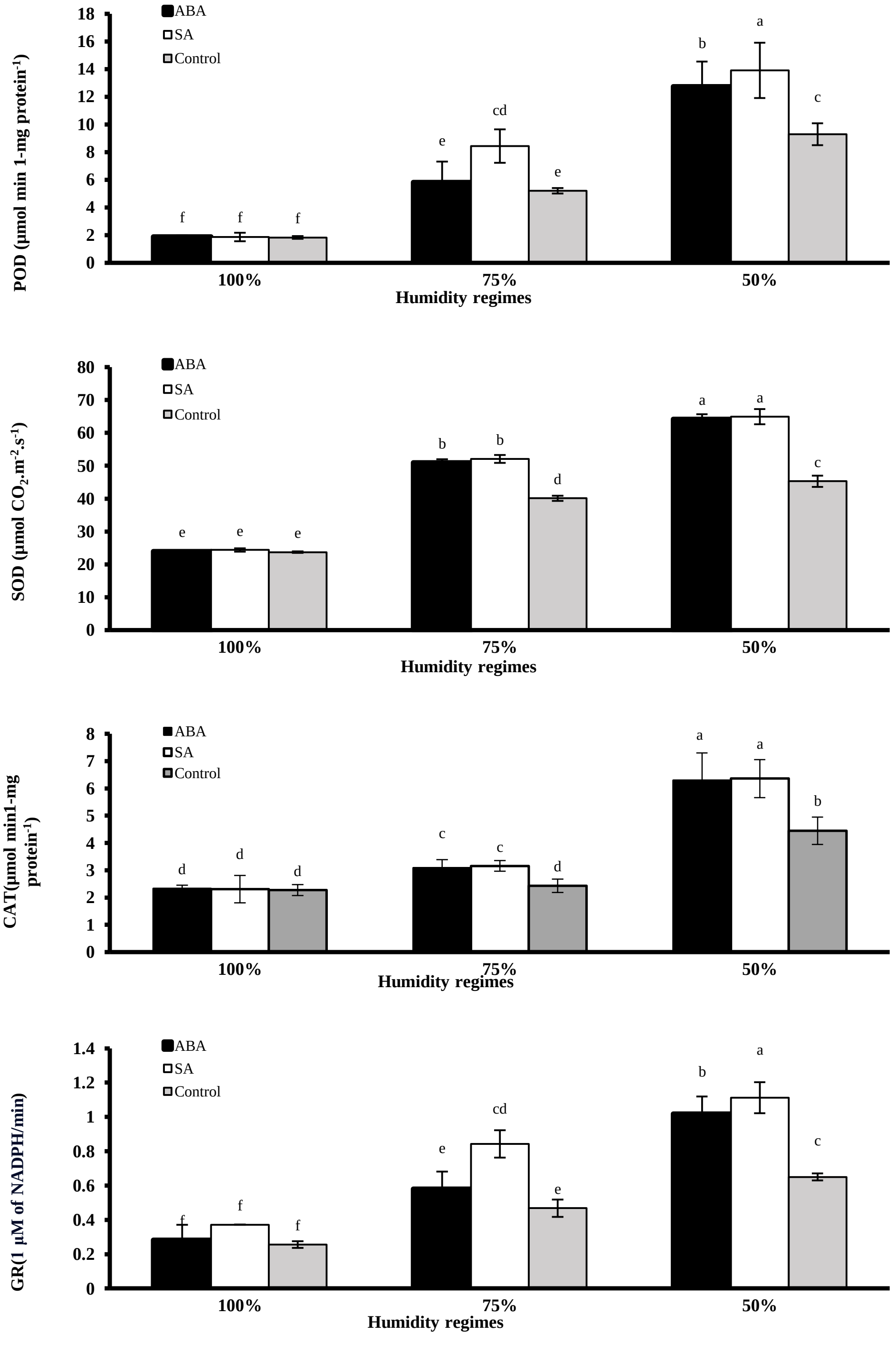
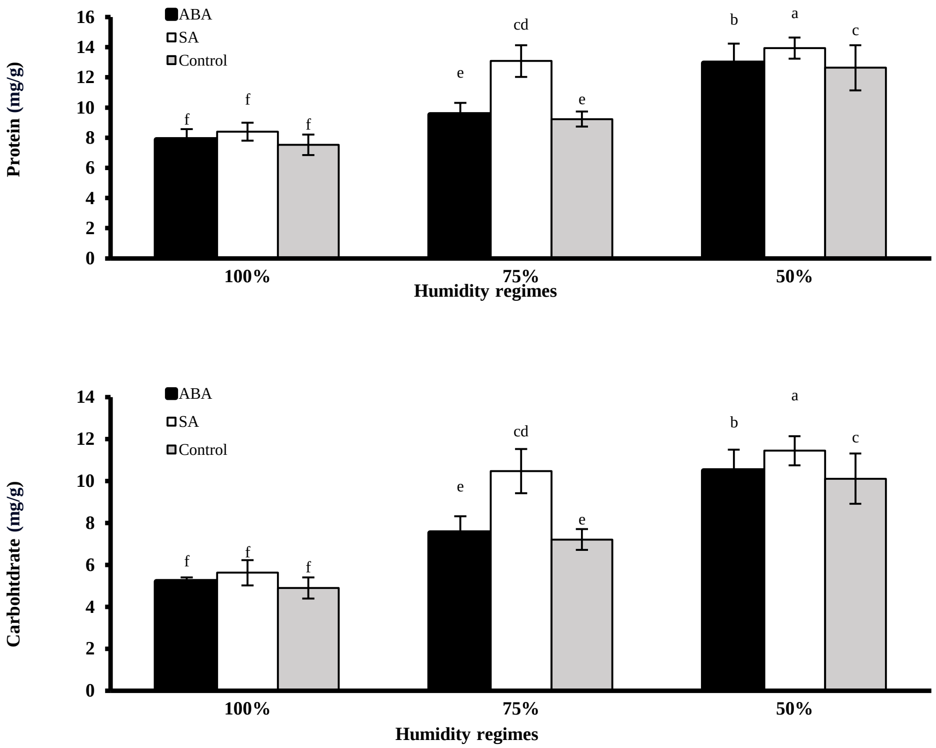


Figure 2 Mean (+SE) concentrations of protein and carbohydrate in maize leaves under combinations of three humidity regimes and three hormone applications situations



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