

Application of Salicylic Acid and Zinc Improves Wheat Yield through Physiological Processes under Different Levels of Irrigation Intervals

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Abstract Water stress, one of the environmental stresses, is the most significant factor restricting plant production on majority of agricultural fields of the world. Wheat is grown on arid-agricultural fields and drought often causes serious problems in wheat production in these fields. So, the present study was conducted to investigate whether the application of salicylic acid (SA) and zinc (Zn) could regulate the relative water content (RWC), membrane stability index (MSI%), electrolyte leakage (EL), soluble carbohydrate content, soluble protein content, proline content, activities of antioxidant non enzymes and enzymes as well as ameliorate the adverse effects of drought stress on wheat. The magnitude of reduction, increased by increasing the drought level. Drought stress at grain filling stage significantly decreased plant height, spike length, number of grains/spike, 1000-grain weight, relative water content, but significant increases were observed in the activities of superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and glutathione reductase (GR) in wheat plants. Both salicylic acid or zinc treatments had greater changes in most of the assayed parameters. The adverse effects of drought as regards the growth characters and yield components were significantly mitigated by SA and Zn supplement. Application of SA or Zn caused great variations in the activities of antioxidant enzymes. Under the normal irrigation condition, addition of SA or Zn markedly increased the activity of both SOD and CAT, however the activity of both POX and GR was significantly decreased. Addition of SA or Zn markedly reduced the increases in the activities of SOD, POX, CAT and GR observed in drought stressed plants. Statistically, Pearson correlation showed there were positive correlations between weight of 1000 grains and morphology, biochemical assay. However, there was no correlation between weight of 1000 grains with proline content and GR activity. Generally, it could be concluded that SA or Zn has (to more extent) a beneficial regulatory role in plants grown under drought stress conditions.

Keywords Irrigation interval, RWC, MSI, Salicylic acid, Zinc, Antioxidant enzyme activity

1. Introduction

Agronomic management factors, for example, irrigation plays important roles in experiencing this the maximum potential of productivity in crop plants. Drought stress can impact the progression of wheat plants and also the accumulation of active substances within their organs [1].

Moderate drought stress may enhance the power of secondary metabolites in plants, while moderate drought stress is known to improve the concentration of secondary metabolites in plants [2, 3].

The main reason from the environmental stress, for example, drought inhibits the growth and photosynthetic abilities of plant may be the disturbance within the balance between the production of reactive oxygen species (ROS)

along with the antioxidant defence, causing accumulation of ROS, which induces, damages protein, membrane lipids, oxidative stress and other cellular components [4].

Wheat is an important crop in Egypt, where its production isn't enough to satisfy the demand for it. The crop is responsive to the timing of a water deficit period rather than the total reduction of applied irrigation water. Exposing wheat plants to high moisture stress, depressed seasonal consumptive use and grain yield [5]. The quantity of wheat yield reduction because of water stress is affected by the stage of grain development, where the early grain development stage is more susceptible to water stress than latter grain development stage. El-Kholy *et al.* [6] stated that, irrigation practices and irrigation water are factors which have always limited wheat productivity. The recommended number of irrigations at the reproductive stages and also the vegetative have to be applied suitably and timely for much better yields.

Salicylic acid (SA) is an endogenous growth regulator of phenolic nature, which participates in the regulation of

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physiological processes in plant [7]. Also, participation in signal regulation of gene expression in the course of leaf senescence [8]. Generally, salicylic acid and its derivatives like acetylsalicylic acid have a significant impact on the various aspects of the plant life [9].

Application grains with acetylsalicylic acid improved the drought tolerance in wheat [10]. Many studies report the protective effects of SA on plants against drought [11] and osmotic stress [12]. Salicylic acid regulates physiological and biochemical properties of plants under abiotic stress and it is also important in disease resistance [13].

Zinc (Zn) is an important plant micronutrient that is involved with many physiological functions, carbohydrate and protein synthesis [14]. The decrease of zinc on plant has been associated with the drought stress caused by decreases in soil, water and therefore, limitation of root growth [15]. The use of zinc under drought conditions would influence crop quality and yield. It plays an important role in ionic balance and regulating stomata in crops to reducing the harmful results of drought [16] and also has protective effects on photo oxidative damage caused by ROS [17].

The influence of drought were more serious during reproductive stage than a vegetative stage in wheat [18]. So, drought conditions coincide with the limit grain yield and grain filling period. The grain of wheat with SA either application SA and Zn can affect the sensitivity of wheat.

The aim of the present work was to investigate the effectiveness of salicylic acid (SA) and zinc (Zn) in alleviating the negative effects of drought stress. Because of this we hypothesized that SA and Zn can mitigate the adverse effects of drought on yield components and physiological characteristics.

2. Materials and Methods

These experiments were carried out in Botanical farm, Fac. of Sci., Al-Azhar Univ. Cairo, Egypt. The grains of wheat plant (*Triticum aestivum* var. Giza 168) were obtained from the Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Soil samples were taken at the depth of 30 cm before planting for physical and chemical analysis as shown in (Tables 1 & 2) according the methods of Nelson and

Sommers [19].

A pot experiment was designed as follows: A homogenous wheat grains (Giza 168) were sown in pots (30 cm in diameter) containing 8.0 K.g. of clay soil and subjected to different level of irrigation intervals namely. The pots were divided into nine groups representing the following treatments,

A) Irrigation treatments

- Irrigation interval (every 7 days tap water).
- Irrigation interval (every 14 days tap water).
- Irrigation interval (every 28 days tap water).

B) Combined treatments

- Irrigation interval every 7 days + SA (100 ppm salicylic acid).
- Irrigation interval every 14 days + SA (100 ppm salicylic acid).
- Irrigation interval every 28 days + SA (100 ppm salicylic acid).
- Irrigation interval every 7 days + Zinc (Zn 75 ppm).
- Irrigation interval every 14 days + Zinc (Zn 75 ppm).
- Irrigation interval every 28 days + Zinc (Zn 75 ppm).

The plants of wheat were treated twice with the above mentioned treatments (as foliage spraying). The first treatment was made when the age of plants was 30 days, while the second treatment was made when the age of plants was 75 days of sowing. The plant samples were collected for analysis when the plants were 85 days old. At the end of the growth season (140 days), analysis of the grains yielded from the different treatments as well as the control was done and the irrigation of water level occurs at 15 days throughout the ages of plant.

2.1. Determination of Relative Water Content (RWC)

Leaf discs of 6 mm diameter were weighed to determine the fresh weight (FW), soaked in distilled water at 25 °C for 4 h to determine the turgid weight (TW), then oven dried at 80 °C for 24 h to determine the dry weight (DW). The relative water content was determined by following the method of Turner and Kramer [20], using the following equation:

$$RWC = [(FW - DW) / (TW - DW)] \times 100$$

Table 1. Physical properties of the used soil land

Gravels	Fine Gravels	Coarse Sand	Medium Sand	Fine Sand	Silt	Clay	Texture Class
1.6	5	4	46	21	7.4	15	Sandy-clay Soil

Table 2. Chemical properties of the used soil

TSS Ppm	pH	E.C. mmhos/cm	Cations meq/L				Anion meq/L			
			Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	Cl ⁻	SO ₄ ⁻⁻	HCO ₃ ⁻	CO ₃ ⁻
678	7.3	2.1	1.75	0.52	2.56	1	2.54	1	1	Zero

2.2. Membrane Stability Index (MSI%)

MSI was estimated using conductivity meter. MSI calculated using the formula described by Sairam [21]. MSI was calculated using the following formula:

$$\text{MSI \%} = \{1 - (C1/C2)\} \times 100$$

2.3. Electrolyte Leakage (EL)

The total leakage of inorganic ions from the leaves was determined using the method of Sullivan and Ross [22]. Twenty leaf discs were placed in a boiling tube containing 10 ml deionized water and the electrical conductivity (EC1) was recorded. The contents were then heated to 45-55 °C for 30 min each in a water bath and the electrical conductivity (EC2) was recorded. The sample was boiled at 100 °C for 10 min and the electrical conductivity (EC3) was recorded. Electrolyte leakage was calculated using the formula:

$$\text{Electrolyte leakage \%} = \{EC2 - EC1\} / EC3 \times 100$$

2.4. Phytochemical Contents

2.4.1. Determination of Soluble Carbohydrate Content

Soluble carbohydrate content was determined in aqueous solution with anthrone sulfuric acid reagent according to Umbriet *et al.* [23] using glucose as a standard. To extract water-soluble carbohydrates, a known weight (0.1 g dry weight) of grains powder was boiled in distilled water in a water bath for 1 h. The extracts were then cooled and filtrated through a centered glass funnel. A total of 0.5 ml of each extract was mixed with 4.5 ml of anthrone reagent (0.2 g anthrone, 8 ml absolute ethyl alcohol, 30 ml distilled water and 100 ml sulfuric acid). The mixture was then boiled in a water bath for 7 min. After cooling, the developed blue green color was measured at 620 nm against the blank.

2.4.2. Determination of Soluble Protein Content

The soluble protein content of grains was determined according to Lowery *et al.* [24] using bovine serum albumin as a standard. Samples (0.1 g dry weight) were extracted in 10 ml distilled water for 2 h at 60 °C. The extracts were centrifuged and the supernatants were collected. One ml of each extract was added to 5 ml of alkaline reagent (50 ml 2% Na₂CO₃ prepared in 0.1 N NaOH and 1 ml 0.5% CuSO₄.5H₂O prepared in 1% sodium potassium tartarate) and mixed thoroughly, then allowed to stand for 10 min. A total of 0.5 ml of folin phenol reagent diluted 1:2 (v/v) was then added and mixed immediately. After 30 min, the extinction against appropriate blank was measured at 700 nm.

2.5. Determination of Stress Response Factors

2.5.1. Determination of Ascorbic Acid (ASC)

The total ascorbic acid content was estimated using folin phenol reagent according to Jagota and Dani [25].

2.5.2. Determination of Glutathione (GSH)

Glutathione was extracted by grinding 0.5 g of plant tissues in 1% picric acid (w/v) under cold condition. After centrifugation at 10,000g for 10 min, the supernatant was collected immediately for assay. Glutathione was estimated according to Beutler *et al.* [26].

2.5.3. Determination of Lipid Peroxidation

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content according to Herna ndez and Almansa [27]. Fresh weight samples (500 mg) were homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000g for 20 min at 4 °C. One ml aliquot of the supernatant was mixed with 3 ml of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA and incubated at 90 °C for 20 min. After stopping the reaction in an ice bath, samples were centrifuged at 10,000 g for 5 min. The supernatant absorbance at 532 nm was then measured. After subtracting the non-specific absorbance at 600 nm.

2.5.4. Determination of Proline Content

Grains were hand-homogenized in 3% of sulfosalicylic acid and centrifuged at 3000g at 4 °C for 10 min. The supernatants were used for proline estimation according to Bates *et al.* [28].

2.5.5. Determination of Total Phenolic Compounds

Total phenolics were measured with the Folin–Ciocalteu reagent according to Dai and Mumper [29]. Twenty five ml of the extract was mixed with 110 ml Folin–Ciocalteu reagent, 200 ml 20% sodium carbonate and 1.9 ml distilled water, and placed at 60 °C for 30 min. Optical density was measured with a spectrophotometer at 750 nm. A standard curve was constructed with different concentrations of gallic acid.

2.6. Determination of Antioxidant Enzymes

Protein enzymes were extracted according to the method of Mu Kherjee and Choudhuri [30]. Superoxide dismutase (SOD), Peroxidase (POX), Catalase (CAT) activity and Glutathione reductase (GR) activities were assayed using the methods of Dhindsa *et al.* [31], Bergmeyer [32], Chen *et al.* [33] and Karni *et al.* [34], respectively.

2.7. Statistical Analysis

The sample size was calculated according to Raosoft and all statistical calculations were done using SPSS (statistical package for the social science version 20.00) statistical program at 0.05 level of probability [35]. Quantitative data with parametric distribution were done using analysis of variance the one-way ANOVA and Post hoc-LSD tests (the least significant difference). The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered non-significant (NS) at the level

of >0.05 , significant at the level of <0.05 , 0.01 and highly significant at the level of <0.001 . The Pearson linear correlation coefficient, automatic and linear modelling and discriminant analysis were estimated to show the relationship between quantitative parameters [36].

3. Results and Discussion

3.1. Growth Parameters

The results of the present work (Table 3) revealed that, different levels of the irrigation interval of wheat plants every 14 and 28 days resulted in significant decreases in shoot length, fresh and dry weight of both shoots and roots and the number of leaves/plant when being compared with plants grown in normal non-stress condition. The magnitude of reduction, increased by increasing irrigated water days. These results are in agreement with those obtained by Raza *et al.* [37] and Yan and Shi [38], they reported that drought stress decrease wheat plant height. Also, Praba *et al.* [39] demonstrated that, in wheat there was a significant decrease in leaf fresh weight, leaf area, dry weight, plant height and shoot dry weight after vegetative stage drought.

The obtained results (Table 3) revealed also that, treated wheat plants with SA or Zn resulted in significant increases in shoot length, fresh and dry weight of both shoots and roots and the number of leaves/plant as being compared with plants grown in non- or stress condition. Since, Anosheh *et al.* [40] reported that after the exogenous applications of salicylic acid reduced the harmful effects of drought. The positive effects of foliar spray of SA and Zn on vegetative growth of wheat under Irrigation interval were in harmony with the results observed by some investigators [16, 41, 42].

3.2. Yield and Yield Components

Results in Figs. (1-5) revealed that, different levels of the

irrigation interval of wheat plants every 14 and 28 days resulted in significant decreases in the number of tillers and spikes/plant as well as the number of grains/plant and weight of 1000-grain when being compared with plants grown in normal non-stress condition. At high number of grains/spike leads to higher grains yield in plants [43], but drought stress significantly reduced grain yield [44]. In the experiment of Kanani *et al.* [18] on wheat, grain yield and many of yield components significantly affected by drought stress on reproductive growth stage. A drought stress induced decrease in 1000-grains weight was previously reported by Farooq *et al.* [10] and Beigzadeh *et al.* [45].

Also, Figs. (1-5) revealed that, the number of tillers and spikes/plant as well as the number of grains/plant and weight of 1000-grains were highly significantly increased in response to the treatments with SA or Zn with normal condition. Except interactive effects irrigation interval every 7 days with Zn revealed an insignificant increase in the number of spikes/plant. The obtained results in Figs. (1-5) revealed also that, treated wheat plants with SA or Zn resulted in significant increases in the number of tillers and spikes/plant as well as of grains/plant and weight of 1000-grains when irrigated water every 14 and 28 days. Except at irrigation interval every 28 days + either SA or Zn resulted in, insignificant increases in the number of tillers/plant. These outcomes are in agreement with Farooq *et al.* [10] and Monjezi *et al.* [16]. According to results, Sharafizad *et al.* [43] also reported that moderate dosage of salicylic acid (1.2 mM) increased spike length/spike. It has been proposed that zinc spray alleviates drought stress effect on one thousand grain weight [16]. Maleki *et al.* [46] also stated that, zinc sulfate can decrease negative effects of drought stress on maize grain yield and yield components. A similar protective effect of zinc sulfate was also observed by Malek-Mohammadi *et al.* [47].

Table 3. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on plant length, F.wt. shoot/plant, D.wt. shoot/plant, F.wt. root/plant, D.wt. root/plant and number of leaves/plant of wheat

Treatments	Different levels of irrigation interval	Plant length (cm)	F.wt. shoot / plant (g)	D.wt. shoot / plant (g)	F.wt. root / plant (g)	D.wt. root / plant (g)	No. of leaves / plant
Tap water	7 days	76.10 \pm 0.61 ^a	18.14 \pm 0.07 ^b	2.01 \pm 0.05 ^a	12.12 \pm 0.06 ^a	2.16 \pm 0.41 ^c	27.07 \pm 0.46 ^d
	14 days	65.20 \pm 0.58 ^c	16.52 \pm 0.09 ^c	1.61 \pm 0.05 ^b	11.11 \pm 0.05 ^b	1.92 \pm 0.04 ^c	21.38 \pm 0.27 ^e
	28 days	51.68 \pm 0.44 ^e	12.60 \pm 0.15 ^f	1.03 \pm 0.03 ^c	8.08 \pm 0.03 ^c	1.64 \pm 0.03 ^d	21.32 \pm 0.23 ^e
SA	7 days	79.67 \pm 0.88 ^f	19.41 \pm 0.23 ^a	2.81 \pm 0.04 ^d	15.05 \pm 0.02 ^d	2.43 \pm 0.04 ^b	31.19 \pm 0.39 ^a
	14 days	69.83 \pm 0.60 ^b	17.79 \pm 0.42 ^b	2.40 \pm 0.03 ^e	14.15 \pm 0.08 ^e	2.53 \pm 0.07 ^b	30.27 \pm 0.14 ^b
	28 days	54.50 \pm 0.58 ^e	14.02 \pm 0.45 ^e	2.15 \pm 0.02 ^f	11.14 \pm 0.07 ^f	2.05 \pm 0.17 ^c	29.50 \pm 0.28 ^{bc}
Zn	7 days	78.23 \pm 0.79 ^a	19.03 \pm 0.09 ^a	3.04 \pm 0.03 ^e	17.18 \pm 0.10 ^e	2.93 \pm 0.03 ^a	31.03 \pm 0.55 ^a
	14 days	68.67 \pm 0.95 ^b	18.02 \pm 0.12 ^b	2.70 \pm 0.06 ^h	16.17 \pm 0.09 ^b	2.80 \pm 0.05 ^a	29.11 \pm 0.15 ^c
	28 days	58.30 \pm 0.85 ^d	15.21 \pm 0.14 ^d	2.61 \pm 0.05 ^k	11.43 \pm 0.12 ^k	2.06 \pm 0.07 ^c	29.47 \pm 0.25 ^{bc}
F ratio		113.352	100.399	221.512	1315.695	76.441	264.574
P value		0.000	0.000	0.000	0.000	0.000	0.000

* Values are means \pm SE (n=10). Mean values in each column with the same letters are not significantly different at $P \leq 0.05$.

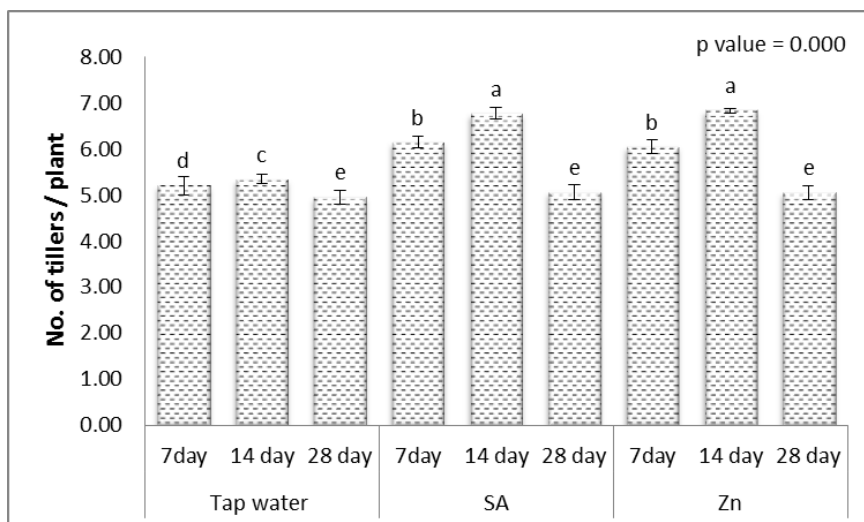


Figure 1. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on No. of tillers/plant of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

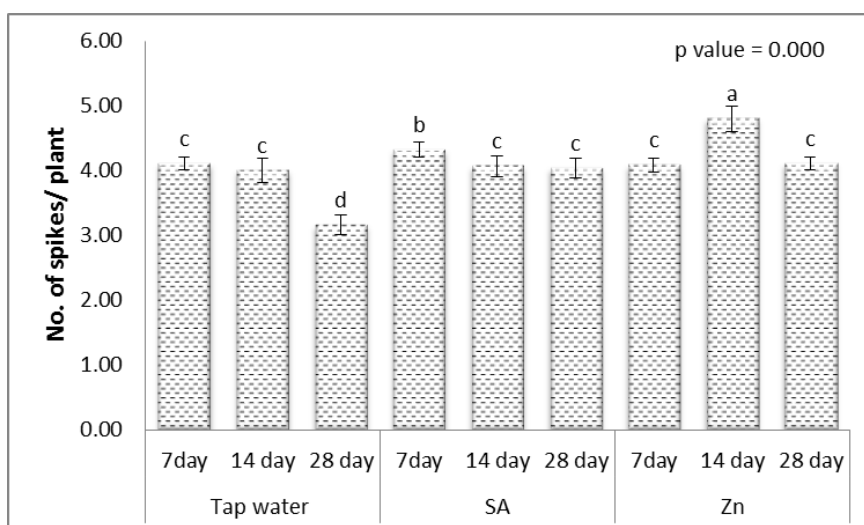


Figure 2. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on No. of spikes/plant of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

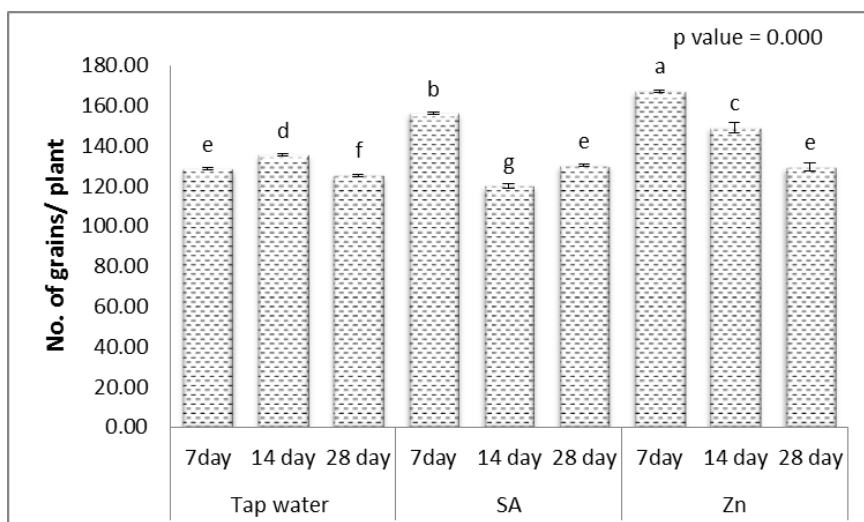


Figure 3. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on No. of grains/plant of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

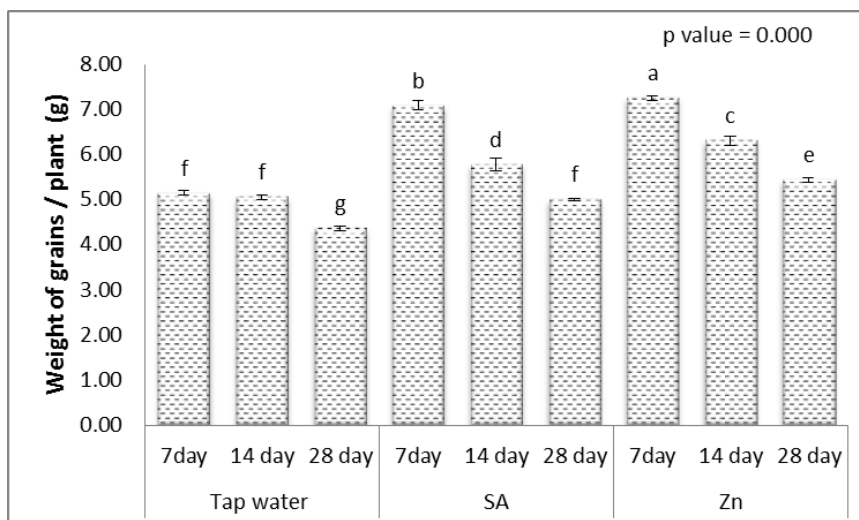


Figure 4. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on weight of grains/plant of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

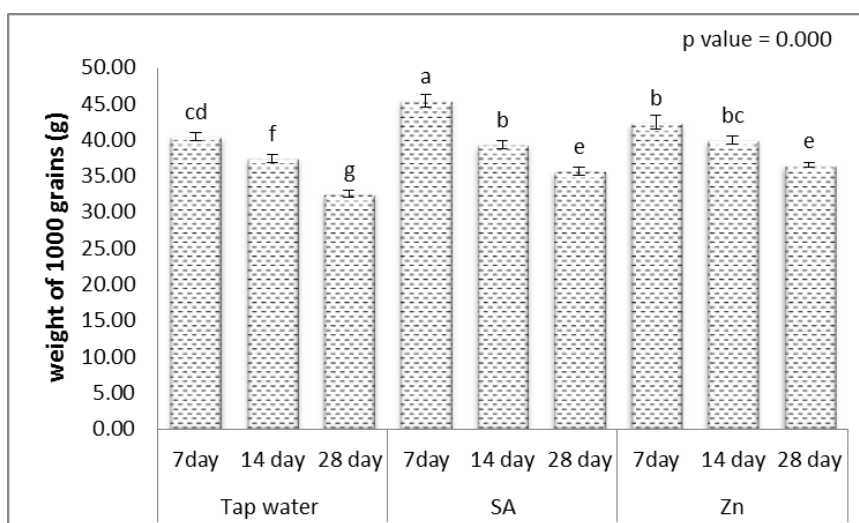


Figure 5. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on weight of 1000-grains of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

3.3. Relative Water Content (RWC %)

Statistical analysis of data for relative water content of leaf demonstrated the effect of different levels at irrigation interval and foliar application was significant on this trait (Fig. 6). With delayed in irrigation, relative water content significantly decreased therefore the highest (31.3%) and lowest (20.8%) RWC were noticed in treatments of irrigation every 14 and 28 days, respectively (Fig. 6). According to the Rahman *et al.*, [48] reports, plants grown under water stress conditions decrease the intracellular water by increasing of osmotic compounds to absorb water from the soil powerfully. It seems there is a direct relationship between the soil moisture content and relative water content of leaf so that reduction in soil moisture and increasing water stress reduces relative water content of leaf.

Salicylic acid (SA) treatment markedly alleviated the effect of drought stress and also increased this trait (Fig. 6). There was significant difference SA applied was more

effective at all different levels of irrigation interval every 14 and 28 days (Fig. 6). The obtained results were concordant with those of obtained by Singh and Usha [49], who reported that increasing of RWC may be related to the role of SA in accumulation of compatible osmolytes in plants subjected to drought stress. Also, Kabiri *et al.* [50] reported that, application of SA as foliar spraying recorded the significant values of RWC of *Nigella sativa* plant grown under drought stress.

Foliar application with Zn increased relative water content of wheat leaves, so that the highest level of RWC (4.1%) was obtained in Zn treatment + irrigation interval every 7 days (Fig. 6). To opinion of the Weisany *et al.* [51] the zinc element have an important role in the regulation of stomatal opening, because this element plays a important role in maintenance of potassium in stomata guard cells and by reducing of leaves water loss, increases the leaf relative water content. Jiang and Huang [52] reported that, difference among leaf relative water contents was significant at Zn

levels, so that the plants which received a higher amount of Zn, had more leaf relative moisture. Maintenance of high RWC in drought resistant cultivars has also been reported to be an adaptation to water stress in several crop species.

3.4. Membrane Stability Index (MSI %)

The results revealed that different levels of irrigation interval significantly decreased the membrane stability index of wheat leaves as compared to unstressed control (Fig. 7). However, SA or Zn application ameliorated the adverse effect of the drought membrane stability index. The most observable indirect effect of drought on plant performance, reduces osmotic potential which results in reduced water availability of plants. These results are in agreement with in agreement with Jaleel *et al.* [53] in *Catharanthus roseus* and Yusuf *et al.* [54] in *Brassica juncea*. Also, Rao *et al.* [55] reported that, the membrane stability index of maize plants was, mostly, highly significantly increased in response to the treatment with SA and drought stress.

3.5. Electrolyte Leakage (EL)

The statistical analysis of data indicated that electrolyte leakage significantly affected by irrigation interval every 14 and 28 days ($p \leq 0.01$) as well as a foliar application ($p \leq 0.01$) (Fig. 8). Mean comparison results showed that the highest electrolyte leakage was recorded by irrigation interval every 28 days and on the contrary, the lowest was seen in the irrigation interval every 14 days (Fig. 8).

In drought stress condition, CO₂ fixation decreases due the stomata closing, while electron transfer and light reactions continue normality. Under such conditions, NADP found limited amounts to simply accept the electrons and thus oxygen can behave as an electron receptor, inducing the manufacture of reactive oxygen species for example peroxide, superoxide radicals and hydroxyl radicals. Improving the reactive oxygen species cause oxidative injuries in lots of cellular components, for example, proteins, lipids, carbohydrates and nucleic acids and result in increasing of electrolyte leakage and cell membrane peroxidation [52]. In this regard Alexieva *et al.* [56] reported that drought stress in pea and wheat plant through enlarging of reactive oxygen species production increased electrolyte leakage.

Foliar application of SA and Zn with irrigated water every 14 and 28 days decreased electrolyte leakage of leaves (Fig. 8). So that, electrolyte leakage of leaves, indicating cell membrane damage is simply because membrane lipid peroxidation in the presence of reactive oxygen species [52]. According to this, the balance between elimination and production of reactive oxygen species determines the survival of plant systems. Such results are in agreement with those recorded by Yadavi *et al* [14] in bean who found that

application of zinc sulfate at 3 mgL⁻¹ concentration decreased electrolyte leakage in irrigation interval every 50, 75 and 100 mm. Also, Kabiri *et al.* [50] found that treatment *Nigella sativa* plants with SA (0, 5, and 10 µM) + interval irrigation at four levels (0, -0.2, -0.4, and -0.6 MPa) caused decrease significant effect on p electrolyte leakage compared to the treated with drought conditions.

3.6. Soluble Carbohydrate and Protein Contents

Results in Figs. (9 & 10) revealed that, the carbohydrate and protein contents in grains of wheat plants were, highly significantly decreased with increasing irrigation level. These results agreed with those of El-Mekawy [57] who mentioned that the effect of irrigation interval on the carbohydrate percentage of black cumin was reduced significantly by decreasing the soil moisture content as a result of increasing the period of irrigation from 2 up to 6 days intervals. Hassan and Ali [58] used three irrigation treatments on *Rosmarinus officinalis* L. The treatments were 100%, 80% and 60% of the field capacity. They found that carbohydrate percentage increased by deficit irrigation treatments. Rabia *et al.* [59] found that the carbohydrate percentage of *Echinacea purpurea* L. significantly decreased as a response to the decrease in irrigation water quantity and reached their minimum value under the lowest irrigation.

On the other hand, data presented in Figs. (9 & 10) revealed that, treatment with either SA or Zn resulted in mostly, highly significant increases in the contents of carbohydrate and protein contents in grains of the treated plants, when the irrigation interval every 14 and 28 days. These results are similar to those obtained, by Yadavi *et al.* [14] and Kabiri *et al.* [50]. So that salicylic acid caused the increase of protein content in wheat [49]. Improving the activation of nitrate contents and nitrate reductase caused the increase of protein content in SA-treated plants [60]. It appears the character of SA impact on the condition from the hormonal system might lead to protective reactions of plants, acceleration of reparative processes, and also the impact on protein contents [9].

The zinc element in stress condition produces an enhancing role in osmotic adjustment process (due to the increase of soluble carbohydrates). Zinc is a vital and occasional consumption element, that have an natural part in protein, and carbohydrate synthesis, cell metabolic process, protection of cell membrane from reactive oxygen species along with other processes connected with adaptation of plants to worry, to ensure that, under drought stress conditions the role of the element is visible like a cause of osmotic regulation, by using intervention within the synthesis of osmotic compounds for compatibility with stress and keep turgor pressure performed their roles [17].

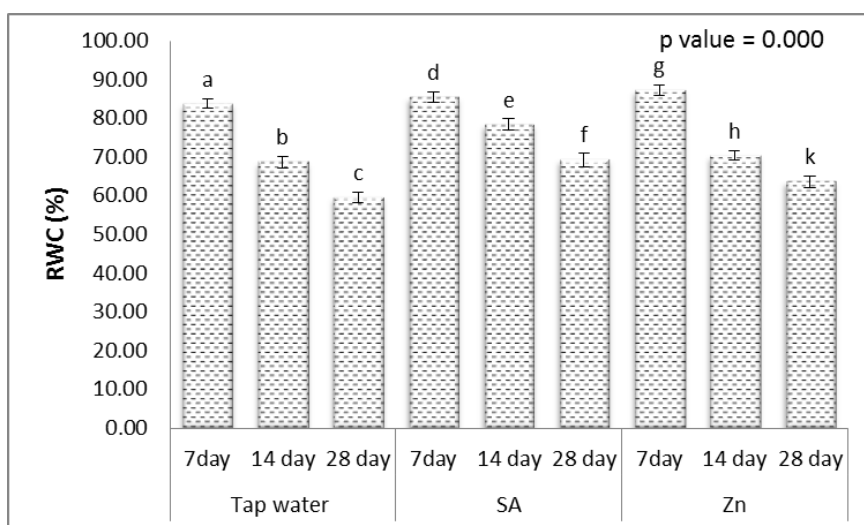


Figure 6. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on relative water content (RWC %) of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

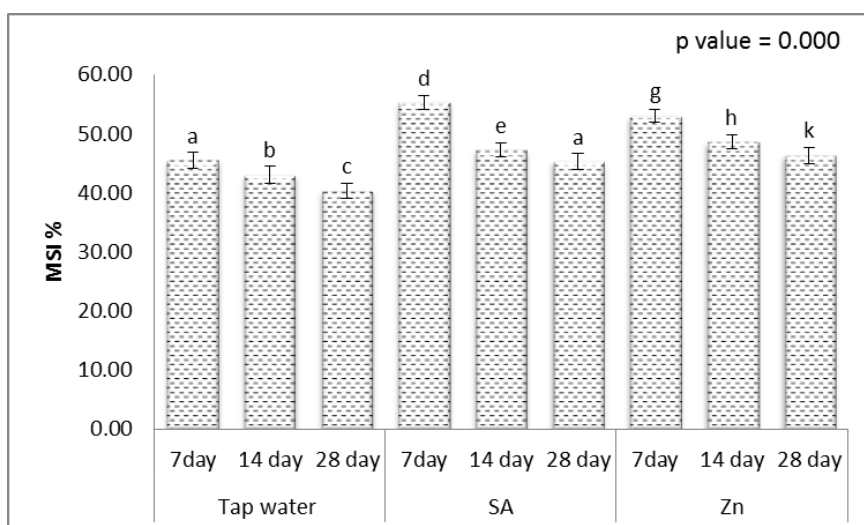


Figure 7. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on membrane stability index (MSI %) of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

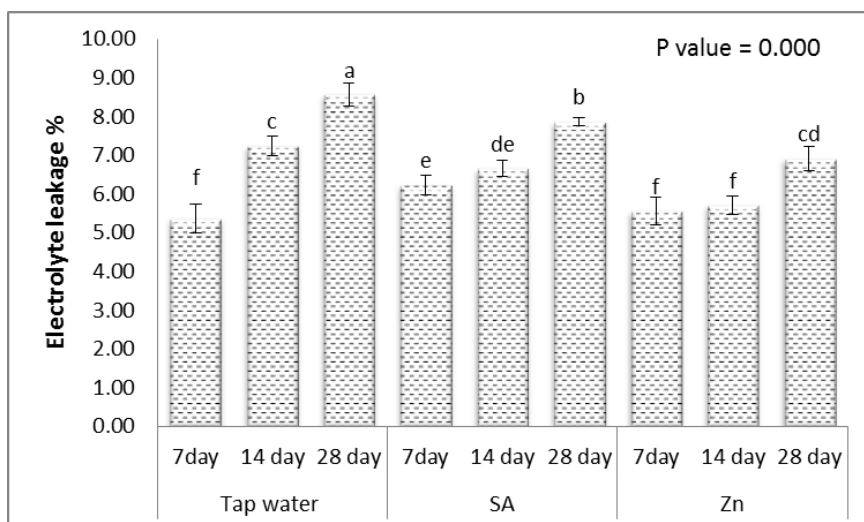


Figure 8. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on electrolyte leakage (EL %) of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

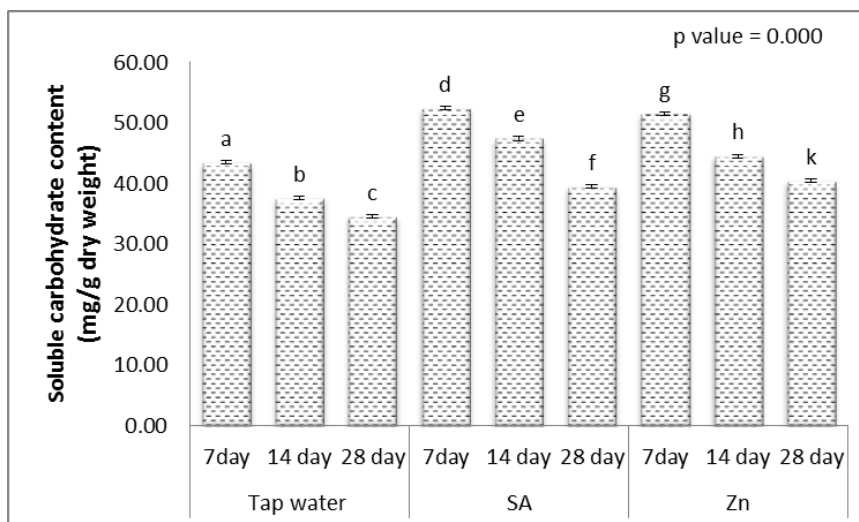


Figure 9. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on soluble carbohydrate content (mg/g dry weight) of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

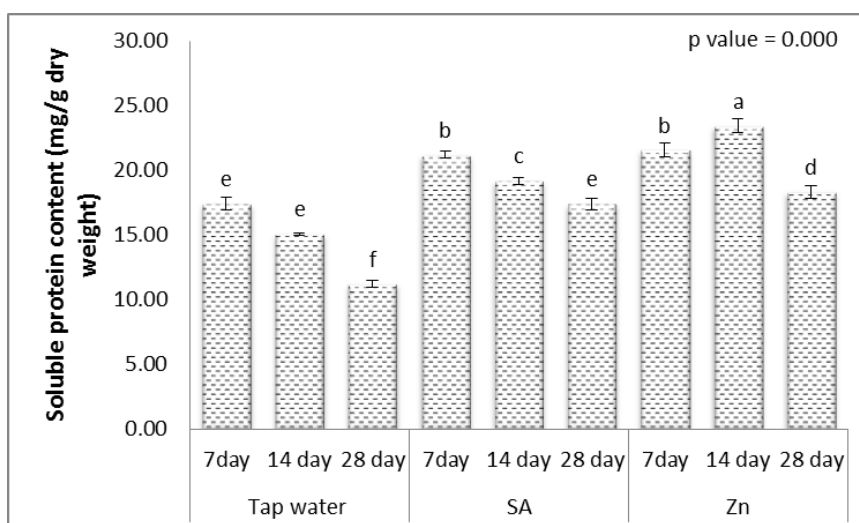


Figure 10. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on soluble protein content (mg/g dry weight) of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

3.7. Determination of Stress Response Factors

3.7.1. Ascorbic Acid, Glutathione, Lipid Peroxidation, Proline and Phenols

Hydrophilic antioxidants such as ascorbic acid and glutathione are indispensable components of the antioxidant system which scavenge ROS [61]. The obtained results (Figs. 11-15) showed that, treating wheat plants with different levels of irrigation interval every 14 and 28 days resulted in, significant decrease in the contents of ascorbic acid and glutathione in shoots, while significant increases in the activities of lipid peroxidation (MDA) and proline when compared to unstressed plant.

Ascorbic acid in collaboration with other aspects of the antioxidant system protects plants against oxidative damage caused by aerobic metabolic process, photosynthesis and a variety of pollutants. So improved manufacture of ascorbic acid and GSH continues to be correlated to reduced ROS production under drought stress [62]. Similarly, decreased

levels of the hydrophilic antioxidants in wheat support the truth that ascorbate and GSH defend the plant against oxidative damage. Compatible solutes, for example glycine-betaine, proline and sugars behave as protein stabilizers, ROS scavengers and therefore are essential aspects of drought tolerance mechanisms [63].

Wheat plant stressed with different levels of irrigation interval every 14 and 28 days showed increase in Malondialdehyde (MDA) and proline content. So that MDA, a product of lipid peroxidation is considered as an indicator of oxidative damage [64]. Huseynova [65] showed that, drought treatments led to an increase in MDA content (lipid peroxidation product) in the drought treated wheat leaves during the vegetative stage, which might be attributed to peroxidation of membrane lipids that could be monitored as increased MDA content. Also, Naureen and Naqvi [66] measured MDA concentrations in abiotic stressed wheat plants that are oxidative stress indicators. H_2O_2 caused membrane damage fasten the Haber-Weiss reaction by

production of hydroxyl radicals to increasing lipid peroxidation. The increase in membrane damage (lipid peroxidation) with increasing water stress levels has been also reported in wheat by Ezzat-Ollah *et al.* [67].

Proline levels are regulated at the Δ^1 pyrroline carboxylate metabolism [68]. From the levels of proline appraised during drought stress, a potential role might be recommended for Δ^1 PCA carboxylase in the regulation of the proline metabolic process in wheat plants. Drought stress caused accumulation of proline is extensively recorded and tolerant species have been shown to express greater amounts of proline and it is precursors compared to susceptible ones [69].

The phenylpropanoid pathway is an important pathway of plant secondary metabolic process, which yields change of phenolics with structural and defense-related functions. These phenolic compounds include anthocyanin, phenolic acidity, flavonoids and phenolic acid, which behave as scavengers of free radicals and other oxidative species through their hydrogen donating (antioxidant) potential [70]. In wheat plant, drought resulted in a decline in phenol compounds (Fig. 15). Among the possible why you should explain the decrease in these compounds under drought stress relates to the antioxidant characteristics of these compounds to scavenging of ROS under drought stress [71].

On the other hand, data presented in Figs. (11-15) revealed that, treatment with either SA or Zn resulted in highly significant increases in the contents of ascorbic acid, glutathione, proline, phenols of the treated plants when different levels of irrigation interval every 7, 14 and 28 days. On the contrary, application of SA or Zn + different levels of irrigation interval every 14 and 28 days caused significant changes decrease in lipid peroxidation (MDA) activities.

These results are in agreement with those of Hayat and Ahmad [9] mentioned that the effect of pretreatment with SA caused a reduction in the amount of lipid peroxidation from plant tissues in addition to more intensive growth processes when compared with control plants. Also Delavari *et al.* [72] showed that pretreatment with SA decreased the level of lipid peroxidation induced by oxidative stress in basil plants. Plants treated with SA accumulated less MDA content compared to control plants under drought stress conditions, suggesting that SA could possess an important role in inducing tolerance to oxidative stress condition in lettuce plants. This is supported by the findings of Agarwal *et al.* [73] who mentioned SA treatment of wheat leaves under water stress conditions, resulted low amounts of MDA. Therefore, the lipid peroxidation caused by drought stress was ameliorated by SA treatments.

In this experiment SA treatment increased proline levels in wheat plants. The data showed that proline content in leaves increased as SA concentrations increased. A higher amount of proline was observed in non-treated plants. These results are in agreement with those of Yazdanpanah *et al.* [74] who found that SA treatment increases the proline content in the leaves. Similarly, SA increases proline levels of the basil plant under water stress [75]. Osmotic adjustment continues to be regarded as one from the crucial mechanisms in plant adaptation to numerous stresses. Besides osmotic adjustment other possible functions of proline include the protection of plasma membrane integrity, the prevention of protein denaturation, being a sink of energy or reducing power being a source of carbon and nitrogen and acting as a hydroxyl radical scavenger [76].

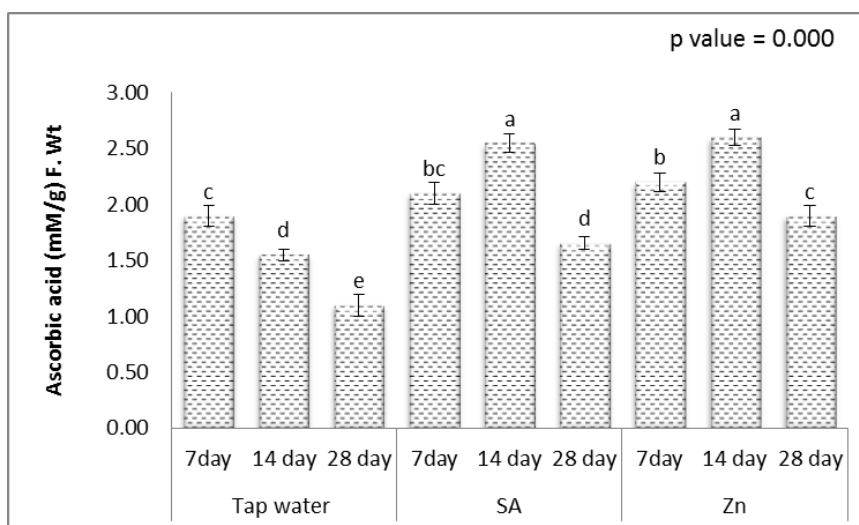


Figure 11. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on ascorbic acid of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

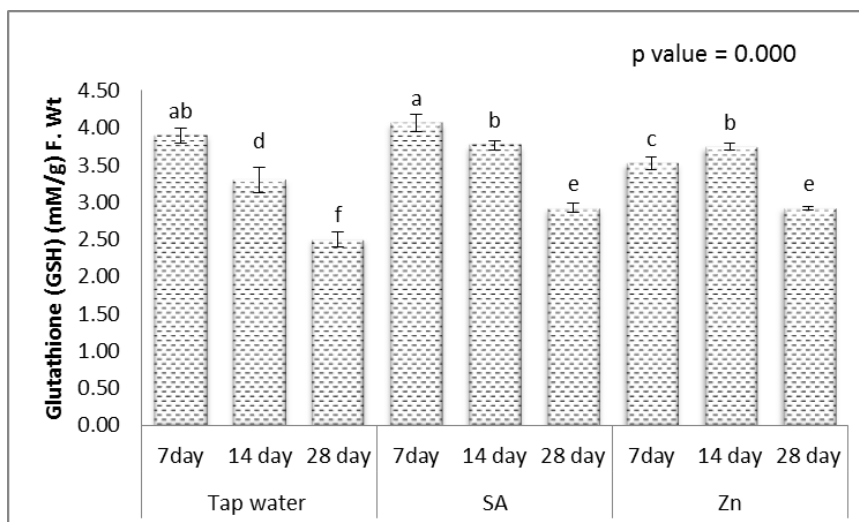


Figure 12. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on glutathione (GSH) of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

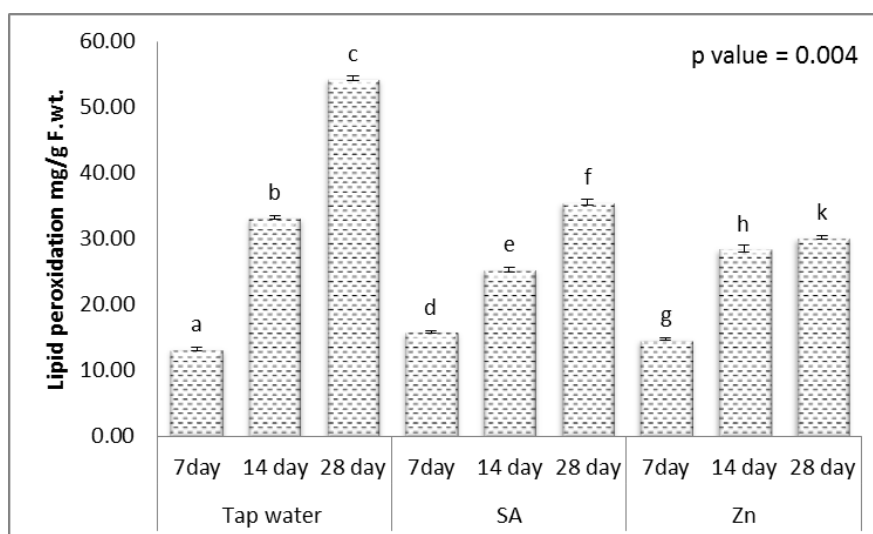


Figure 13. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on lipid peroxidation (MDA) of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

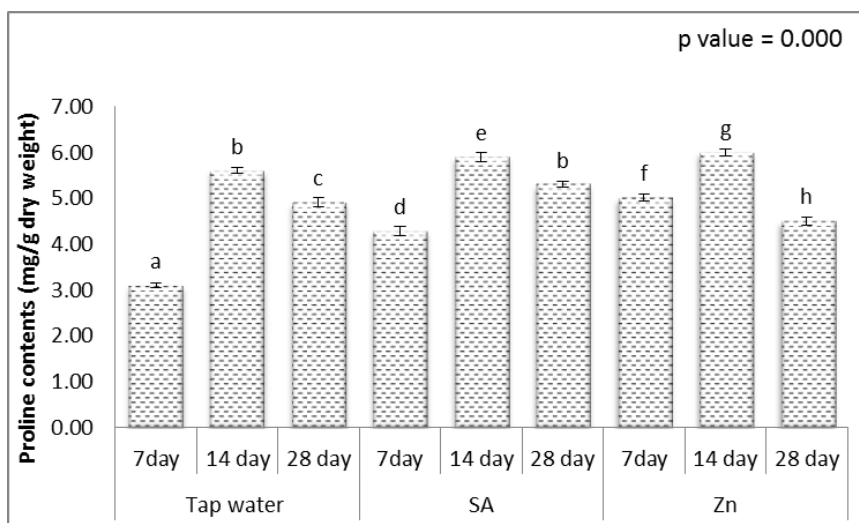


Figure 14. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on proline contents of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

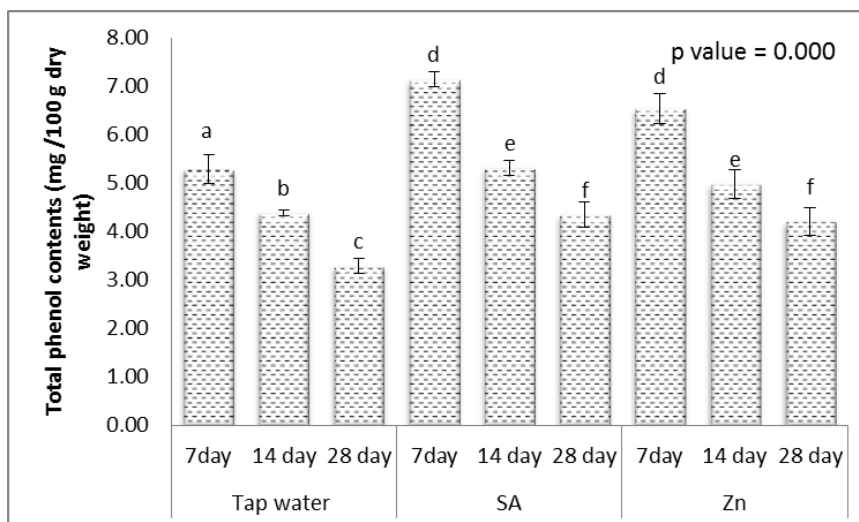


Figure 15. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on total phenols content (mg/100 g dry weight) of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

Salicylic acid (SA) is considered as a phenolic compound synthesized throughout the plant kingdom via the phenylpropanoid pathway, so increases the phenol content [77]. Treatment with SA or Zn increases significantly the total phenolic content in wheat plants under drought conditions. Polyphenols represent a great family of plant secondary metabolites. The synthesis of these compounds is stimulated antioxidants to defend the plant against oxidative stress. Enlarge in the total phenolic content by application of SA or Zn in wheat plants can be explained by enzyme activation. It had been reported that treatment with SA or Zn caused significantly improved activities of phenylalanine ammonia lyase (PAL) the most essential enzyme accountable for the biosynthesis of polyphenols [78].

3.8. Antioxidant Enzymes

Tolerance to drought-stress in higher plants correlates to the amount of antioxidant systems and substrates [79]. To combat the results of drought-caused oxidative stress, plants creates a complex mechanism of the antioxidant system. Relatively greater activities of ROS-scavenging enzymes happen to be reported the antioxidant system plays a huge role in plant tolerance against ecological stress.

To prevent the effects of drought-generated oxidative stress, plants create a complex mechanism of the antioxidant system. Relatively higher activities of ROS-scavenging enzymes happen to be reported the antioxidant system plays a huge role in plant tolerance against ecological stress. This indicated plants will produce more SOD, POX, CAT, GR and APX enzymes under drought conditions to eliminate the extra ROS in cells. Within this study, SOD, POX, CAT, GR and APX activities elevated markedly within the drought tolerant (Figs. 16-20). This demonstrated that drought-tolerant were efficient scavenger of H_2O_2 , which may result in better protection against H_2O_2 . The SOD detoxifies superoxide anion free radicals (O_2^-) by forming H_2O_2 and so the H_2O_2 could be reduced by POX and CAT

[80].

The tolerance of wheat plant to environmental stresses continues to be connected with greater activities of antioxidant enzymes. For instance, the drought-tolerant wheat (*Triticum aestivum*) [80, 81] and black gram (*Phaseolus mungo*) [82] had greater activities of SOD, POX and CAT compared to drought-sensitive species.

Peroxidase (POX) also involved with several plant processes, including lignification, oxidation of phenolics, regulation of cell elongation and detoxing of harmful toxins for example H_2O_2 [82]. Furthermore, the CAT is one of the highest turnover rates for those enzymes using the possibility to directly dismutate H_2O_2 into H_2O and O_2 and is essential for ROS detoxing in peroxisomes during stress condition [83].

Among antioxidant enzymes, GR plays a vital role in regeneration of reduced glutathione, which, could be a reducing equivalent donor for decrease in dehydroascorbate. Concentration dependent elevation of GR under drought stress (Fig. 19) in wheat plant correlated with reduced GSH levels, thus contributing to the efficient operation of GSH-ASC cycle. Similar rises in GR activity under water stress was formerly reported for barley [84].

Ascorbate peroxidase (APX) activity, which is an important component of the antioxidant system, plays an important role in eliminating H_2O_2 molecules and in the modulation of its steady-state levels in various plant subcellular compartments [85]. These observations in wheat are in good agreement with drought stressed in liquorice plant [86].

The obtained results (Figs. 16-20) showed that, activities of SOD, POX, CAT, GR and APX were increased in response to the treatment with either SA or Zn + irrigation interval every 7 days. On the other hand, significant decrease, regarding the activities of SOD, POX, CAT, GR and APX was resulted due to the treatment with SA, or Zn + different levels of irrigation interval every 14 and 28 days. Previous

reports have also shown that salicylic acid application enhanced the activity of POX in barley plants under drought conditions [87].

The increased Zn efficiency by foliar application may be due to its direct absorption and accumulation in the plants by diffusion from the surface of the leaves to epidermal cells [88]. Therefore, concentration of a solution for fertigation and foliar application of Zn should be chosen with care, based on previous recommendations of different workers. The activation of SOD, POX, CAT and APX enzymes has been reported in several Zn-treated plants subjected to diverse stresses, e.g. in rapeseed seedlings under drought [46] and in wheat, Zn phytotoxicity [47]. The possible mechanisms that might be responsible for enzymes activation by the addition of appropriate doses of Zn include the spontaneous dismutation of O_2^- into H_2O_2 [89] or the direct quenching of O_2^- and OH^- by Zn compounds [90].

3.9. Correlation

The result of the correlation analysis under water stress condition showed that weight of 1000-grains and morphology (Weight of grains/plant (g), No. of grains/plant) and biochemical (RWC, MSI, EL, soluble carbohydrates, soluble protein, proline content, SOD, POX, CAT, GR and MDA) parameters had significant correlation (Table 4). There were positive and significant correlation among weight of 1000-grains and morphology, biochemical (RWC, MSI, soluble carbohydrates and soluble protein). In contrast, there were negative and significant correlation between weight of 1000-grains and biochemical (EL, POX, CAT and MDA) parameters. While, non-significant correlation among weight of 1000-grains, SOD and GR. Similar results were reported by Attarbashi *et al.* [91] and Munir *et al.* [92] which showed that most of the physiological traits significantly affected the grain yield in wheat crop.

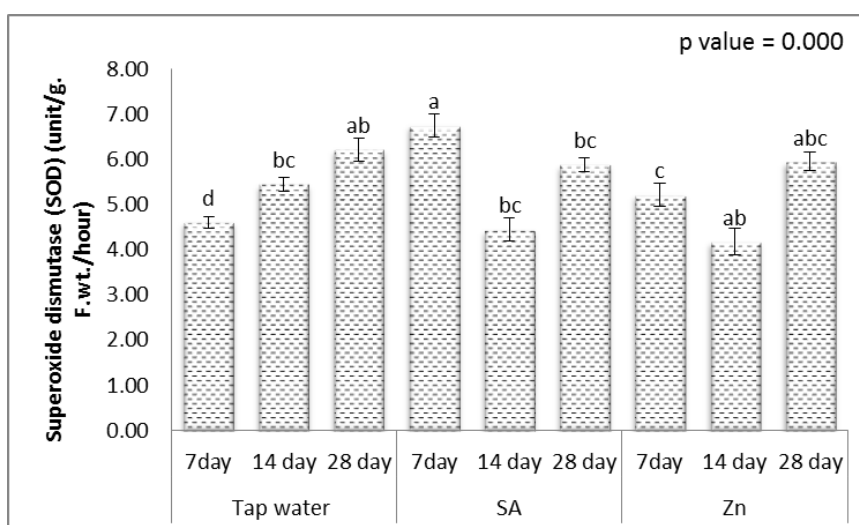


Figure 16. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on superoxide dismutase (SOD) of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

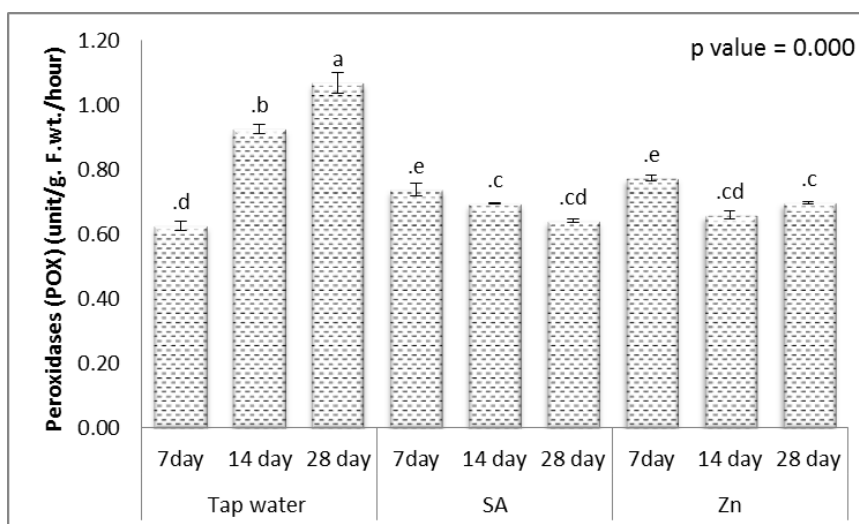


Figure 17. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on peroxidase (POX) of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

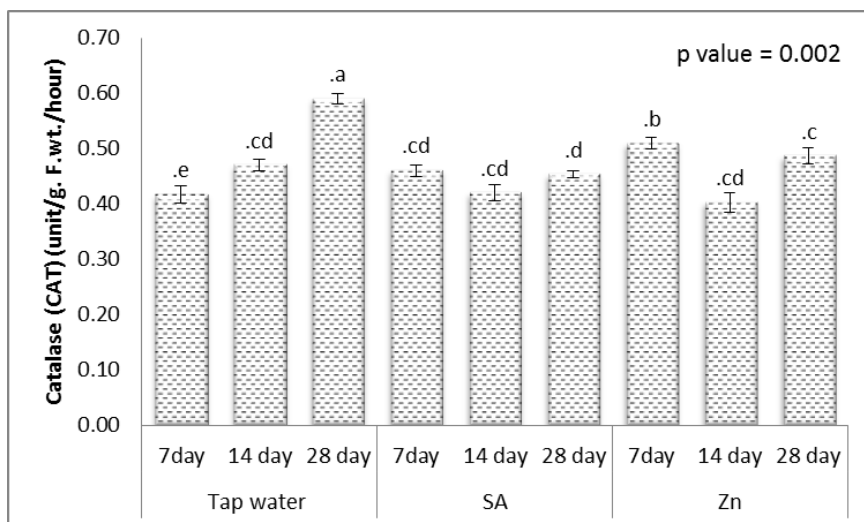


Figure 18. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on catalase (CAT) of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

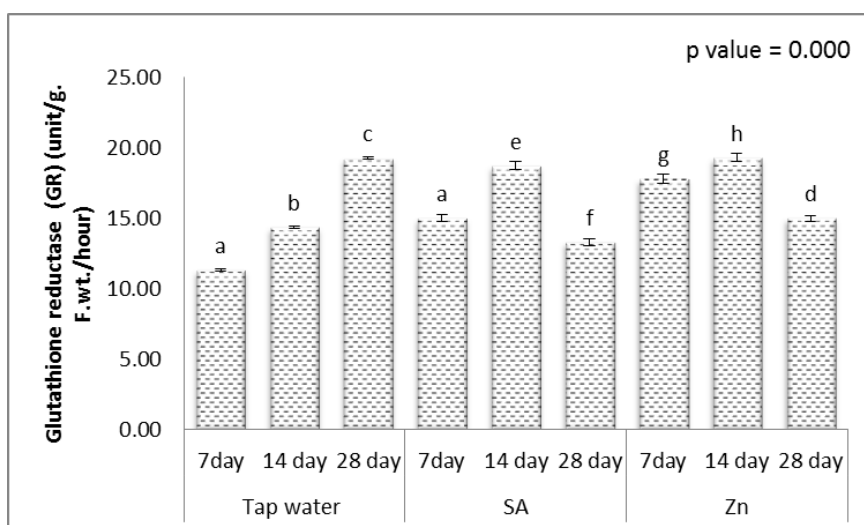


Figure 19. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on glutathione reductase (GR) of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

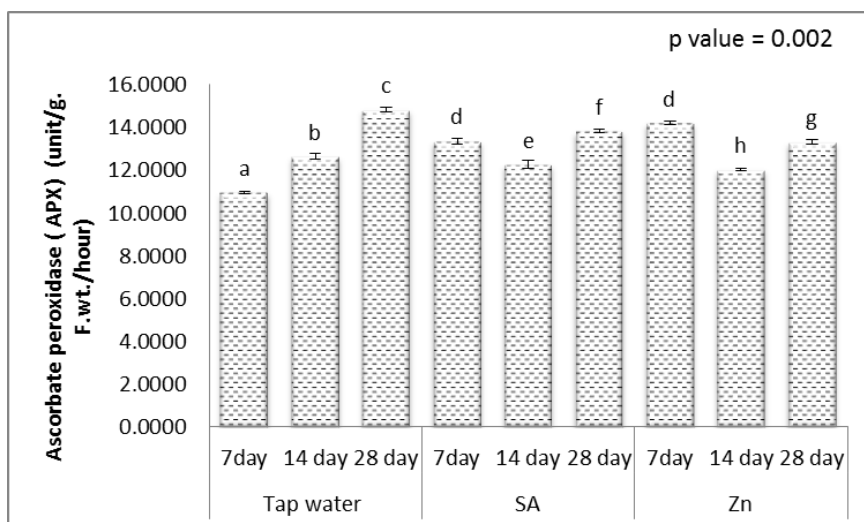


Figure 20. Effect of salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval on ascorbate peroxidase (APX) of wheat (*Triticum aestivum* L. var. Giza 168). The bars with the different letters are significantly different at $P \leq 0.05$

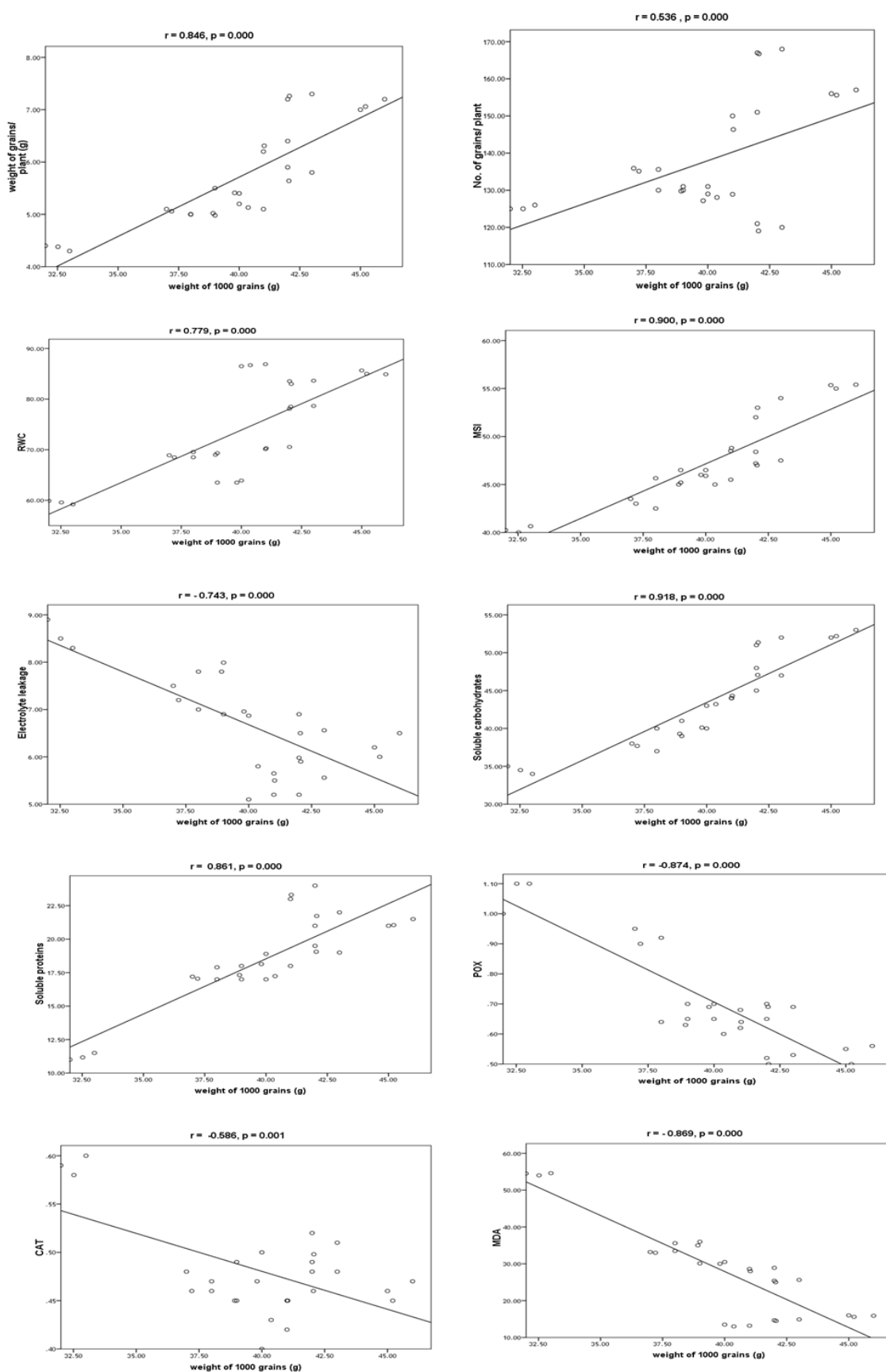


Figure 21. Linear correlation between weight of 1000-grains and items of morphology, biochemical and their statistical significance

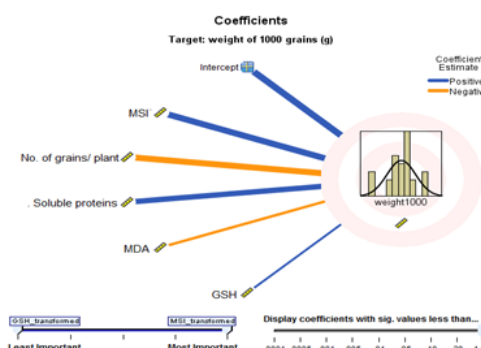
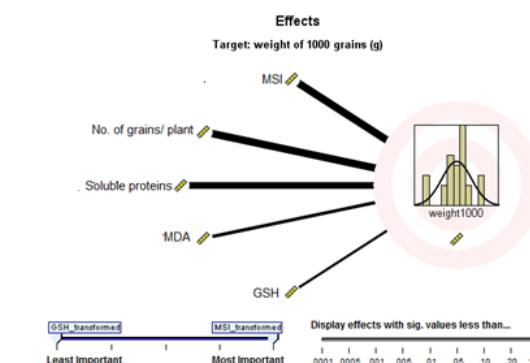
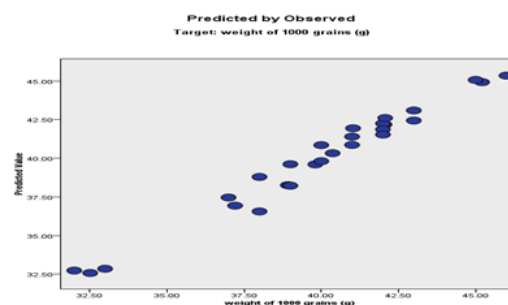
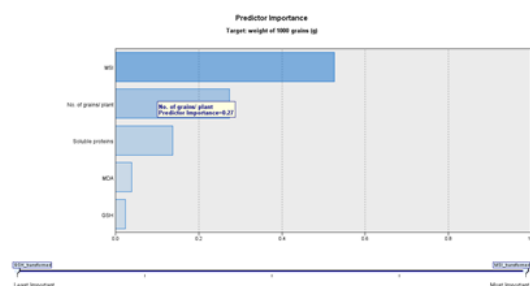
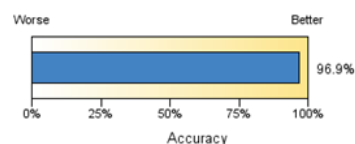
Table 4. The correlation between weight of 1000-grains and items of morphology (Weight of grains/plant (g), No. of grains/plant) and biochemical (RWC, MSI, EL, soluble carbohydrates, soluble protein, proline content, SOD, POX, CAT, GR and MDA) parameters among the studied

Item	Pearson correlation coefficient (r)	P. value	Significance
Weight of grains/plant (g)	0.846	0.000	Significant
No. of grains/plant	0.536	0.000	Significant
RWC	0.779	0.000	Significant
MSI %	0.900	0.000	Significant
Electrolyte leakage	- 0.743	0.000	Significant
Soluble carbohydrates	0.918	0.000	Significant
Soluble proteins	0.861	0.000	Significant
SOD	0.956	0.464	Non-significant
POX	-0.874	0.000	Significant
CAT	-0.586	0.001	Significant
GR	-0.218	0.325	Non-significant
MDA	-0.869	0.000	Significant

Model Summary

Target	weight of 1000 grains (g)
Automatic Data Preparation	On
Model Selection Method	Forward Stepwise
Information Criterion	15.923-

The information criterion is used to compare to models.
Models with smaller information criterion values fit better.

**Figure 22.** Automatic linear modeling of the yielded grains of the wheat in response to salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval

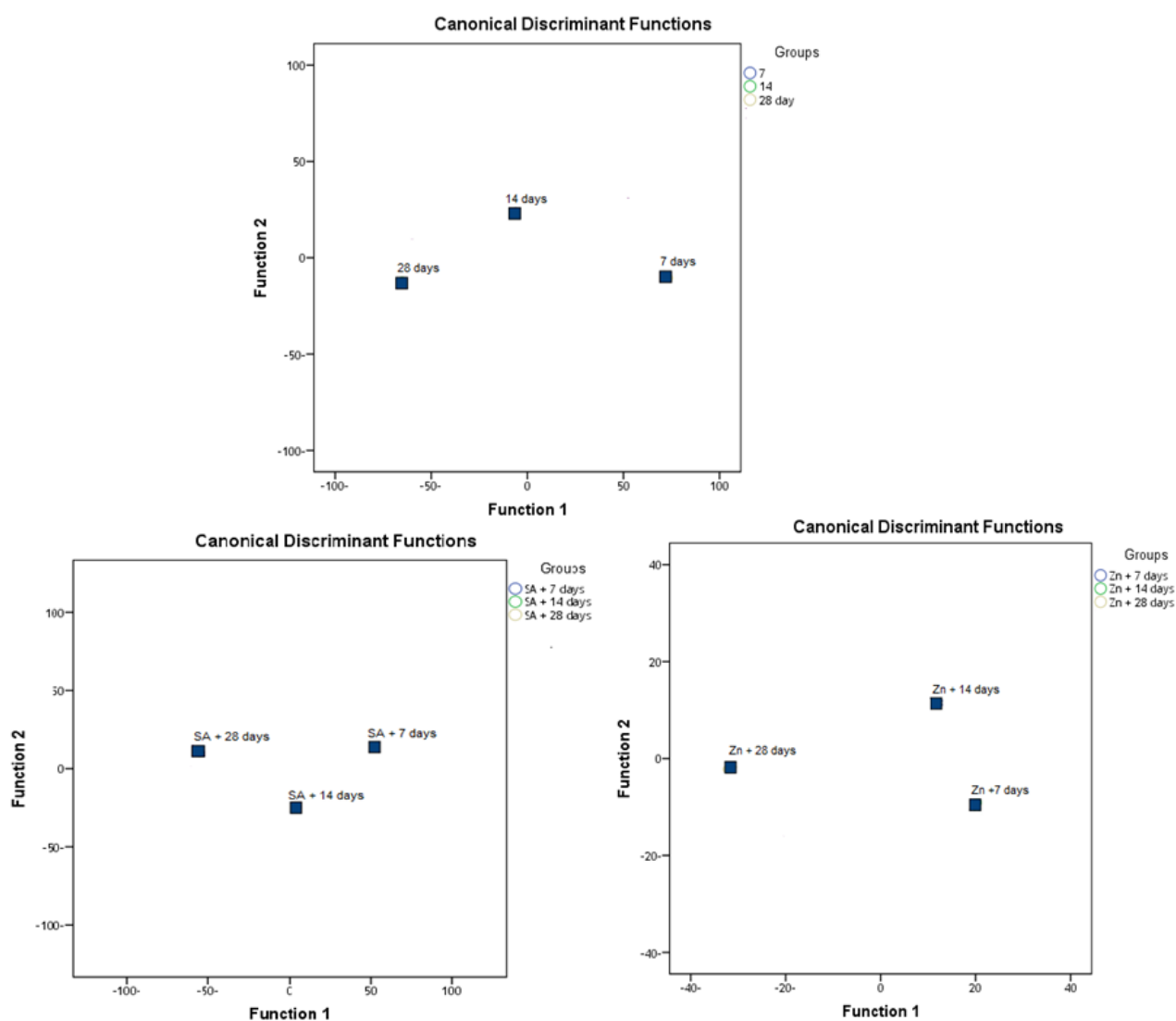


Figure 23. Discriminant of the yielded grains of the wheat in yielded grains in response to salicylic acid (SA), Zinc (Zn) and different levels of irrigation interval

3.10. Automatic Linear Modeling and Discriminant Analysis

Finally, when used the yielded weight as a target; result observed that the accuracy of all data about 96.9%. The effect of target weight of 1000-grains, the wide line is very effective as MSI, then the number of grains/plant, then soluble protein, then MDA and then GSH. Also, the coefficient of target yield weight is the positive blue color wide line which effective as MSI, soluble protein and GSH. While, negative orange color wide line is effective as the number of grains/plant and MDA (Fig. 22). The discriminant analysis that led to the conclusion when foliar application with SA or Zn on wheat plants, we resulted that the SA or Zn is the best treatment to alleviate the stress condition as shown in figure (23).

4. Conclusions

In conclusion, water stress negatively affected the growth, yield, biochemical and antioxidant enzymes of the wheat plant. However, application with salicylic acid or zinc application had a beneficial effect on growth and chemical constituents as well as yield quality of wheat plants under different levels of irrigation interval.

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