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Exogenous Silicon Ameliorate Alkalinity Stress in Sorghum *(Sorghum Bicolor L.)* Plants by Up-Regulating the Membrane Characteristics and Antioxidant Capacity Defense

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Abstract

The protective role of silicon in relation to growth vigor of flag leaf, membrane characteristics (%), antioxidant enzymes, lipid peroxidation, membrane stability index (%) and membrane leakage, as well as non-enzymatic antioxidants and non-photosynthetic pigment contents were investigated in alkalinity stressed sorghum plants. In the majority of cases, alkalinity stress induced significant reduction ($p \leq 0.05$) in leaf area and degree of succulence (non-significant increase in case of sensitive cultivar) of both sorghum cultivars during grain-filling. On the other hand, alkalinity stress induced a clear increase ($p \leq 0.05$) in the degree of leaf sclerophylly of tolerant cultivar and a non-significant increase in case of sensitive cultivar. Alkalinity stress reduced bio-membranes stability through increasing its lipid peroxidation resulting in an increase in membrane leakage (ML) with a simultaneous decrease in membrane stability index (MSI) in leaves of both sorghum cultivars. Moreover, it was obvious that alkalinity significantly increased the activity of catalyse, ascorbic acid oxidase (AAO) and peroxidase (POD) activities and induced a non-significant reduction in polyphenol oxidase (PPO) activity in leaves of both sorghum cultivars comparing with the control plants. Among cultivars, tolerant one showed higher enzymes activity than sensitive one. Application of silicon markedly increased AAO and POD activities as well as a non-significant decrease in PPO activity in leaves of alkalinity stressed sorghum plants. Alkalinity stress caused a non-significant increase ($p \leq 0.05$) in the amount of total phenols and flavonoids as well as non-photosynthetic pigment content (anthocyanin, β-carotene and lycopene contents) in flag leaf of both cultivars. In addition, application of silicon induced an additional increase ($p \leq 0.05$) in total phenols and flavonoids as well as anthocyanin and β-carotene contents except for lycopene content which induced a non-significant decrease. These results suggest that the exogenous application of silicon assisted the plants to become more tolerant to alkalinity stress induced oxidative damage by up-regulating the membrane characteristics and enhancing their antioxidant defense system.

Keywords: Sorghum; Alkalinity; Silicon; Membrane characteristics; Antioxidant enzymes defense

Abbreviations: ROS: Reactive Oxygen Species; MDA: Malondialdehyde; AAO: Ascorbic Acid Oxidase; POD: Peroxidase; PPO: Polyphenol Oxidase; CAT: Catalase; ML: Membrane Leakage; MSI: Membrane Stability Index; TCA:

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Trichloroacetic Acid; Si: Silicon; SOD: Superoxide Dismutase; APX: Ascorbic Acid Peroxidase; EC: Electric Conductance; MII: Membrane Injury Index; TV: Total Volume; VU: Volume Used; POX: Peroxidase.

Introduction

Efforts are underway to enhance the production of different crops to meet the food requirements of rapidly increasing population of Egypt. Selecting and cultivating the crops that can tolerate water salinity is potentially an important strategy to save fresh water resources and maximize the crop yield in salt affected areas. Sorghum *(Sorghum bicolor L.)* is an example of a so-called ancient whole grain cereal that is better known to Western societies as an animal feed rather than a human food source. Sorghum is grown around the world, and ranks fifth in global cereal production after maize, rice, wheat and barley [1]. In many countries of Africa and Asia, sorghum is widely cultivated due to its adaptability to semi-arid and arid conditions and high temperature. Sorghum-based foods are a good source of both iron and zinc [2].

Alkaline stress, which is defined as the existence of alkaline salts (Na_2CO_3 or $NaHCO_3$) in the soil [3] is one of the most crucial abiotic stressors which plants encounter in the era of climate change. A number of studies have shown that alkaline stress is more dangerous than saline stress, owing to its additional high pH stress [4]. High pH value may lead to reduction in seed germination, destruction of the root cell structure, and change in the nutrient availability and disorder in nutrient uptake and thus resulting in a significant decrease in the yield of agricultural plants [5]. Egyptian soils are in general distinguished by a little alkaline to alkaline pH values (7.5-8.7) which are mainly due to its arid ambience [6]. A few studies have been carried out on the effects of alkaline stress on plant growth and productivity. However, only scant information is available about the morphological, physiological, biochemical, and anti-oxidative responses in plants under alkaline stress.

Silicon (Si) is the 2nd most abundant element on the earth crust after oxygen. It is accumulated in plants at a rate comparable to those of macronutrient elements like calcium, magnesium and phosphorous [7]. It is evident that Si is beneficial for growth of many plants under various abiotic (e.g. salt, drought and metal toxicity) and biotic (plant diseases and pests) stresses [8]. A number of possible mechanisms are reported through which Si may increase salinity tolerance in plants including increased plant water status 9] and stimulation of ROS scavenging system [10].

Priming of seeds with silicon (Si) is one of the major techniques, which can improve abiotic stress tolerance in plants [11]. Si is recognized as quasi essential element for plants because its deficiency results in various dysfunctions with respect to plant growth, evolution, and proliferation [12]. Si, as a fertilizer, bio-stimulator plant protectant, plays a pivotal role in enhancing the plants growth and productivity, especially in stress regimes [13]. *Zea mays* are classified as a Si accumulator and are relatively susceptible to alkaline stress. Si plays a pivotal role in alleviating the negative effects of alkaline stress on maize growth by improving water status, enhancing photosynthetic pigments, accumulating osmoprotectants rather than proline, activating the antioxidant machinery, and maintaining the balance of K+/Na+ ratio [14].

Membranes are thought to play a central role in cell viability as they participate in metabolic activities of the plant either directly or indirectly. The degree of cell membrane injury induced by water stress may be easily estimated through measurements of membrane stability index (MSI) [15]. In addition, membrane leakage (ML) to cell electrolytes is an indicator of cell membrane integrity [16]. Furthermore, malondialdehyde (MDA) content is usually used to measure the extent of lipid peroxidation resulting from oxidative stress under water deficit conditions [17].

Reactive oxygen species (ROS) are highly cytotoxic and can seriously react with vital biomolecules such as lipids, proteins, nucleic acid, etc., causing lipid peroxidation, protein denaturing and DNA mutation, respectively [18]. To minimize the deleterious effects of stress and to complete their life cycle under adverse conditions, plants have evolved different adaptive responses. Hence, the tolerance may be because of ion homeostasis, osmotic adjustment, efficient and synchronous action of various components of antioxidant defence system [19].

Antioxidants are the first line of defence against free radical damage [20]. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbic acid peroxidase (APX) and peroxidase (POD) [21]. There are several compounds which contribute to the anti-oxidative properties; these include anthocyanin, polyphenols, vitamin C, flavonoids and carotene [22]. Under salt stress, the level of non-enzymatic antioxidant was increased, due to their capacity to protect itself against oxidative stress [20]. This work was undertaken to explain the impacts of silicon (Si) on growth vigor of flag leaf, membrane characteristics, the antioxidant enzyme activities and non-

enzymatic antioxidants as well as non-photosynthetic pigment of sorghum plants grown in soil irrigated with different concentrations of alkaline salt.

Materials and Methods

Plant material and experimental design

This experiment was conducted in a greenhouse at Botany Department, Faculty of Science, Mansoura University, Egypt. Pure strains of sorghum (*Sorghum bicolor L.* (*Moench)* (Alkalinity sensitive and tolerant cultivars) was kindly supplied by the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. Grains of sorghum were surface-sterilized with mercuric chloride (0.01 M $HgCl_2$) for 3 min, and then rinsed three times with distilled water. The sterilized grains from each cultivar were divided into two sets (≈ 500 g per set for each cultivar). Furthermore, the sterilized grains of each set were divided into two groups; the first group was primed with distilled water, and the second group with 1.5 mM of freshly prepared Si (as sodium meta-silicate $Na_2O_3Si.5H_2O$) solution for 6h, thereafter air-dried. The grains of both groups were sown in plastic pots (five seeds/pot) filled with 5.5 kg of dried soil (clay/sand 2/1, v/v). The pots were arranged in completely randomized design in factorial arrangement. At the time of sowing, the grains were irrigated at field capacity with various alkaline salt concentrations of 0 (control), 25, 50, and 75 mM Na_2CO_3 based on the method of Radi et al. [4] with each pot receiving 400 ml of a designated saline solution. The Na_2CO_3 concentrations used were equivalent to 0 (control), 0.528, 1.056, and 1.584 g Na_2CO_3 kg−1 soil, respectively. Leaching was avoided by maintaining soil water below field capacity at all times. The Si and Na_2CO_3 concentrations were selected based on our preliminary tests. The pots were then irrigated at field capacity with normal water through the whole experimental period.

The pot of the 1st set was allocated to four groups (45 pots per each group) as follows: control (Cont.), control Silicon, 25% Na_2CO_3, Silicon+25% Na_2CO_3, 50% Na_2CO_3, Silicon+50% Na_2CO_3, 75% Na_2CO_3, Silicon+75% Na_2CO_3 (for sensitive cultivar). The 2nd set groups were allocated to four groups as follows: control (Cont.), control Silicon, 25% Na_2CO_3, Silicon+25% Na_2CO_3, Silicon+50% Na_2CO_3, Silicon+75% Na_2CO_3 (for tolerant cultivar). After thinning and at heading, the plants received 36 kg N ha-1 as urea and 25 kg P ha−1 as superphosphate.

Plant studies at heading stage

At heading stage and when the plants were 60- day old, sampling was carried out for the first time to follow up the impact of alkalinity stress on the morph-physiological traits of the considered sorghum plants (i.e. growth vigor of flag leaf, antioxidant machinery and membrane characteristics were all investigated. For measurements, only the flag leaves of the main shoots were implemented because of its significance as a supply of photosynthetic products for grains (source- sink relationship).
Leaf area = Length X Breadth X 0.75 [23]
Degree of succulence = Water amount / Leaf area [24]
Degree of sclerophylly = Dry mass / Leaf area [24]

Assessment of membrane features

The membrane parameters including lipid peroxidation, membrane injury index and membrane stability index were determined in the present study. For estimating these features, fresh sorghum flag leaves were caught up after being carefully washed via de-ionized water to eliminate every salt molecule that might be attached to the leaves surface.

Determination of membrane lipid peroxidation

The idea of estimating the membrane lipid peroxidation of the studied sorghum varieties was mainly depend on determining malondialdehyde (MDA) content following Heath & Packer. A known fresh weight of the flag leaves, particularly one gram, was homogenized via a porcelain mortar in 5 ml of 0.1% trichloroacetic acid (TCA). After that, the resulted homogenate from maceration was spin via centrifuge at high speed, mainly 10,000 rpm for about 5 minutes. Every one ml of the supernatant was reacted with 4 ml of 20% TCA containing 0.5%. The reaction mixture was kept in water bath adjusted at 95°C for 30 minutes incubation period then allowed to cool rapidly. The forming mixture was then centrifuged at high speed, mainly 10,000 rpm for about 15 minutes and the optical density of TBA-MDA complex in the supernatant was spectrophotometrically determined at 532 nm (OD532) and 600 nm (OD600). The optical densities were adjusted for unspecific turbidity by deducting OD600 from the values at maximum absorption OD532. The concentration of MDA was calculated using 155 × 10-3 µM-1 cm-1 as an extinction coefficient to be expressed as µmol MDA g-1 fresh mass.

Determination of membrane injury index

The method used for the estimation of membrane injury index was that of Deshmukh *et al*. In two groups, a known fresh weight of flag leaves, particularly 0.2 gram, was sliced into minute sections with regular size to be transferred into test tubes containing 20 ml of de-ionized water. The first group was stored in water bath adjusted at 40°C for half an hour incubation period whereas the other group was incubated in boiling water bath for only 15 minutes. After cooling, the electric conductance (EC) of

sample in each group was read via a conductivity meter (model CD-4301). The percentage of membrane injury index (MII) was calculated according to the subsequent relation:

$$MII = (EC1 / EC2) \times 100$$

Where, EC1 and EC2 refer to the electric conductance in mS at 40 and 100°C; respectively.

Determination of membrane stability index

Many methods were earlier designed in other investigations to estimate membrane stability index (MSI). Nevertheless, an easy technique was disguised in the recent study to straightforwardly derive the value of MSI from that selected for MII. The percentage of membrane stability index (MSI) was calculated according to the following equation:

$$MSI = 100 - MII$$

Assessment of enzymatic antioxidant defense system

In the current study, sex antioxidant enzymes; including catalase, peroxidase, polyphenol oxidase, ascorbic peroxidase, glutathione reductase and superoxide dismutase were assayed. The way followed for extraction of these enzymes was described by Agarwal & Shaheen [25]. A known fresh biomass, particularly two grams, of flag leaves of the various sorghum varieties was firstly washed with deionized water. After that, the leaves were handily homogenized via a porcelain mortar in 20 ml of refrigerated 0.1 M phosphate buffer. The buffer used for extraction of both ascorbic peroxidase and superoxide dismutase was prepared at pH 7.8. Meanwhile, for catalase, peroxidase, poly-phenol oxidase and glutathione reductase; the extraction buffer was prepared at pH 6.8. After that, the homogenate was filtered via a specie type of filters mainly 4 layers of cheesecloth as well as the filtrate was spin in a cooling centrifuge at 10,000 rpm for 20 minutes. Finally, the supernatant was raised up to 20 ml and thus stored as enzyme extract for assaying.

Estimation of Catalase (CAT; EC 1.11.1.6)

Enzyme extraction

Homogenize a known weight of plant material with M/150 phosphate buffer (assay buffer diluted 10 times) in a pre-chilled mortar. The homogenate is then centrifuged at 5000 rpm for 15 min at 4 °C. Enzyme assays were conducted immediately following extraction. CAT activity was assayed in a method following Aebi [26]. Activity was determined by following the decomposition of H2O2 at 240 nm. On decomposition of H_2O_2 by catalase, the optical density decreases with time. Absorbance was read against a control cuvette containing enzyme solution as in the experimental cuvette, but with H_2O_2 - free PO4 buffer (M/15). Into the experimental cuvette, 3 ml of H_2O_2 - PO_4 buffer) were transferred and mixed with 0.01 - 0.04 ml sample. Δt required for the optical density decrease from 0.45 to 0.40 was recorded and used in the calculations.

The enzyme specific activity units g-1 f wt = [17/ Δt] x [TV / VU] x D x [1/ f wt];

Δt = time change in second; TV = total volume of the extract (ml); VU = volume used (ml); D = dilution; f wt = weight of the fresh leaf tissue (g)

Assay of peroxidase (POX; EC 1.11.1.7.)

Peroxidase activity was assayed following the method adopted by Devi [27]. The principle of assaying POX activity depends on the production of purpurogallin due to the peroxidation of pyrogallol. The activity was monitored by recording the increment of optical density at 420 nm. The reaction mixture was prepared as 3 ml of pyrogallol phosphate buffer (0.05 M pyrogallol in 0.1 M phosphate buffer at pH 6.0), 0.5 ml of H_2O_2 (1%) and 0.1 ml of the enzyme extract. The blank tube contained the same contents except replacing the enzyme extract with de-ionized water.

Assay of polyphenol oxidase (PPO; EC 1.14.18.1.)

Following Devi [27], PPO activity was assayed as the change in optical density at 420 nm. The idea of assaying PPO activity relies on the oxidation of pyrogallol into purpurogallin. The reaction mixture contained 1 ml pyrogallol, 2 ml of 0.02 M phosphate buffer at pH 7 and 0.1 ml of the enzyme extract. The blank tube contained the same contents except replacing the enzyme extract with de-ionized water.

Assay of ascorbic peroxidase (APX; EC 1.11.1.11.)

Ascorbic peroxidase activity was determined following the strategy adopted by Nakano & Asada. The activity of APX was assayed as the decrement in optical density at 290 nm as a result of the ascorbate peroxidation into dehydroascorbate. The reaction mixture was prepared as 2.5 ml of ascorbic acid (0.5 mM in phosphate buffer at pH 7.0), 0.4 ml of H_2O_2 (2 mM) and 0.1 ml of the enzyme extract. The blank tube contained the same contents except replacing the enzyme extract with de-ionized water.

Phenolic constituents

To determine the phenolic compounds synthesized by sorghum plants under the studied conditions, the following methods were followed to estimate total phenols and flavonoids.

Estimation of flavonoids

The total flavonoid content was determined following the spectrophotometric method of Dewanto *et al.* [28]. To the methanolic extracts of the plant tissue, 0.3 ml of 5% $NaNO_2$ solution was added; at 5 minutes, 0.3 ml of 10% $AlCl_3$ was added; and at 6 minutes, 2 ml of 1 N NaOH was added. About 2.4 ml of distilled water was then added and mixed well, and the absorbance of the reaction mixture was measured at 510 nm.

Estimation of total phenols

Total phenols were estimated using the method of Malik & Singh [29], depending on the reaction of phenols with phosphomolybdic acid in Folin-Ciocalteau reagent in an alkaline medium to produce blue-colored complex (molybdenum blue). A known dry weight of the plant tissue was ground in 10 ml of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was saved and the residue was re-extracted with 5 ml of 80% ethanol, centrifuged and the supernatant was collected. The supernatant was evaporated to dryness and the obtained residue was dissolved in 5 ml of distilled water. Half ml aliquots from each sample were pipetted into test tubes. The volume in each tube was made up to 3 ml with distilled water, followed by the addition of half ml of Folin-Ciocalteau reagent. After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added and the tubes were incubated in a boiling water bath for exactly one minute. After cooling, the optical density of the colour developed was measured spectrophotometric ally at 650 nm.

Non-photosynthetic pigment

The medically-active non-photosynthetic pigment determined in this study includes anthocyanin, β-carotene and lycopene.

Estimation of lycopene and β-carotene

Lycopene and β-carotene were determined according to the method of Nagata & Yamashita [30]. An aliquot volume of the methanolic extract was vigorously shaken with 10 ml of an acetone: hexane mixture (4: 6, v/ v) for a minute and filtered through Whatman No. 4 filter paper. The absorbance (A) was measured at various wavelengths; namely 453, 505 and 663 nm. The contents of lycopene and β-carotene were calculated according to the following equations:

Lycopene = 0.0458 (A663) + 0.372 (A505) - 0.0806 (A453)

β -carotene = 0.216 (A663) - 0.304 (A505) + 0.452 (A453)

Estimation of anthocyanin

Anthocyanin were extracted from the oven-dried ground tissues by suspending in 10 ml of acidified methanol (methanol: water: HCl, 79: 20: 1, v/ v) and auto extracting at 0°C for 72 hours in dark with continuous shaking. The extracts were then centrifuged for 10 minutes at 5000 rpm and the absorbance was measured at 530 and 657 nm for each supernatant [31].

Anthocyanin = A530 - 1/3 A657

Statistical analysis

It should be mentioned that the sample numbers which were taken for investigation were as follows: ten for growth parameters and three for all chemical analyses and only the mean values were represented in the respective figures. The data were subjected to one-way analysis of variance (ANOVA), and different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311.

Results

Changes in flag leaf growth

Perusal of the data shown in Figure 1 cleared that, in general, all concentrations of alkalinity caused noticeable decreases ($P \leq 0.05$) in growth vigor of flag leaf (i.e. flag leaf area, leaf fresh mass, leaf dry masse as well as degree of succulence and the degree of leaf sclerophylly) as compared to the control. On the other hand, application of silicon (without alkalinity) leads to increase the previous parameters significantly than that of control of both sorghum cultivars. The applied silicon plus alkalinity stress also increase the previous parameters in compared with alkalinized plants but still less than control except in case of pre-soaking with silicon with low alkalinity (25%) which significantly increase (leaf fresh mass) of sensitive sorghum cultivar and also except in case of pre-soaking with silicon with high alkalinity (75%) in sensitive cultivar recorded a clear reduction and in tolerant one recorded a non-significant reduction. Generally, tolerant cultivar induced better results than sensitive one.

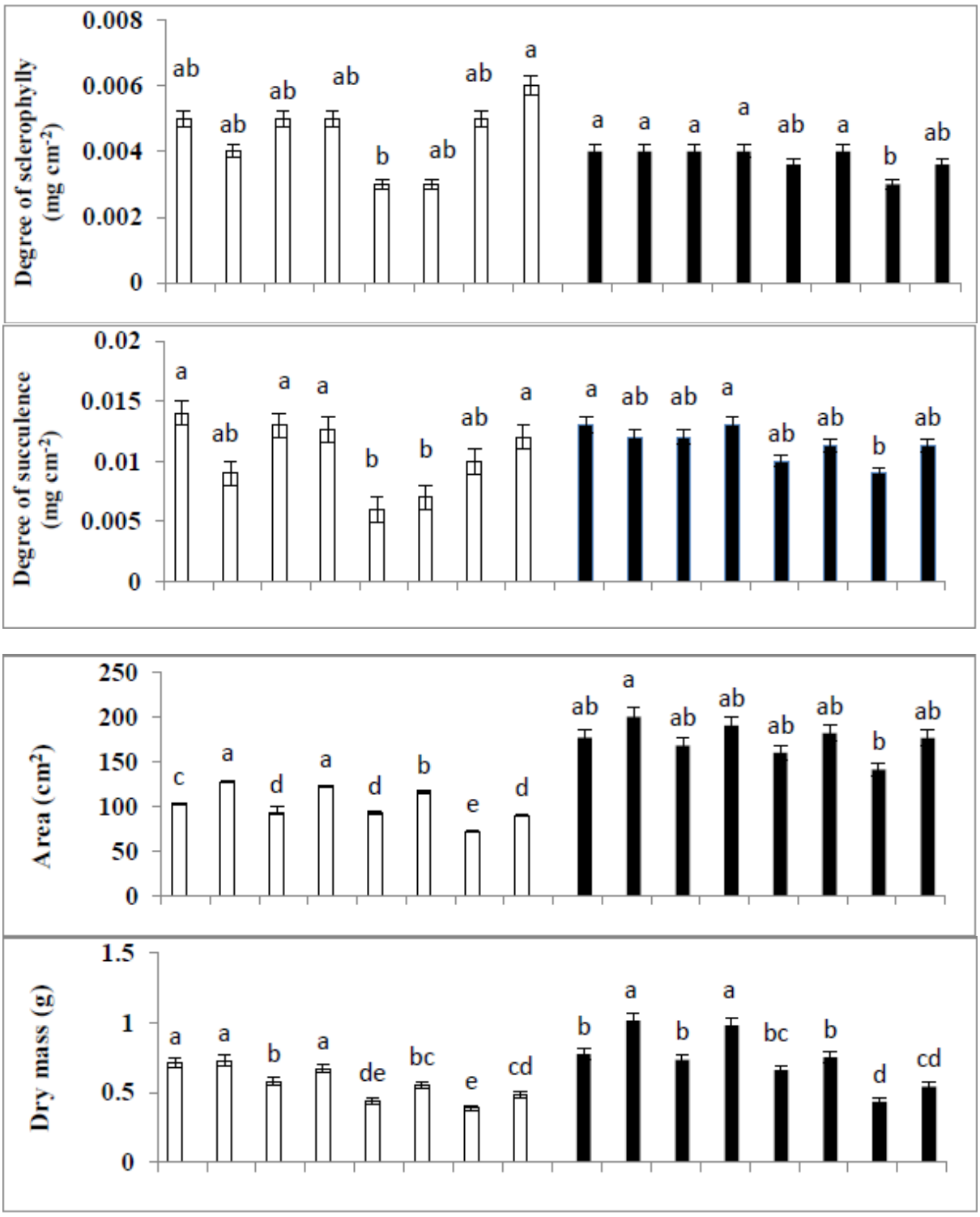

Figure 1: Effect of sodium meta-silicate on growth vigor of flag leaf (flag leaf fresh, dry masses (g), flag leaf area (cm^2), degree of succulence (mg cm^2) and degree of sclerophylly (mg cm^2)) of alkaline sorghum cultivars. Vertical bars represent standard error of the mean (n=3). Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311.

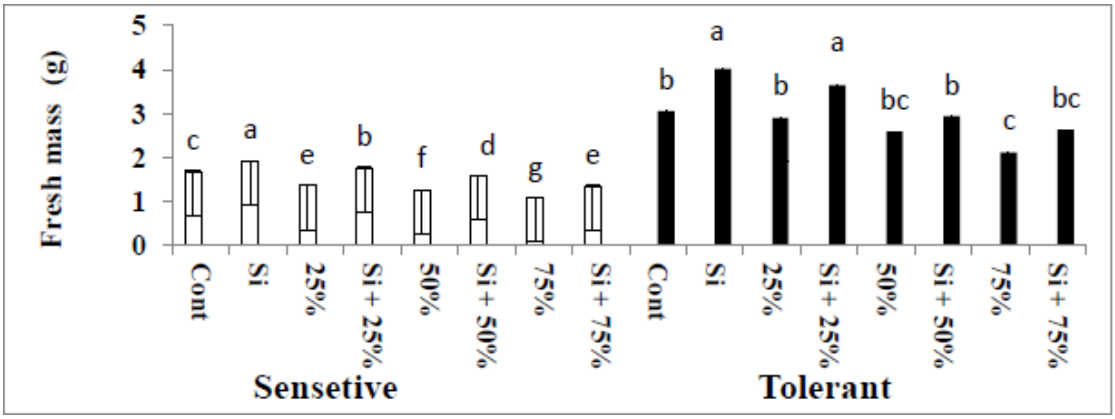

Figure 1: Effect of sodium meta-silicate on growth vigor of flag leaf (flag leaf fresh, dry masses (g), flag leaf area (cm^2), degree of succulence (mg cm^2) and degree of sclerophylly (mg cm^2)) of alkaline sorghum cultivars. Vertical bars represent standard error of the mean (n=3). Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311.

Changes in membrane characteristics and antioxidant capacity

This experiment was designed to investigate the positive effect of grain priming with silicon (Si) on membrane characteristics and antioxidant capacity by determining enzymatic antioxidant and non-enzymatic antioxidant as well as non-photosynthetic pigment in flag leaf of both alkalinity sorghum cultivars.

Changes in lipid peroxidation

As compared to the control values, the results in Figure 2 reflected that all concentrations of alkalinity increased lipid peroxidation of both sorghum cultivars. Moreover, application of silicon caused a significant decrease ($p \leq 0.05$) in lipid peroxidation of tolerant sorghum cultivar and a non-significant decrease in sensitive cultivar as compared with control plants. The applied silicon plus alkalinity stress increase lipid peroxidation as compared with control plants except in case of pre-soaking with silicon with low alkalinity (25% in sensitive cultivar; 25% and 50% in tolerant one) which recorded a non-significant decrease.

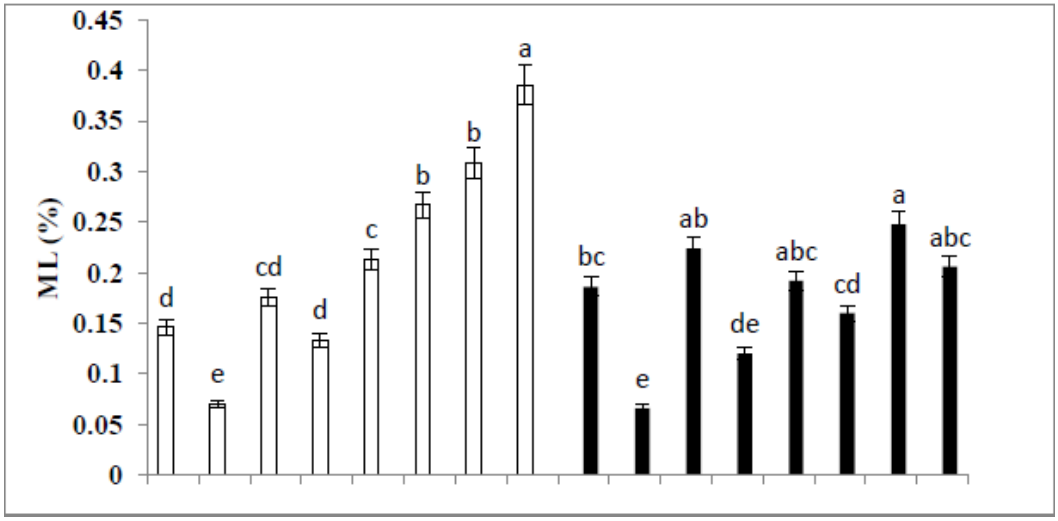

Figure 2: Effect of sodium meta-silicate on lipid peroxidation as malondialdehyde (MDA) content (mmole g^{-1} d wt) as well as membrane stability index (MSI) % and membrane leakage (ML) % in flag leaf of alkaline sorghum cultivars. Vertical bars represent standard error of the mean (n=3). Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311.

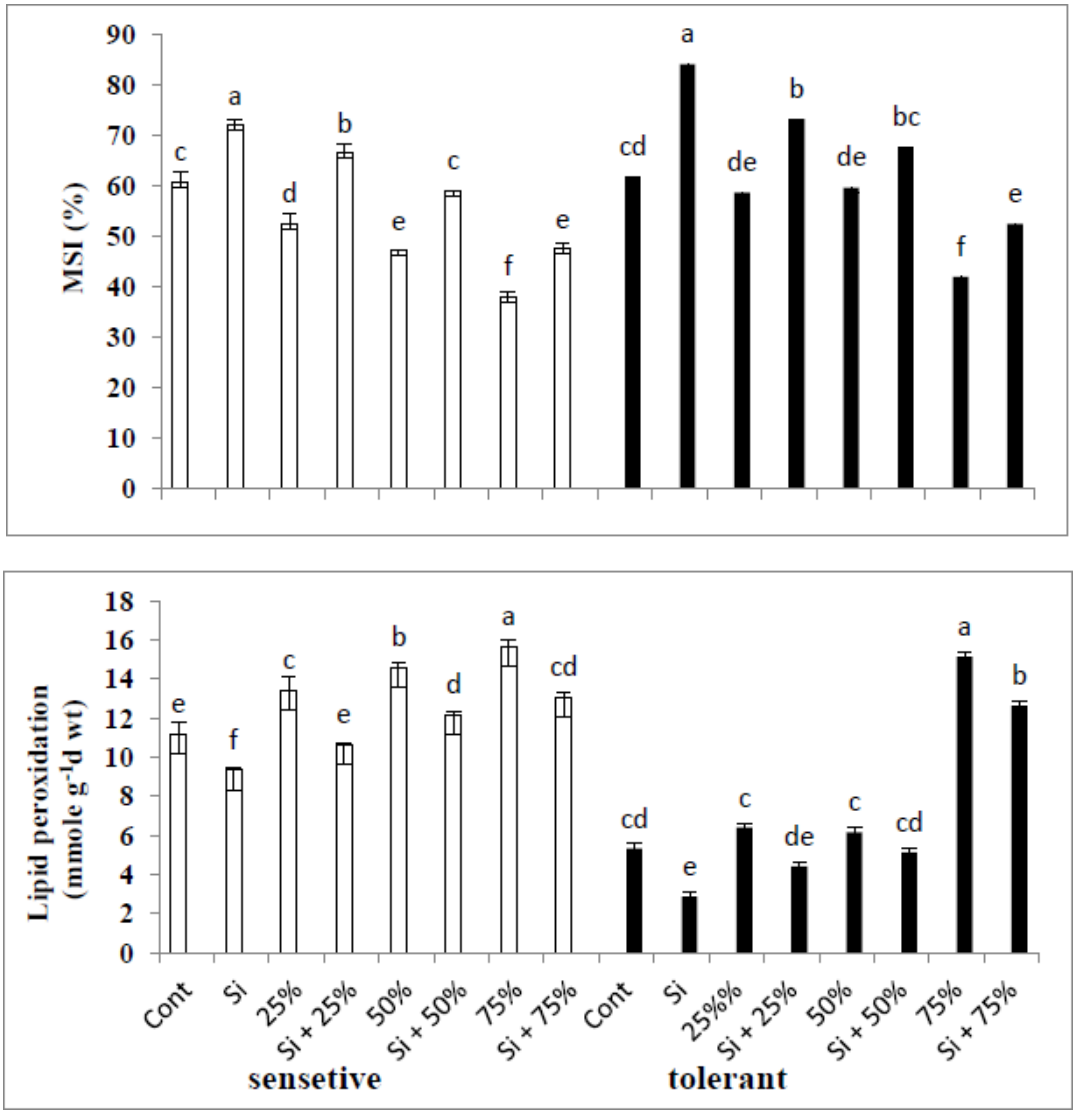

Figure 2: Effect of sodium meta-silicate on lipid peroxidation as malondialdehyde (MDA) content (mmole g^{-1} d wt) as well as membrane stability index (MSI) % and membrane leakage (ML) % in flag leaf of alkaline sorghum cultivars. Vertical bars represent standard error of the mean (n=3). Different letters indicate significant differences between treatments at p ≤0.05, according to CoHort/ CoStat software, Version 6.311.

Changes in membrane stability index (MSI) and membrane leakage (ML)

Data shown in Figure 2 cleared that, the pattern of change in MSI % is opposite to that in ML %. As compared to control plants, the significant reduction ($p \leq 0.05$) recorded in MSI % in response to alkalinity stress was accompanied with an increase in ML % of both sorghum cultivars. The effect was more pronounced with the sensitive one. The applied silicon plus alkalinity stress induced marked decrease ($p \leq 0.05$) in MSI as compared with control plants except in case of pre-soaking with silicon with low alkalinity (25% in sensitive cultivar; 25% and 50% in tolerant one) which recorded a significant increase. Generally, tolerant cultivar induced more response results than sensitive one.

Changes in antioxidant enzymes activity

The data presented in Figure 3 showed that, all concentrations of alkalinity increased catalase, ascorbic acid oxidase (AAO) and peroxidase (POD) activities except in case of high alkalinity (75% which recorded a non-significant decreased). Alkalinity stress caused a

significant decrease in PPO activity in flag leaf of both sorghum plants except for 25% alkalinity in case of tolerant cultivar recorded a significant increase. Silicon application induced marked increase ($p \leq 0.05$) in catalase, ascorbic acid oxidase (AAO) and peroxidase (POD) activities and induced a non- significant reduction in polyphenol oxidase (PPO) activity in flag leaves of both sorghum cultivars comparing with the control plants. Among cultivars, tolerant one showed higher enzymes activity than sensitive one. The applied silicon plus alkalinity stress induced marked increase ($p \leq 0.05$) in catalase, ascorbic acid oxidase (AAO) and peroxidase (POD) activities in comparing with control plants except in case of pre-soaking with silicon with low alkalinity (25% in sensitive cultivar; 25% and 50% in tolerant one) which recorded a non- significant decrease. The applied silicon plus alkalinity stress caused a non -significant decrease in PPO activity in flag leaf of alkalinity stressed sorghum plants except for 75% alkalinity in case of tolerant cultivar recorded a non- significant increase.

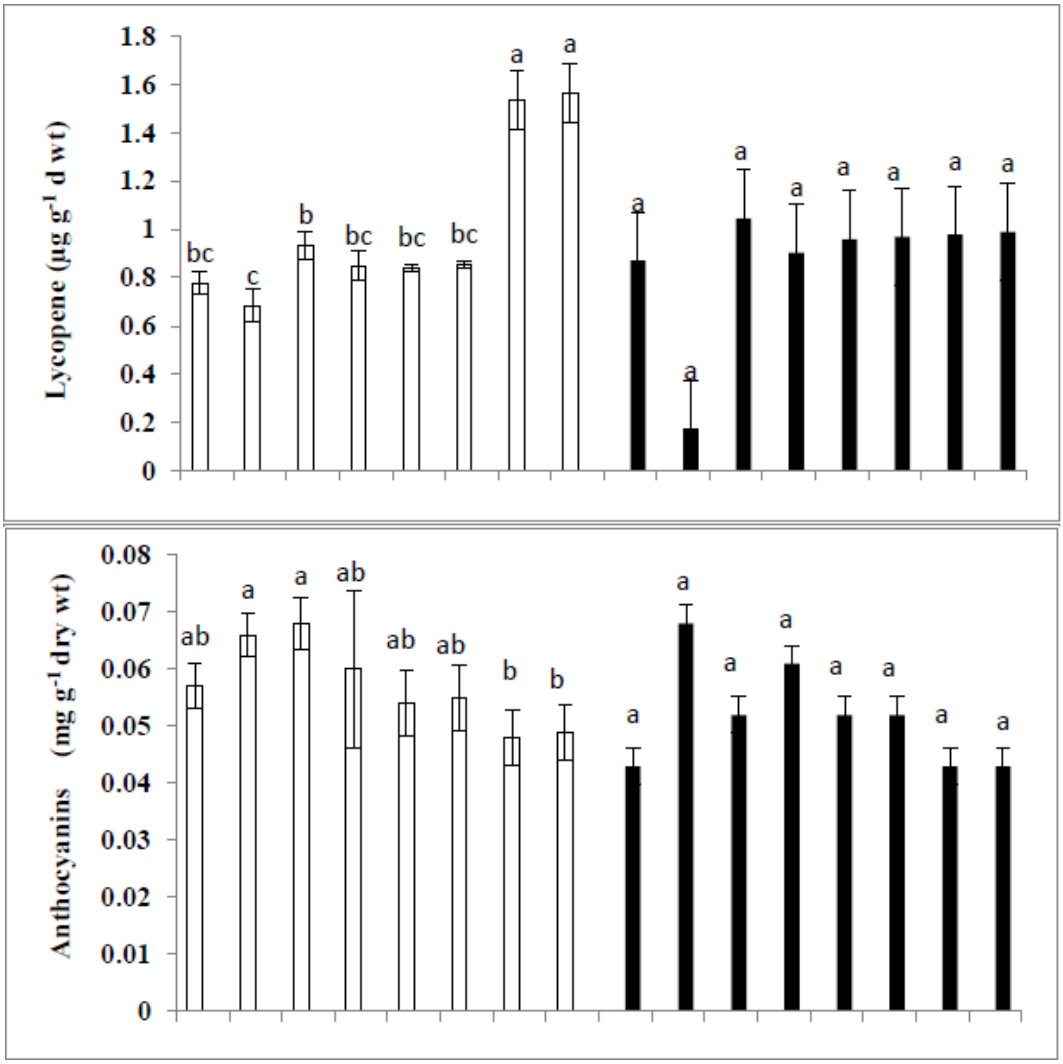

Figure 3: Effect of sodium meta silicate on non-photosynthetic pigment; β-carotene (µg g^{-1} d wt)), anthocyanins (mg g^{-1} dry wt) and lycopene (µg g^{-1} d wt) and of flag leaf of alkaline sorghum cultivars. Vertical bars represent standard error of the mean (n=3). Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311.

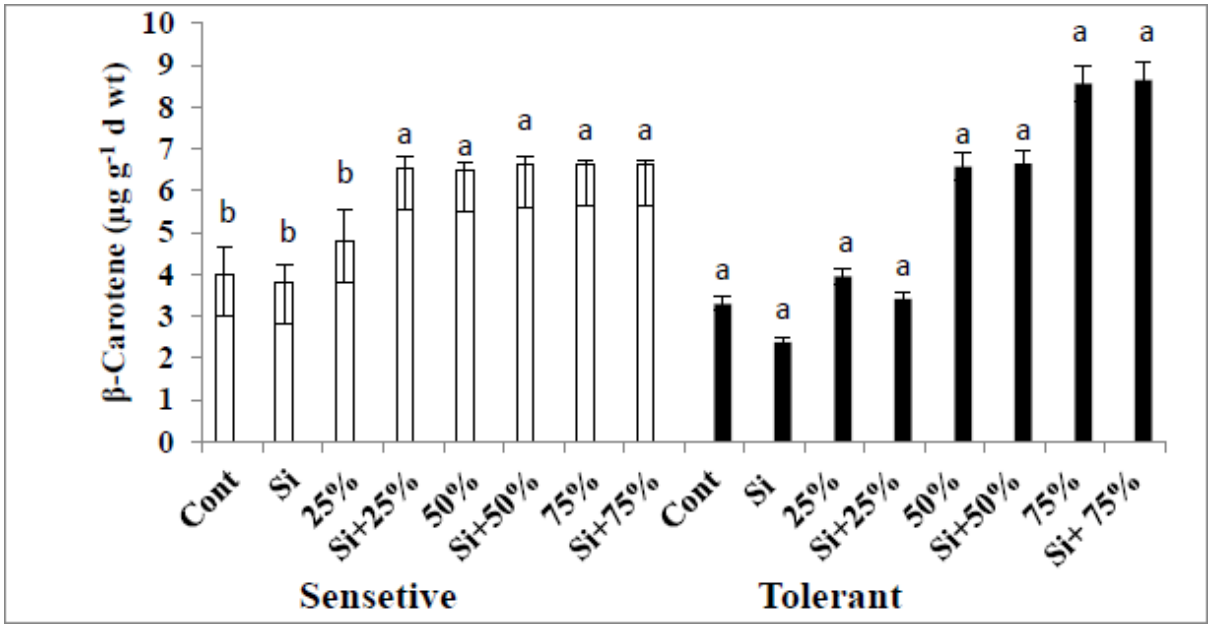

Figure 3: Effect of sodium meta silicate on non-photosynthetic pigment; β-carotene (µg g^1 d wt)), anthocyanins (mg g^1 dry wt) and lycopene (µg g^1 d wt) and of flag leaf of alkaline sorghum cultivars. Vertical bars represent standard error of the mean (n=3). Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311.

Changes in phenolic compounds

Data in Figure 4 revealed that all concentrations of alkalinity increased the amount of total phenols and flavonoids in flag leaf of both cultivars except in case of pre-soaking with silicon with high alkalinity (75% in sensitive cultivar; 50% and 75% in tolerant one) which recorded a non- significant decrease. Moreover, all alkalinity stress levels plus silicon caused a non-significant increase ($p \leq 0.05$) in the amount of total phenols and flavonoids in flag leaf of both cultivars except in case of pre-soaking with silicon with high alkalinity (75% in sensitive cultivar; 50% and 75% in tolerant one) which recorded a non- significant decrease.

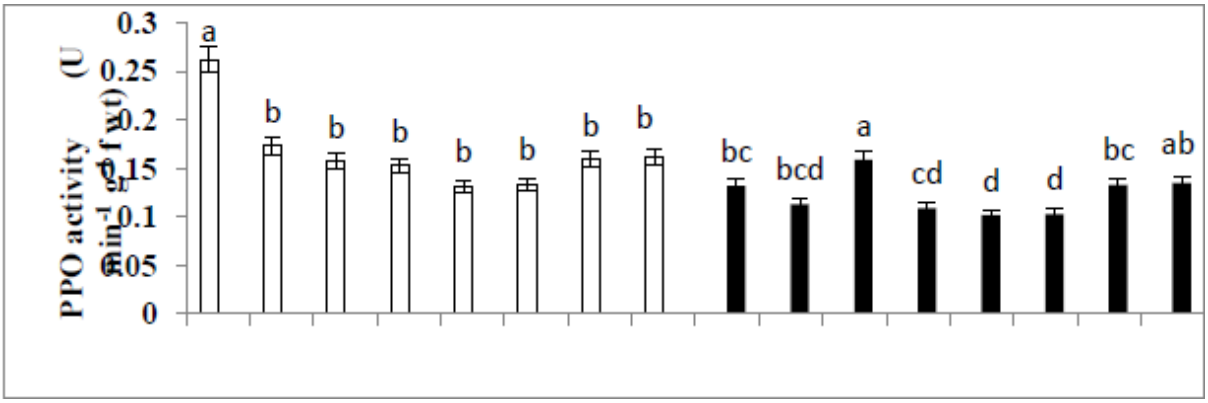

Figure 4: Effect of sodium meta-silicate on enzymes activity (Catalase (U min^{-1} g^{-1} f wt), POD activity (U min^{-1} g^{-1} f wt), AAO activity (U min^{-1} g^{-1} f wt) and PPO activity (U min^{-1} g^{-1} f wt)) in flag leaf of alkaline sorghum cultivars. Vertical bars represent standard error of the mean (n=3). Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311.

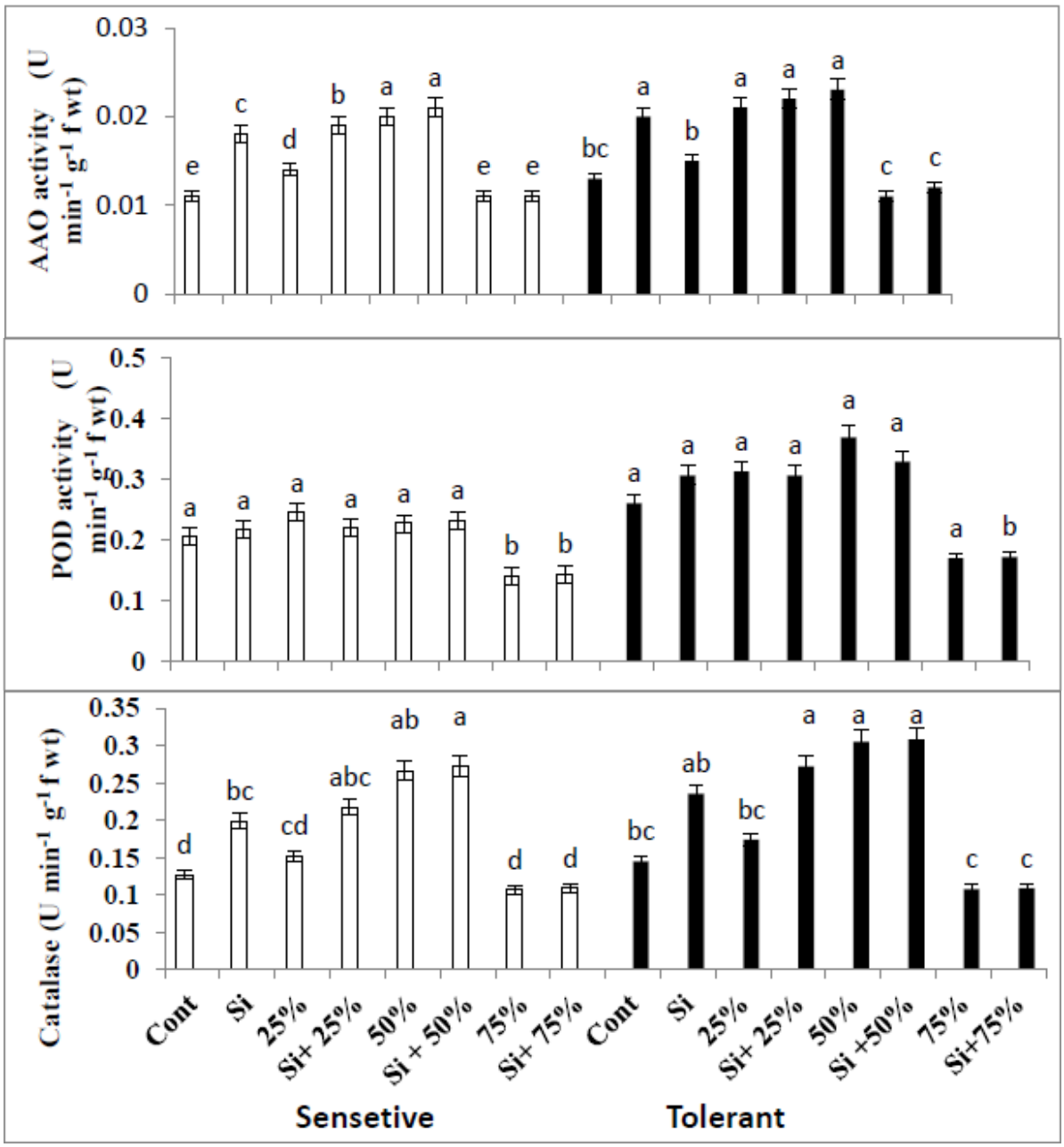

Figure 4: Effect of sodium meta-silicate on enzymes activity (Catalase (U min^{-1} g^{-1} f wt), POD activity (U min^{-1} g^{-1} f wt), AAO activity (U min^{-1} g^{-1} f wt) and PPO activity (U min^{-1} g^{-1} f wt)) in flag leaf of alkaline sorghum cultivars. Vertical bars represent standard error of the mean (n=3). Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311.

Changes in non-photosynthetic pigment

In relation to sorghum cultivar, the flag leaves of the control tolerant plants had higher non-photosynthetic pigment (anthocyanin and lycopene) contents than the sensitive one (Figure 5). All concentrations of alkalinity increased non-photosynthetic pigment (anthocyanin, lycopene and β-carotene) contents except in case of low alkalinity (25% in sensitive cultivar in case of anthocyanin) which recorded a non- significant increase. Moreover, application of silicon caused a non-significant increase in anthocyanin and a non-significant decrease ($p \leq 0.05$) in lycopene and β-carotene contents in both

sorghum cultivars. Alkalinity stress plus silicon application resulted in a non- significant increase ($p \leq 0.05$) in anthocyanin and β-carotene contents of the two sorghum cultivars except in case of pre-soaking with silicon with high alkalinity (75% in sensitive cultivar) which recorded a non- significant decrease. Application of silicon induced a non- significant decrease ($p \leq 0.05$) in lycopene content. While, alkalinity stress plus silicon application resulted in a non- significant increase ($p \leq 0.05$) in lycopene content of the two sorghum cultivars.

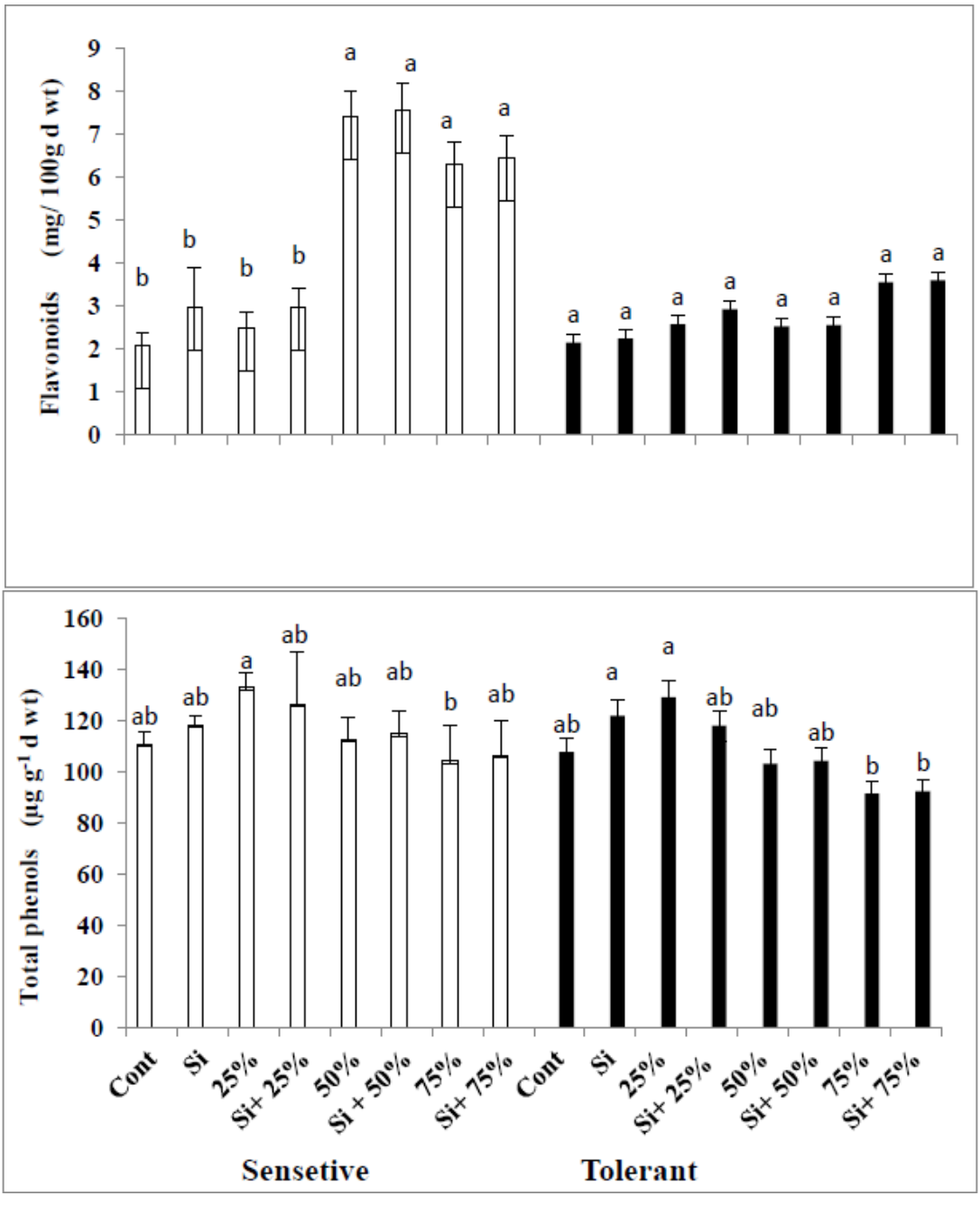

Figure 5: Effect of sodium meta silicate on phenolics content (total phenols (µg g^1 d wt) flavonoids (mg/ 100g d wt)) of flag leaf of alkalinity sorghum cultivars. Vertical bars represent standard error of the mean (n=3). Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311.

Discussion

In this study, the impacts of Si as an agriculturally effective fertilizer element on mitigation of alkalinity pressure were studied in sorghum. The detrimental influences caused by alkaline salt on different growth parameters of sorghum plants could occur due to the raise in pH, reduction in cell enlargement and cell division, metabolic disorders, nutritional damage and ion imbalance. Alkalinity is one of the major abiotic stress factors that affect plant growth and productivity, especially in arid and semi-arid areas. Grain sorghum after rice and wheat is the third important food grain for many people. The plant responses to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of growth [32]. The response and adaptation of plants to such conditions are very complex and highly variable [33]. Of all these methods, exogenous application of Si an effective method to alleviate the adverse effects of alkalinity stress.

Flag leaf plays the key and the most important role in plant life as it transport assimilates to spike and developing grains. The performance of flag leaf under certain growth condition reflects the overall viability and development of the whole plant. The results obtained in the present study, as shown in Figure 1, revealed that alkalinity caused marked reduction in leaf biomass, area and degree of succulence. On the other hand, Si caused massive increase in the cumulative degree of leaf scleraphylly. In agreement with our results, Sankar *et al*. [34] also reported that total leaf area as well as leaf fresh and dry weight in *Abelmoschus esculentus* plants were significantly reduced under drought stress. The general pattern of plant response to stress is a reduction in the rate of leaf surface expansion, followed by a cessation of expansion as the stress intensifies [35]. The retardation of leaf growth in stressed plants could be attributed to decreased turgor that may diminish cell production within the leaves.

Stressing the studied sorghum plants by alkalinity caused marked reduction in growth vigor of flag leaf (i.e. flag leaf area, leaf fresh mass, leaf dry masse as well as degree of succulence and the degree of leaf sclerophylly) (Figure 1). Hence, Netondo *et al*. [36] attributed the decrease in leaf area under stress to early leaf senescence and death, reduced growth rate or delayed emergence. They also concluded that the reduction in leaf area could be considered as one of the major reasons for lowered carbon gain and growth under stress conditions. Moreover, plant tried to cope with the water stress by reducing its leaf area in order to allow the conservation of energy, minimize the deleterious effects of water deficit alkalinity and to complete their life cycle under stress conditions (i.e. avoidance and/or tolerance mechanisms).

In the present study, the cumulative degree of leaf succulence also decreased under stress conditions (Figure 1). These results agree with those obtained by Welch & Rieseberg [37,38] working with three varieties of sunflower plants. Therefore, greater leaf succulence can be recorded as a means of increasing stress tolerance Welch & Rieseberg [37,38]. On contrary to the trend recorded for the degree of leaf succulence, the cumulative degree of leaf sclerophylly was found to increase under stress conditions (Figure 1). In accordance with these results, leaf sclerophylly was found to increase by stressing wheat plants [33].

The current work showed that the enhancement in leaf growth was more pronounced showing beneficial effects of Si on alkalinity stressed sorghum plants. Si application ameliorated the adverse effects of alkalinity by increasing flag leaf area. This indicated that Si application enhanced the crop growth not only under alkalinity but also under non alkaline conditions. These results are supported by Gong *et al*. [39], who observed the similar results in barely crop. The possible mechanisms responsible for better crop growth in the presence of Si under stressful conditions might be the prevention of loss of water from aerial parts of plant by keeping the water status maintained by the plant [40].

The improvement of degree of leaf succulence in stressed maize plants observed under Si treatment was perhaps due to the deposition of Si as silicate crystals in epidermal tissues, which composes a barrier to water transpiration through the cuticles and stomata [41] resulting in higher leaf area of maize plants as recorded in Si-primed plants under non-stressed and alkaline stress conditions (Figure 1). Furthermore, Si pre-treatment as seed-priming improved other growth parameters of alkaline- stressed maize seedlings (Figure 1). Thus, the results of this study and previously published reports collectively indicate the protecting role of Si against a wide range of environmental pressures [42].

Cell membrane is one of the main cellular targets common to different stress conditions [43]. In this regard, lipid peroxidation, membrane stability index (MSI) and membrane leakage (ML) could be considered as widely used stress indicators of plant membranes. In the present study, alkalinity stress conditions caused significant increase in lipid peroxidation and ML with marked decrease in MSI of sorghum plants (Figure 2). These results are in agreement with those obtained by Fayyaz

[44] who demonstrated that the amount of electrolyte leakage from the leaves of poplar plants was increased under water stress conditions. It is well known that water stress enhances free radical production, which induces the lipid peroxidation of bio-membranes, reflecting the stress-induced damage in tissues [45]. Evidence suggests that membranes are the primary sites of injury to cells and organelles because ROS can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids in plasma lemma or intracellular organelles leading to leakage of cellular contents, rapid desiccation and cell death [46].

In the present study, lipid peroxidation was estimated as malondialdehyde (MAD) content which is one of the decomposition products of polyunsaturated fatty acids (PUFA) of biomembranes [47]. Under alkaline regimes, MDA content accumulated in maize plants (Figure 2), clearly suggesting ROS burst and prospective oxidative damage to plant cells. This result is supported by the study of Ahmad *et al*. who found an increase in MDA content in two mulberry *(Morus alba L.)* cultivars with increase in exogenous $NaHCO_3$ level. Thus, the increase in MDA content might result from stomata closure causing a decrease in leaf CO_2 concentration. This, in turn, might cause a decrease in the concentration of $NADP^+$ available to accept electrons from PSI and/or PSII and thus initiate O_2 reduction with the concomitant generation of ROS [48]. Under water stress conditions, electrolyte leakage could be attributed to the damage of cell membranes which become more permeable due to less water availability [49]. The data obtained by Masoumi *et al*. [50] also indicated water stress-induced membrane injury, indicated by higher membrane leakage in *Borujerd Kochia* plants.

Exogenous application of Si in growth medium reduces electrolyte leakage in salt-stressed plants by maintaining the integrity and functions of membrane, thus mitigating salt toxicity [51]. The present study showed that addition of Na-silicate decreased electrolyte leakage under saline condition in both cultivars in comparison to control. This ameliorative effect of Si may be due to its hydrophilic nature by maintaining plant water status and by protecting the plants from physiological drought [52]. On the other hand, treatment with Si significantly hampered MDA accumulation in stressed maize plants compared with that of control and alkaline- treated alone plants (Figure 2), suggesting that Si triggers mechanisms to mitigate oxidative damage in stressed plants.

Alkalinity not only imposes the osmotic stress, but also marked as an oxidative stress which can stimulate accumulation of reactive oxygen species (ROS).

Hence, ROS levels in cells need to be tightly regulated via ROS-scavenging. Plants scavenge ROS by various protective mechanisms such as enzymatic antioxidant and non-enzymatic antioxidant. The antioxidant defense machinery that protects plants against oxidative stress damages includes both enzymatic and non-enzymatic antioxidant defense systems that work in concert to control the cascades of uncontrolled oxidation and protect the cells from oxidative damage by scavenging the ROS [53]. The results presented in Figure 3 revealed that alkalinity stress induced marked increase ($p \leq 0.05$) in AAO, POD and PAL activities and induced a non-significant reduction in PPO activity in flag leaves of both sorghum cultivars during grain-filling comparing with the control plants. Among cultivars, tolerant one showed higher enzymes activity than sensitive one. Similar responses to stress conditions were reported in maize [54]. Ascorbic acid is considered as the most abundant, powerful and water soluble antioxidant that helps to reduce the oxidative damages caused by ROS in plants [53]. Changes of ascorbic acid or oxidized ascorbic acid under drought stress are a part of antioxidant defense mechanism of plants [55,56]. In addition, POD is one of the major systems for the enzymatic removal of H_2O_2 in plants [57].

Our results suggested that Si triggers mechanisms to mitigate oxidative damage in stressed plants. Indeed, Si pre-treatment significantly increased the content of antioxidant phenols in *sorghum* plants plants under alkaline stress (Figure 4). Furthermore, seed-priming with Si also resulted in a significant increase in catalase, ascorbic acid oxidase (AAO) and peroxidase (POD) activities in stressed sorghum plants relative to plants treated with alkalinity alone. These results indicate that Si enhances antioxidant system to protect plants against alkalinity-induced oxidative damage, as evidenced by the observed reduced MDA level. Moreover, Si moderately offsets the negative effects of salt stress by enhancing SOD and CAT activities and soluble proteins in tomato [58]. Si application in sorghum excited the scavenging system and promoted the production of SOD and CAT in both cultivars.

Conclusion

We could suggested that addition of Si improved plant defense system to detoxify ROS induced under alkalinity stress, which in turn helped to increase leaf growth, improve membrane characteristics and enhanced the antioxidant capacity efficiency. It is also confirmed that scavenging system is primary defense line against oxidative stress induced by alkalinity stress.

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