

ARSENIC APPLICATION CHANGED GROWTH, PHOTOSYNTHETIC PIGMENTS AND ANTIOXIDANT ENZYMES ACTIVITY IN SORGHUM (*SORGHUM BICOLOR* L.) UNDER SALINITY STRESS*

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The elevation of arsenic (As) content in soils is of considerable concern with respect to its uptake by plant and subsequent entry into wildlife and human food chains. The treatment of sorghum seedlings with As as $\text{NaH}_2\text{As}_4\text{O}_7 \cdot 7\text{H}_2\text{O}$ at various concentrations ($A_1=0$, $A_2=20$, $A_3=40$ and $A_4=60$ mg As kg^{-1} soil) and salinity at four different levels ($S_1=0$, $S_2=3$, $S_3=6$ and $S_4=9$ dS m^{-1}) reduced fresh and dry weights of sorghum plants. The co-application of As and salinity increased the guaiacol peroxidase (GPX) activity in shoot and root tissues. The highest GPX activity in shoot and root tissues was obtained at S_2A_4 and S_3A_3 treatments, respectively. The activity of catalase (CAT) in shoot was not changed, but unlike the GPX activity, salinity and As decreased the CAT activity in root tissues. Concerning the photosynthesis pigments, salinity had no effect on the chlorophyll 'a', chlorophyll 'b' and carotenoid content in leaves, but the As treatment significantly decreased the content of both chlorophyll types. Salinity increased the anthocyanin content in leaves. There were negative correlation between soluble carbohydrates ($r^2 = -0.78^{**}$) and stomata conductance ($r^2 = -0.45^{**}$) and dry weight of the plant biomass in this study. By increasing the salinity and As concentration in root medium, soluble carbohydrate in leaves increased but salinity decreased the leaf stomata conductance.

Heavy metal, Metabolic processes, Physiological parameters, Salinity, Sorghum



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INTRODUCTION

In recent years, heavy metal pollution in soil has gained major concern due to its negative impact on agricultural production and human health. Heavy metals and metalloids are released into the environment by many anthropogenic activities (Rahman et al., 2014). Arsenic (As) contamination in soil and groundwater is a worldwide problem, resulting from natural geologic activity and anthropogenic sources such as mining, heavy industry, semiconductor manu-

facturing, forest products, landfill leachates, fertilizers, pesticides and sewage (Francisco et al., 2002).

The serious arsenic harm has become one of the problems attracting the attention of researchers worldwide. Large numbers of studies indicated that low concentrations of arsenic stimulated the growth of plants; but excessive arsenic is harm to them (Han et al., 2002). Han et al. (2002) stated that at higher concentration, arsenic interfered with metabolic processes and inhibited plant growth and development through arsenic induced phytotoxicity. Johana et al.

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(2003) has confirmed that when plants were exposed to excess arsenic, either in soil or in solution culture, they exhibited toxicity symptoms such as inhibition of seed germination, decrease in plant height, depression in tillering, reduction in root growth, decrease in shoot growth, lower fruit and grain yield, and sometimes this exposure led to plant death. However, little is known about the effect of arsenic on photosynthesis, the basis of plant bio-chemical system.

Requejo, Tena. (2005) reported on the effect of arsenic exposure on maize (*Zea mays* L.) root proteome and concluded that the induction of oxidative stress is the main process underlying arsenic toxicity in plants. Plants respond to oxidative stress by increasing the production of antioxidant enzymes such as superoxide dismutase (SOD) or ascorbate peroxidase (APX) (Heidari, Jamshidi, 2011). In this case, SOD is one among the primary induced enzymes, and is responsible for the detoxification of the active superoxide radicals. In plants several isomeric forms of SOD catalyze the dismutation of the superoxide radicals (O_2^-) to hydrogen peroxide (H_2O_2). The conversion of H_2O_2 into water in peroxisomes is carried out by catalase (CAT), while that in cytosol and chloroplasts by ascorbate glutathione cycle, which involves APX, ascorbate (ASA), and glutathione reductase (GR) (Foyer, Noctor, 2003).

Guaiacol peroxidase (GPX) are ubiquitous enzymes that catalyze the reduction of H_2O_2 or organic peroxides to water or the corresponding alcohols using glutathione (GSH) or thioredoxins (Trxs) as electron donors (Herbette et al., 2007; Brigelius-Flöhé, Maiorino, 2013). In different plants, GPX may protect membranes from peroxidative damage (Herbette et al., 2007) and some *Arabidopsis thaliana* GPX isoforms may play additional roles in redox transduction and stress signaling (Chang et al., 2009).

The adverse impact of arsenic on growth and physiological reactions in crop plants can be intensified by the high salinity conditions. Therefore, similar to arsenic, salinity is one of the most important stress factors which limit the growth and development of plants. More than 1000 million hectares of land throughout the world are salt-affected (Munns, Tester, 2008). Salinity can affect in several physiological and metabolic processes in plants such as photosynthesis, protein synthesis, respiration, nitrogen assimilation, and phytohormone turnover. Plant responses to stress are complex and depend on a number of interrelated factors based on morphological, biochemical, and physiological processes (Qasim et al., 2003). Salinity stress, like other abiotic stresses, can also lead to oxidative stress through the increase in reactive oxygen species (ROS). To be able to endure oxidative damage, plants must possess efficient antioxidant system (Apel, Hirt, 2004).

Salinity affects plants through osmotic stress and ion imbalance and toxicity. Osmotic effects are due to

salt-induced decrease in the soil water potential. Salts for some time accumulate inside a plant before the plant function is affected (Heidari, Jamshidi, 2011). Plants have developed a wide range of mechanisms to sustain productivity under salt stress environment. These mechanisms are osmotic adjustment, Na^+ and/or Cl^- exclusion, and tissue tolerance of high concentrations of Na^+ and/or Cl^- . The synthesis and accumulation of low molecular weight metabolites, known as compatible solutes, is a ubiquitous mechanism for osmotic adjustment in plants. Their main role is to increase the ability of cells to retain water without affecting the normal metabolism. Amino acids, sugars, betaines and proline compounds may accumulate, as compatible solutes, in many plant species. Research on salt tolerance of various crops has indicated that salt tolerance depends largely on genera and species and even on cultivars within certain species (Munns, Tester, 2008).

Sorghum (*Sorghum bicolor* L.) was characterized as moderately tolerant to salinity. Selection and breeding have always been conducted to achieve high yield and better quality of crops under stressful conditions. In recent year, sorghum has been considered is a major grain and forage crop and a potential bioenergy crop. Large variations in salt tolerance among genotypes have been reported for sorghum (Krishnamurthy et al., 2007). Understanding the physiological and mechanisms to cope with environmental stresses can be very useful for the selection of plants suitable for cultivation under high salinity conditions. Therefore, the aim of this study was to determine the interaction between salinity and arsenic soil content on growth, antioxidant enzyme activity and photosynthesis pigment in sorghum. In Iran, high salinity of agricultural soils is frequent and a simultaneous use of mineral fertilizers and irrigation with wastewaters can result in the elevated As contents in the soils.

MATERIAL AND METHODS

Plant culture

A plot experiment was laid out as a completely randomized 4×4 factorial design with three replicates in a greenhouse at the Agricultural Shahrood University of Technology, Iran during 2015. Totally 48 plots (26×20 cm) were filled with sandy loam soil (electrical conductivity (EC)= 1.1 dS m^{-1} and $pH=7.56$). Four different quantities ($A_1=0$, $A_2=20$, $A_3=40$ and $A_4=60 \text{ mg As kg}^{-1}$ soil) of sodium arsenate ($NaH_2As_4O_7 \cdot 7H_2O$) were applied as separate treatments per plot and mixed into the soil. The amount of arsenic based on the weight of the soil in each plot was calculated based on the treatment and then mixed into the soil at depth of 10 cm. In this study, eight sorghum seeds were sown at uniform depth

(2 cm). Following emergence, the plants were thinned to three per plot.

The sorghum plants were subjected to four different salinity treatments ($S_1=0$, $S_2=3$, $S_3=6$ and $S_4=9$ dS m^{-1}) using NaCl applied in increments and dissolved in the irrigation water. Air temperatures in the greenhouse were controlled between 25-35°C during the day and 20-25°C during the night. Relative humidity ranged from 50 to 80%. Radiation intensity averaged 1200 $\mu\text{mol m}^{-2} \text{s}^{-2}$, with a minimum of 344 and a maximum of 1500 $\mu\text{mol m}^{-2} \text{s}^{-2}$ at solar noon. The light cycle during the experiment was 14.30 hours light and 9.30 darkness.

The plants were harvested 30 days after initiation of the salinity treatments. At harvest, the shoots of each plant were individually cut, freshly weighed, each plant was put to an oven (SH-Scientific-SH-DO-54NG, South Korea) at 70°C for 48 h, and then weighed again to obtain the dry shoot biomass weight per plant. Soil was carefully removed from the root samples only for determine the present antioxidant enzymes activity.

Chlorophyll, carotenoid, anthocyanin, soluble carbohydrate and Stomata conductance

Thirty days after the initiation of the salinity treatments, one of plant from the plot was harvested and the organic and inorganic solutes were extracted from the mature leaf blades. Chlorophyll 'a', Chlorophyll 'b' and carotenoid in the leaves were extracted with 80% acetone and determined according to the method by Arnon (1967), wherein the chlorophyll spectrum absorptions were measured at 645 and 663 nm, respectively, and the carotenoid calculated at 440 nm.

Anthocyanin contents was determined using the method of Mancinelli et al. (1975) and using the formula, $A=A_{530}-(1/3A_{657})$. Soluble carbohydrate was determined according to Horwitz (1975). Stomata conductance was measured by prometer (SC1 model, USA).

Enzyme Assays

Antioxidant enzyme activities in the tissues of leaves and roots were assayed from leaf and root samples collected in an ice bucket and brought to the laboratory. These samples were washed with distilled water and their surfaces wiped clean of moisture. The samples (0.5 g) were then homogenized in near zero degree 0.1 M phosphate buffers (pH 7.5) containing 0.5 mM EDTA using a pre-chilled pestle and mortar. Transferred to centrifuge tubes, the homogenate was centrifuged at 4 °C in a refrigerated Beckman unit for 15 min. at 15,000 rpm. The supernatant was transferred to 30 ml tubes and used sequentially as the enzyme extract.

Total CAT was assayed by measuring the residual H_2O_2 by titanium reagent (Taranishi et al., 1974). Reaction mixture (3 ml) consisted of 1 ml of 6 mM H_2O_2 and 1.9 ml of 0.1 M phosphate buffer (pH 7.0) in test tubes, and the reaction was initiated by adding 0.1 ml of diluted enzyme extract. The reaction was stopped after 5 min by addition of 4 ml of titanium reagent, which also formed a coloured complex with residual H_2O_2 . The reaction mixture without enzyme served as control and developed maximal colour with titanium reagent. Aliquots were centrifuged at 10000 rpm for 10 min and absorbance of supernatant was recorded at 415 nm in spectrophotometer (JENWAY 6105). The residual H_2O_2 content in samples was computed with the help of standard curve.

Total GPX activity was determined as described by Urbánek et al. (1991) in a reaction mixture (2.0 ml) containing 100 mM phosphate buffer (pH 7.0), 0.1 μM EDTA, 5.0 mM guaiacol, 15.0 mM H_2O_2 and 50 μl of the enzyme extract. The addition of enzyme extract started the reaction and the increase in absorbance was recorded at 470 nm for 1 min. Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient ($26.6 \text{ mM}^{-1} \text{ cm}^{-1}$). The results were expressed as $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1}$ dry matter, taking into consideration that 4 mole H_2O_2 are reduced to produce 1 mole of tetraguaiacol (Plewa et al., 1991).

Statistical Analyses

All data were analyzed with the SAS software (Version 9.2) after first undergoing an ANOVA to determine statistical significance for the treatment effects ($P=0.05$ or less). Significant differences between individual means were determined using the Least Significant Difference test (LSD). Data points in the figures represent the means \pm standard errors of three independent experiments with at least three replications per treatment.

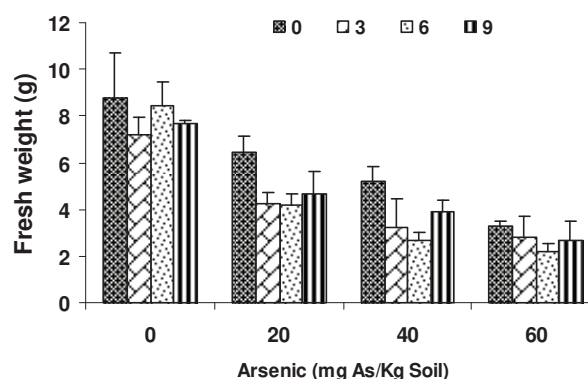


Fig. 1. Effect of the salinity and arsenic on fresh weight

Table 1. Results of variance analysis (ANOVA) of NaCl concentrations (S), Arsenic (As) and their interaction for growth, antioxidant activity, soluble carbohydrate, stomata conductance and photosynthesis pigments

Dependent variable	Independent variable		
	Salinity (S)	Arsenic (As)	S*As
Fresh weight	9.39*	51.65**	21.09**
Dry weight	0.67*	2.81**	0.091 ^{ns}
Chlorophyll 'a'	47.01 ^{ns}	250.02**	117.1 ^{ns}
Chlorophyll 'b'	6.85 ^{ns}	26.42**	13.6 ^{ns}
Carotenoid	0.148 ^{ns}	0.382 ^{ns}	0.181 ^{ns}
Anthocyanin	0.081 ^{ns}	1.85**	0.79*
GPX activity in root	0.0291**	0.0156**	0.0215**
GPX activity in shoot	0.000213*	0.000413**	0.000137*
CAT activity in root	0.0211**	0.0127**	0.0135**
CAT activity in shoot	0.0167 ^{ns}	0.0133 ^{ns}	0.0105 ^{ns}
Stomata conductance	458316.95 ^{ns}	2989259.8**	3181962.9**
Soluble carbohydrate	4.61 ^{ns}	0.571 ^{ns}	6.85*

GPX = guaiacol peroxidase, CAT = catalase; ns = not significant

F-values at 5% level are presented; significant at *P < 0.05 and **P < 0.01

RESULTS

Fresh and dry weight and photosynthesis pigments

The change in fresh and dry weight may reflect the growth rate reaction of a plant to its living environment. Statistical analysis of the data in Table 1 showed that, interaction between salinity and arsenic treatments had significantly affected fresh weight of sorghum plants.

By increasing salinity levels from control to 9 ds m⁻¹, an additional decreased occurred. In the presence of arsenic, the reduction was greater. The maximum fresh weight was obtained at the S₁A₁ and the lowest at the S₃A₄ treatments (Fig. 1). Salinity decreased dry weight of plant biomass by about 42.2 % at the 9 ds/m compared to the control treatment and the reduction of dry weight at the arsenic treatment was about 32.1% (Table 2)

Salinity and arsenic significantly reduced fresh and dry weight, but salinity treatment had no significant effect on photosynthetic pigments in sorghum plants. Salinity had no significant effects on chlorophyll 'a', chlorophyll 'b' and carotenoid content in leaves of sorghum (Table 1). However by increasing salinity levels from 0 to 9 ds m⁻¹ the content of these pigments was altered but the change was not significant (Table 2).

Arsenic treatment in this study, except carotenoid, significantly decreased the content of chlorophyll 'a' and chlorophyll 'b' in leaves (Table 2). However, by increasing arsenic concentration from A₁ to A₃, the reduction of chlorophyll 'a' and 'b' was not high, but at the highest level of arsenic (A₄), the reduction of chlorophyll types made about 48.7% and 41.4% re-

spectively, as compared to the A₁ treatment (Table 2). Anthocyanin is another photosynthetic pigment in this experiment significant effected by interaction between salinity and arsenic (Table 1). As can be seen in Fig. 2, the highest amount of anthocyanin was obtained at the S₄A₁ treatment and the lowest at the S₁A₄ treatment.

Antioxidants enzyme activities

Sorghum grown at various NaCl and arsenic concentrations showed changes in antioxidant activities of H₂O₂ scavenging enzymes in shoot and roots tissues. The interaction between salinity and arsenic treatments in this study except CAT activities in shoot, had significant effect on GPX activity in root and shoots and CAT activities in root tissues (Table 1).

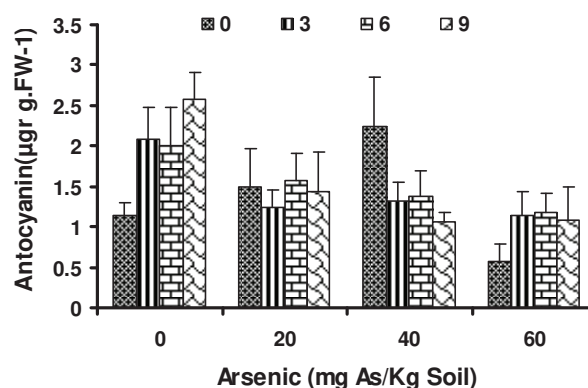


Fig. 2. Effects of salinity and arsenic on anthocyanin pigment in leaves of sorghum plants

Table 2. Growth, antioxidant activity, soluble carbohydrate, stomata conductance and photosynthetic pigments of sorghum plants at different salinity and arsenic concentration

Treatment	Fresh weight (gr)	Dry weight	Photosynthesis pigments ($\mu\text{g/g.FW}$)			Stomata c onductance (mmol.m-2.sec)	Soluble carbohydrate ($\mu\text{mol Glucose/g FW}$)	Antioxidant activity ($\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$)			
			chlorophyll 'a'	chlorophyll 'b'	Carotenoid			Anthocyanin	GPX		CAT
								Root	Shoot	Root	Shoot
Salinity (ds/m)											
0	5.94a	1.35a	19.1a	7.71a	1.18a	1.36a	20.47a	0.271a	0.015b	0.313a	0.574a
3	4.79b	0.989b	16.61a	6.71a	1.155a	1.44a	20.93a	0.283b	0.023ab	0.225b	0.654a
6	4.38bc	1.015b	17.28a	6.83a	1.175a	1.53a	21.88a	0.238a	0.023ab	0.238b	0.652a
9	3.86c	0.785b	14.22a	5.86a	0.952a	1.54a	22.51a	0.165b	0.024a	0.227b	0.628a
Arsenic (mg AS/Kg Soil)											
0	7.58a	1.67a	20.71a	8.21a	1.20a	1.95a	20.01a	0.221a	0.0141b	0.298a	0.648a
20	4.87b	1.11b	19.81a	7.35ab	1.23a	1.43b	21.44a	0.239a	0.0191b	0.242b	0.642a
40	3.75c	0.78c	15.91ab	6.82ab	0.85a	1.50b	21.31a	0.235a	0.0200b	0.240b	0.577a
60	2.77d	0.56c	10.69b	4.72b	1.18a	0.99c	22.02a	0.161b	0.0283a	0.223c	0.640a

FW = fresh weight, DW = dry weight, GPX = guaiacol peroxidase, CAT = catalase
Means followed by the same letter are not significantly different within rows and column according to Least Significant Difference (LSD) ($P \leq 0.05$)

At the end of the experimental period, by increasing salinity levels from S1 to S4 the GPX activities in shoot, and until S3 level in root, were increased but arsenic intensified the activities in both shoot and root tissues (Table 2). The highest GPX activity in shoot and root at the combined arsenic and salinity treatment were obtained at S2A4 and S3A3 respectively (Figs. 3 and 4). In this study, the results showed that a negative relationship between fresh and dry weight with the GPX activity in shoot ($r^2 = -0.52^{**}$).

Generally, the CAT activity in root tissues was decreased not only by increasing salinity levels from 0 to 9 ds m⁻¹, but also by the arsenic treatment. The CAT activity was affected by both stresses (Table 2). By the combined arsenic and salinity treatment, the highest CAT activity in root tissues was obtained by the S1A3 treatment (Fig. 5).

Stomata conductance and carbohydrate content

The content of soluble carbohydrate in the leaves of sorghum was determined. The results of this study

indicated that the interaction between salinity and arsenic treatments had a significant effect on the soluble carbohydrate content in sorghum plants (Table 1). The results showed that by increasing salinity levels from control to 9 ds m⁻¹, the accumulation of carbohydrates in the leaves was increased. In this case, the presence of arsenic concentrations also increases the accumulation of carbohydrates in leaves. So that by increasing the concentration of arsenic from 0 to 60, the content of soluble carbohydrates in the leaves was enhanced. The highest amount of soluble carbohydrates was obtained at S4A3 treatment (Fig. 6). A significant negative correlation was observed between soluble carbohydrate and plant dry weight ($r^2 = -0.78^{**}$). Thus the accumulation of carbohydrates in plant tissues to regulate osmotic adjustment under abiotic stress is useful, but its synthesis requires energy.

The effects of salinity and arsenic on stomata conductance in leaves of sorghum are showed in Table 1. The interactions between salinity and arsenic had significant effect on stomata conductance in leaves. However, by increasing salinity levels in root me-

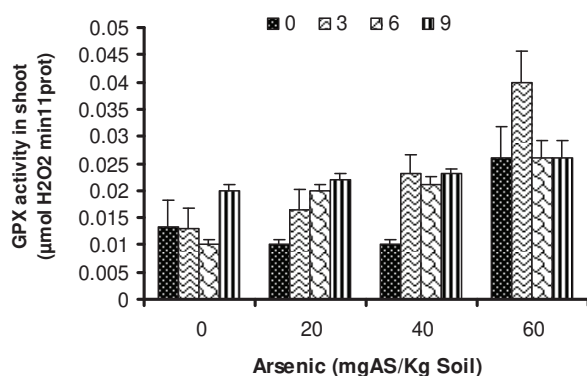


Fig. 3. Effects of salinity and arsenic on GPX activity in shoot

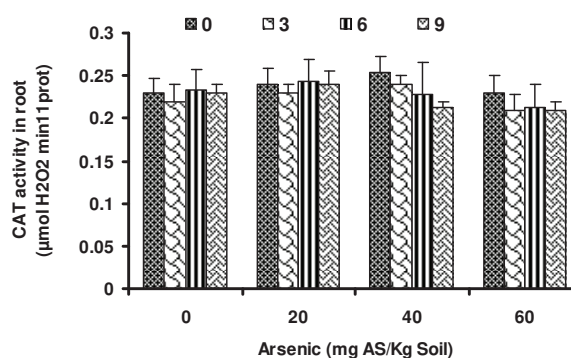


Fig. 5. Effects of salinity and arsenic on CAT activity in root

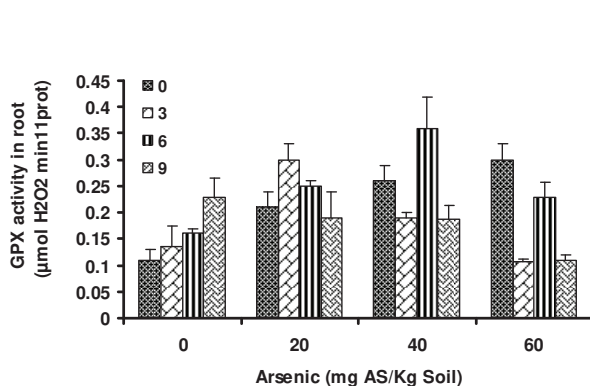


Fig. 4. Effects of salinity and arsenic on GPX activity in root

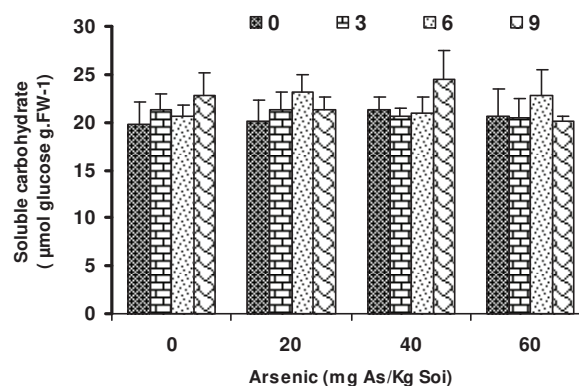


Fig. 6. Effects of salinity and arsenic on soluble carbohydrate in leaves of sorghum plant

dium, the leaf stomata conductance in sorghum was decreased, but the application of arsenic in the root medium increased the stomata conductance in the plants (Table 2). At the combined effect of arsenic and salinity, the highest of stomata conductance was obtained at S1A4 treatment (Fig. 7). In this study, negative correlation between stomata conductance and dry weight ($r^2 = -0.45^{**}$) were obtained.

DISCUSSION

Environmental stress like drought, temperature, salinity and heavy metals are the major constraint that limits plant growth and productivity. Previous studies have mostly been devoted to the physiological response of plants in the environment single stressed by e.g. salinity (Nawaz et al., 2010) and heavy metals (Jele et al., 2004). Of the environmental stresses, both salinity and arsenic stress can lead to changes in growth and development and oxidative damage by ROS increase in plants. Indeed, Jaleel et al. (2009) reported that the reduction of plant growth under salt stress is the result of the alteration of many physiological activities in the plant, such as photosynthetic activity, mineral uptake and antioxidant activity. This experiment confirmed that both salinity and arsenic can negative impact many physiological parameters such as photosynthetic pigments, nutrient uptake and antioxidant enzymes activities. The results in Table 1 showed that the interaction between salinity and arsenic had not significant effects on photosynthetic pigments, by increasing salinity and arsenic concentration in root media, the content of photosynthetic pigments and fresh weight decreased.

In addition, salinity and arsenic supported the development of oxidative stress in sorghum plants. The interaction between salinity and arsenic treatments had

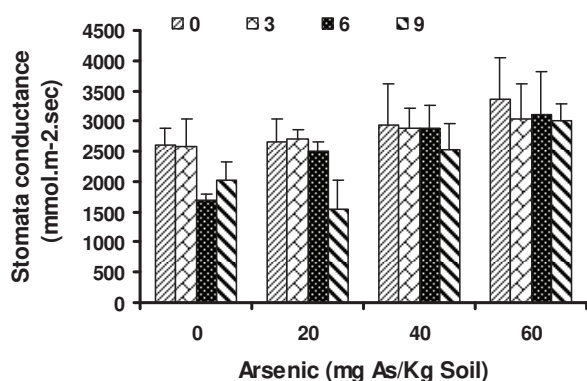


Fig. 7. Effects of salinity and arsenic on stomatal conductance in leaves of sorghum plant

significant effect (except CAT activities in shoot) on the GPX activity in root and shoots and CAT activities in root tissues (Table 1). The highest GPX activity in shoot and root were obtained at S₂A₄ and S₃A₃ treatment respectively (Figs. 3 and 4). Unlike GPX activity in root and shoot tissues, salinity and arsenic decreased the CAT activity in root tissues. By increasing the concentration of salinity and arsenic in root medium the activity of CAT was decreased (Table 2). There is significant evidence that exposure to inorganic arsenic species results in the generation of ROS. This probably occurs through the conversion of arsenate to arsenite, a process which readily occurs in plants, and leads to the synthesis of enzymatic antioxidants such as SOD, CAT and glutathione-S-transferase, and nonenzymatic antioxidants, e.g. glutathione and ascorbate (Hartley-Whitaker et al., 2001).

The plant roots are the first contact point with nutrient elements and salinity. In this study, the increasing antioxidant enzymes activities were higher in roots than in shoots when exposed to both arsenic and salinity treatments (Figs. 3 and 4). To minimize the effects of oxidative stress, plant cells have evolved a complex antioxidant system, which is composed of low-molecular mass antioxidants (glutathione, ascorate and carotenoids) as well as ROS-scavenging enzymes, such as SOD, CAT, APX, GPX, and GR (Apel, Hirt, 2004).

Besides salinity and arsenic presence in soil, plant growth is basically affected by photosynthesis – the most important biochemical process which converts massive amount of sunlight into electrical and then chemical energy. The most important photosynthetic pigment is chlorophyll, consists of two types – chlorophyll ‘a’ and chlorophyll ‘b’. Miteva, Merakchyska. (2002) reported that arsenic concentrations of 25 mg kg⁻¹ soil did not have negative affect the photosynthetic process in bean plants (*Phaseolus vulgaris* L.), while the higher doses (50 and 100 mg of As kg⁻¹ soil) inhibited the photosynthesis by 42 and 32%, respectively. In this study, the arsenic treatment, except carotenoid, significantly decreased the content of chlorophyll ‘a’ and chlorophyll ‘b’ in leaves of sorghum plants (Table 2). However, by increasing the arsenic concentration from A₁ to A₃, the reduction of chlorophyll ‘a’ and ‘b’ was not high, but at the highest level of arsenic (A₄), the reduction of both chlorophyll types was about 48.7% and 41.4% respectively compared to the A₁ treatment (Table 2).

Salinity showed no significant effects on chlorophyll ‘a’, chlorophyll ‘b’ and carotenoid content in leaves of sorghum (Table 1). However by increasing salinity levels non-significant changes in the pigment content occurred (Table 2). According to the data obtained from the experiments, anthocyanin production was influenced by both salinity and arsenic treatment. The data analysis showed that the interaction between of salinity and arsenic affected it significantly (Table 1).

The highest amount of anthocyanin was obtained at the S_4A_1 treatment and the lowest at the S_1A_4 treatment (Fig. 2). As reported by Winkel-Shirley (2002) anthocyanin synthesis is one of the subsequent production and its localization in root, stem and especially leaf tissues may allow the plant to develop resistance to a number of environmental stresses. Ali, Abbas (2003) found that saline (NaCl) stress in barley seedlings caused an increase in total phenolic compounds such as flavonoids. Salinity also enhanced peroxidase and then decreased the growth rate of seedling plants.

Stomata conductance is one of the factors in the process of photosynthesis. In this experiment, however, by increasing salinity levels, the stomata conductance in leaves was reduced, but the arsenic treatment increased the stomata conductance in leaves (Fig.7). Under these conditions, salinity and arsenic increased the accumulation of soluble carbohydrates in the leaves tissues (Table 2). Metabolic acclimation via the accumulation of compatible solutes is often regarded as a basic strategy for the protection and survival of plants under abiotic stress. The synthesis and accumulation of low molecular weight metabolites, known as compatible solutes, is an ubiquitous mechanism for osmotic adjustment in plants. Their main role is to increase the ability of cells to retain water without affecting normal metabolism. Amino acids, sugars, betaines and proline compounds may accumulate as compatible solutes in many plant species (Shabala, Cui, 2006).

CONCLUSION

The results obtained from this experiment showed that, salinity and arsenic can adversely affect some of physiological and biochemical traits in sorghum plants. Our results indicate that by increasing salinity levels from 0 to 9 $ds\ m^{-1}$ in the culture media, the content of chlorophyll 'a', chlorophyll 'b' and carotenoid in leaves changed, but these change were not significant. In this study, salinity accelerated the soluble carbohydrate formation and the activity of antioxidant enzymes such as GPX in root and shoot tissues of sorghum plants. The CAT activity in root tissues and stomata conductance in leaves were decreased by increasing salinity levels of the culture media. In the presence of salinity, with the arsenic concentration increase from 0 to 60 $mg\ As\ kg^{-1}$ soil, the content of chlorophyll 'a' and 'b' in leaves and the amount of CAT in root tissues were decreased. Arsenic also increased the GPX activity and stomata conductance in leaf tissues of sorghum plants. Generally, the results proved the resistance of sorghum plants to salinity, but at the level higher than 6 $dS\ m^{-1}$ the presence of arsenic exceeding 20 $mg\ As\ kg^{-1}$ soil resulted in the changes in physiological characteristics.

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