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Research Article

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Morpho-Anatomical Responses of Alkalinized Sorghum (*Sorghum Bicolor L.*) Cultivars to Silicon

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Abstract

Alkalinity is likely the most important factor that adversely affects plant growth and development. In this study two sorghum (*Sorghum bicolor* L.) cultivars (alkalinity sensitive cultivar Giza 15 and alkalinity tolerant one ICSR 92003) were subjected to various alkaline salt concentrations of 0 (control), 25, 50, and 75 mM Na_2CO_3 and compared in terms of growth vigor of root, shoot and flag leaf as well as flag leaf anatomical features of both cultivars. The experiment was conducted in pots to evaluate the beneficial effect of grain presoaking in sodium meta-silicate ($Na_2Sio_3.5H_2O$ at 1.5 mM) on the two sorghum cultivars. In general, alkalinity stress caused noticeable reduction in almost all growth criteria of root, shoot and flag leaf which was consistent with the progressive alteration in flag leaf anatomical features of both cultivars during grain filling. In relation to sorghum cultivar, the sensitive was more affected by alkalinity stress than the tolerant one. Generally, the application of silicon induced marked increase in growth vigor of root, shoot and flag leaf. Moreover, the anatomical features in alkalinized plants were stimulated by silicon in both sorghum cultivars since silicon induced marked increase in conducted canals (xylem area and phloem tissue area), leaf thickness, ground tissue thickness, number of vascular bundle tissues, proto-xylem vessel area as well as meta-xylem vessel area and tracheids area of both sorghum cultivars either exposed or not exposed to alkalinity stress.

Keywords: Sorghum; Alkalinity; *Growth vigor;* Anatomical features; Xylem; Phloem; Vascular bundle

Abbreviations: Si: Silicon; Cont: Control; S: Sensitive; ANOVA: Analysis Of Variance; LSD: Least Significant Difference

Introduction

Sorghum is a major contributor to the staple diets of local populations [1]. A great efforts have focused on sorghum grain and its co-products as a source of functional and nutraceutical components with human health promoting actions including phenolic [2], particularly they have a high antioxidant activity provided by phenolic acids, condensed tannins and anthocyanin [3], that can accelerate phase II detoxifying enzymes and attenuate proliferation of carcinoma cells [4]. Alkalinity is a major constraint for sorghum production in Egypt [5].

Alkalization and salinization induce severe effects on the natural grass lands and farming lands. The presence of alkaline salts (Na_2CO_3 or $NaHCO_3$) in the soil caused mainly alkaline stress [6]. which is one of the most crucial abiotic stressors. Many studies have been showed that alkaline stress is more harmful than saline stress, and this is mainly due to its additional high pH stress [7]. Increase in pH value may reduce seed germination, destruction of the root cell structure, alteration in the nutrient availability and disturbance in nutrient uptake, and consequently induces a significant reduction in the yield of crop plants [8]. The adverse effects of alkalinity on plant growth are stated by a high pH value, which can directly destroy plant roots, alteration the availability of nutrients, and disturbance the balance of ions and mineral nutrition [9].

Increasing salinity level is injurious to plant growth and caused marked changes in its morphological features such as decrease in shoot and root lengths, leaf area and total biomass production [10]. Many investigators have noticed that salinity caused marked reduction in plant height, fresh and dry weights of shoot and root, the relative growth rate as well as leaf area of wheat plants [11].

It is clear that there are numerous changes in leaf anatomy of the plants growing under salt stress [12]. Plants encountering stressful environmental conditions, such as high salinity, respond to this external stressor with characteristic modifications in their anatomy. Irrigation of wheat plants with NaCl, especially at 99 mM, induced marked increases in flag leaf blade, mesophyll tissue thickness, as well as the number of motor cells and hairs on lower epidermis. Furthermore, treatment with NaCl generally decrease the peduncle diameter, xylem tissue thickness, number of hairs as well as number of stomata on the peduncle of the main shoot [13]. Well developed motor cells for extensive leaf rolling was apparent in *Festuca novae* [14], and *Deschampsia antarctica* [15] plants grown under drought.

Salinity was found to increase epidermal thickness, mesophyll thickness, palisade cell length, palisade diameter and spongy cell diameter in leaves of bean and cotton plants [16]. In leaves of potato, salt stress caused narrow intercellular spaces and a reduction in chloroplast number [17]. Moreover, salinity reduced stomatal distribution in the leaves of tomato plants [18]. Recently, it was shown that seed pretreated with plant growth regulators (PGRs) could counteract the harmful effects of salinity on germination and plant growth, since the major effect of salinity is the reduction of crop growth by the reduced hormone delivery from roots to leaves [19]. These PGRs have been found to play a primary role in the integration of the responses expressed by plants under stress conditions [20].

Silicon (Si) has been verified to play an important role in enhancing plant resistance to abiotic stress [5]. Si plays an important role in plant-environment relationships because it can enhances plants' abilities to withstand edaphoclimatic and/or biological adversities by acting as a "natural anti-stress" mechanism that enables higher yields and a better-quality end product. Silicon has a large number of diverse roles in plants, and does so primarily when the plants are under stressful conditions, whereas under precious conditions, its role is often minimal or even nonexistent [21].

Silicates most often benefit plants grown in Si-poor soils and during adverse years, including prolonged periods of drought, frost, high incidence of pests and/or diseases [22]. Also, under such conditions, plants with an enough good supply of Si tolerate a lack of water for a longer period because they more efficiently use the absorbed water and lose it at a lower speed than plants with a low Si level. Si increases crop yield and improves technological quality, while the lack of this element can reduce the plants' biological ability to withstand adverse environmental conditions [22].

Materials and Methods

Plant material and experimental design

A homogenous lot of Sorghum bicolor L. (i.e. either alkalinity sensitive cultivar Giza 15 or alkalinity tolerant cultivar ICSR 92003) grains were selected. The grains were separately surface sterilized by soaking in 0.01 M $HgCl_2$ solution for three minutes, then washed thoroughly with distilled water. The sterilized grains from each cultivar were divided into two sets (≈ 300 g per set for each cultivar). Grains of the 1st set were soaked in distilled water to serve as control, while those of the 2nd were soaked in 1.5 mM of freshly prepared Si (as sodium meta-silicate $Na_2O_3Si.5H_2O$) solution for 6 hrs, thereafter air-dried. The grains of both groups were sown in plastic pots (ten 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 .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 according to 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 eight groups (64 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 eight 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.

The agronomic traits

To estimate the morphological features of sorghum plants grown under different growth conditions, ten plant replicates from each treatment were harvested then their shoots were separated from roots. Leaf area was measured, that besides estimating leaf biomass. Agronomic traits were calculated using the following formula:

Leaf area **=** Length X Breadth X 0.75 [23]

Degree of succulence = Water amount / Leaf area [24]

Degree of sclerophylly = Dry mass / Leaf area [25]

Distribution = Fresh mass / Length [26]

Density = Dry mass / Length [26]

Anatomical studies

For anatomical studies, samples from fresh flag leaves were used. Samples were killed and fixed in Formalin-Acetic acid-Alcohol for at least 48 hours. Dehydration, sectioning staining and mounting procedures was followed according to the method described by [27]. Sections were cut at thickness 15µm, and then stained with safranin and light green combination. Canda Balsam was used as mounting medium. Sections were estimated by the aid of light microscope. Measurements of all anatomical parameters were calculated in keel region to the nearest µm.

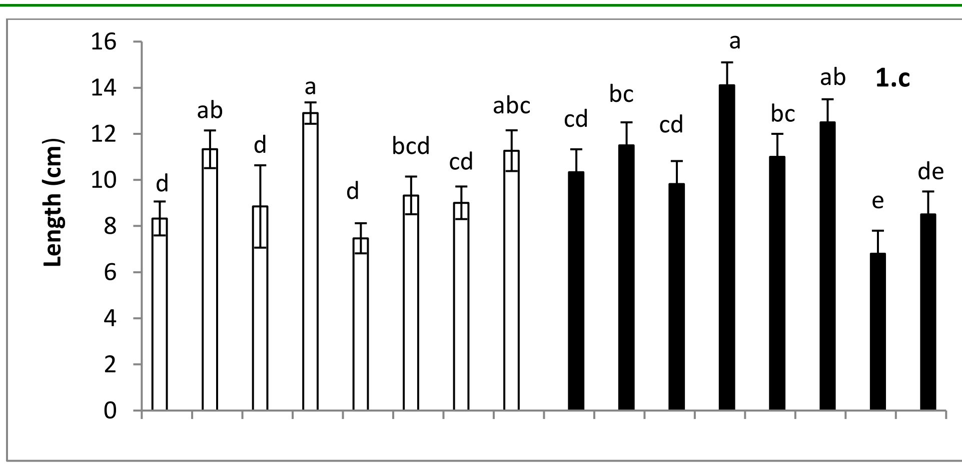
Statistical analysis

It should be mentioned that the sample numbers which were taken for investigation were as follows: ten for growth parameters, ten for agronomic traits 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 growth vigor of root

The pattern of results in Figures 1a-1d and 2a-2c showed that, in relation to sorghum cultivars, the tolerant plants had higher root criteria values than the sensitive ones. In the majority of cases, alkalinity stress caused marked decrease ($p \leq 0.05$) in root biomass (fresh and dry masses) in both cultivars as compared to the control values during grain -filling (i.e. 60 days after planting). On the other hand, alkalinity stress plus silicon treatment generally induced a noticeable increase ($p \leq 0.05$) in the root length and root shoot ratio of both sorghum cultivars during grain-filling. The magnitude of reduction was greater in alkalinity sensitive cultivar than alkalinity tolerant one. In general of cases, application of silicon induced a marked increase ($p \leq 0.05$) in the values of root biomass (fresh and dry masses), root length as well as root/shoot ratio, root density and distribution of both sorghum cultivars.



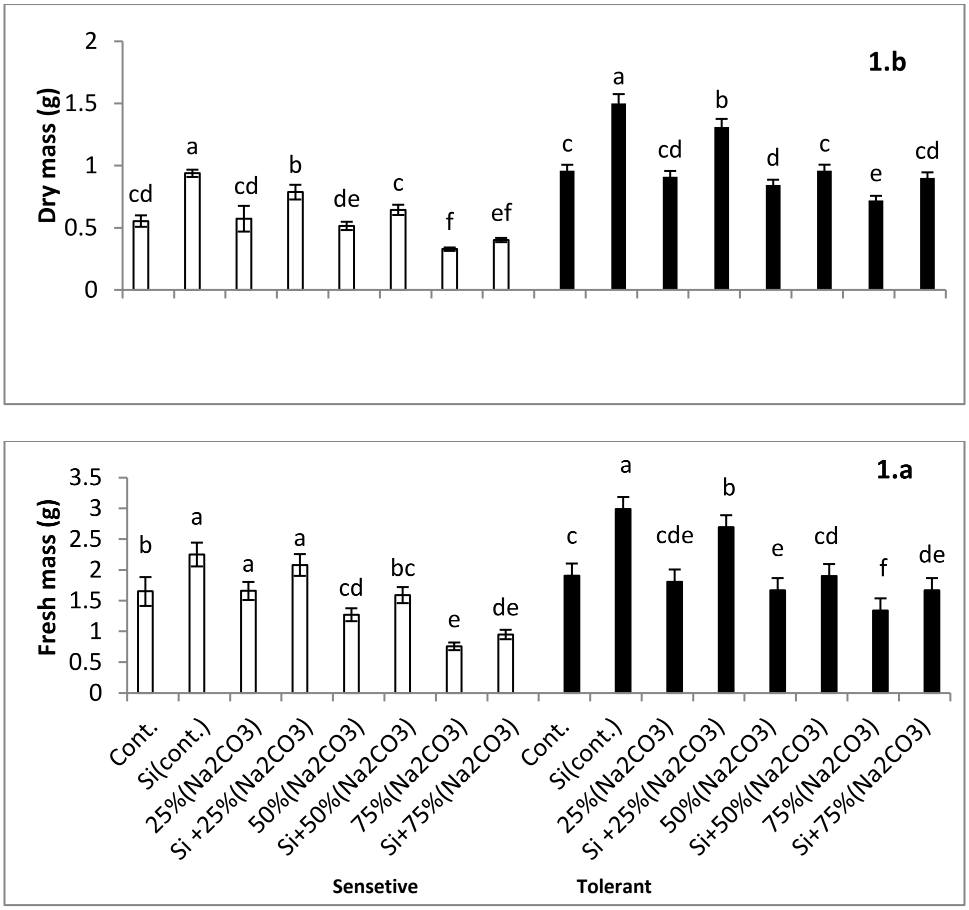
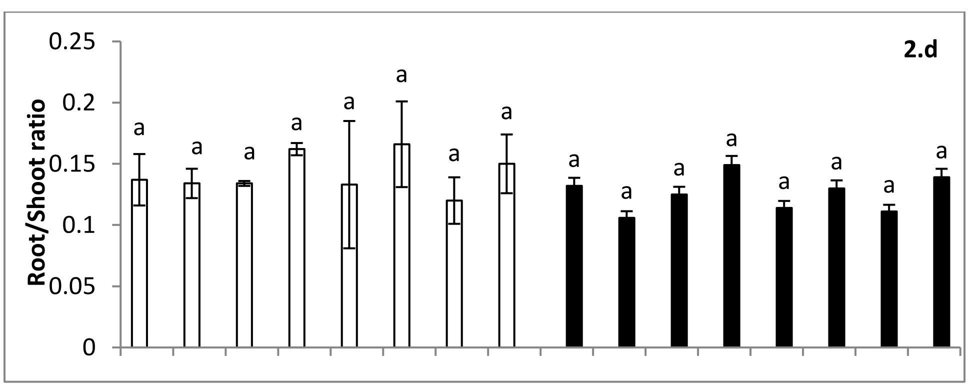


Figure 1: Effect of sodium meta-silicate on growth vigor of root [root fresh mass (g) 1.a., dry masse (g) 1.b. As well as root length (cm) 1.c.] Of alkalinity stressed sorghum plants. Vertical bars represent standard error of the mean (n=10). Different letters indicate significant differences between treatments at p≤0.05, according to CoHort/ CoStat software, Version 6.31.



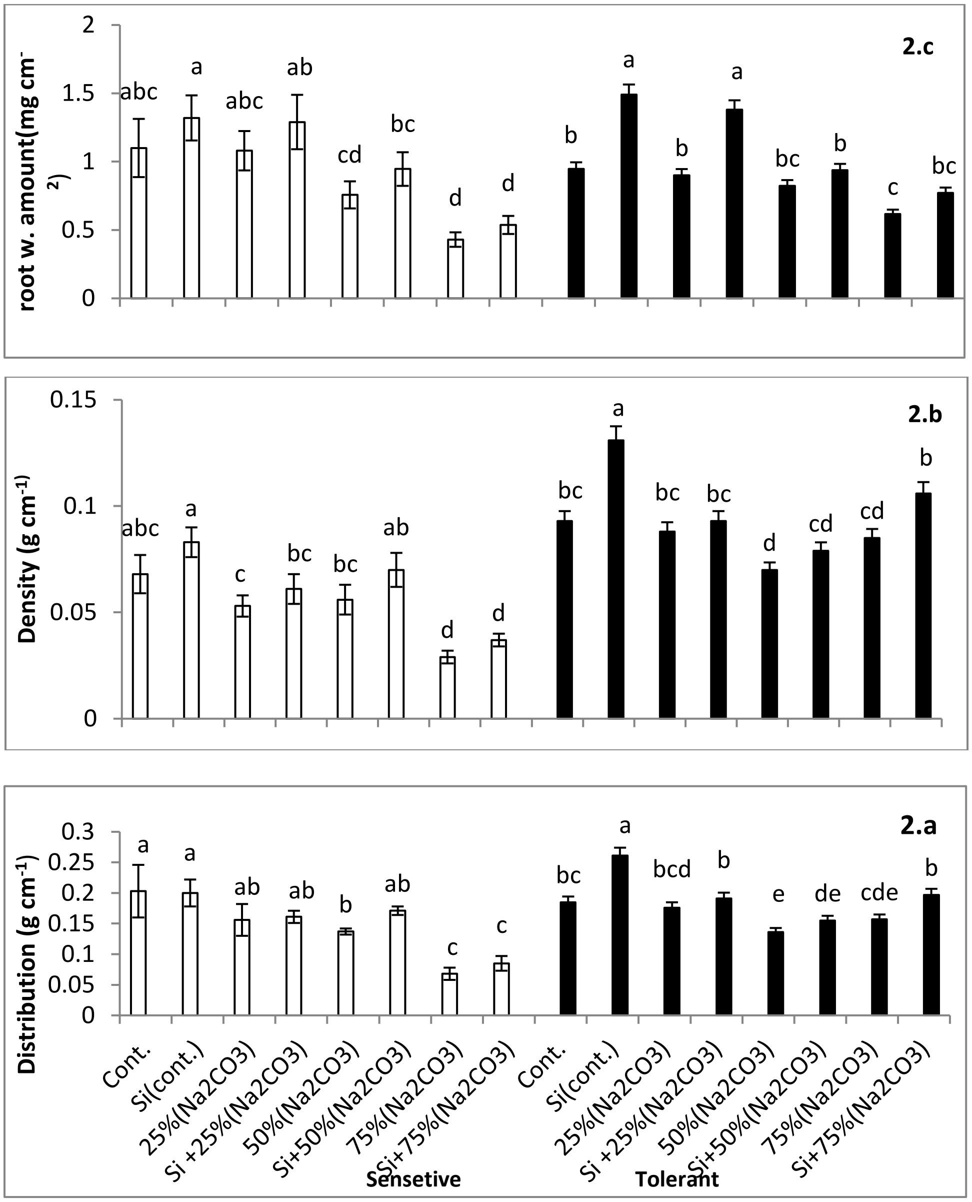


Figure 2: Effect of sodium meta-silicate on growth vigor of root (root distribution 2.a., root density 2.b., root water amount 2.c. and root /shoot ratio 2.d.) Of alkalinity stressed sorghum plants. Vertical bars represent standard error of the mean (n=10). Different letters indicate significant differences between treatments at $p \le 0.05$, according to CoHort/ CoStat software, Version 6.311.

Changes in growth vigor of shoot

The data represented in Figures 3a-3c and 4a- 4c revealed that, in relation to control values, alkalinity stress caused noticeable decrease ($p \le 0.05$) in all shoot characteristics (i.e. shoot biomasses, shoot length, shoot density and distribution as well as shoot water amount) during grain-filling in both sorghum cultivars. The alkalinity sensitive cultivar appeared to be more affected than the tolerant one.

In comparison with control plants or with alkalinity stressed ones, application of silicon resulted in obvious increase in all above mentioned shoot growth criteria, except high alkalinity concentration (75%), silicon alleviated the stress effect on all growth vigor of shoot.

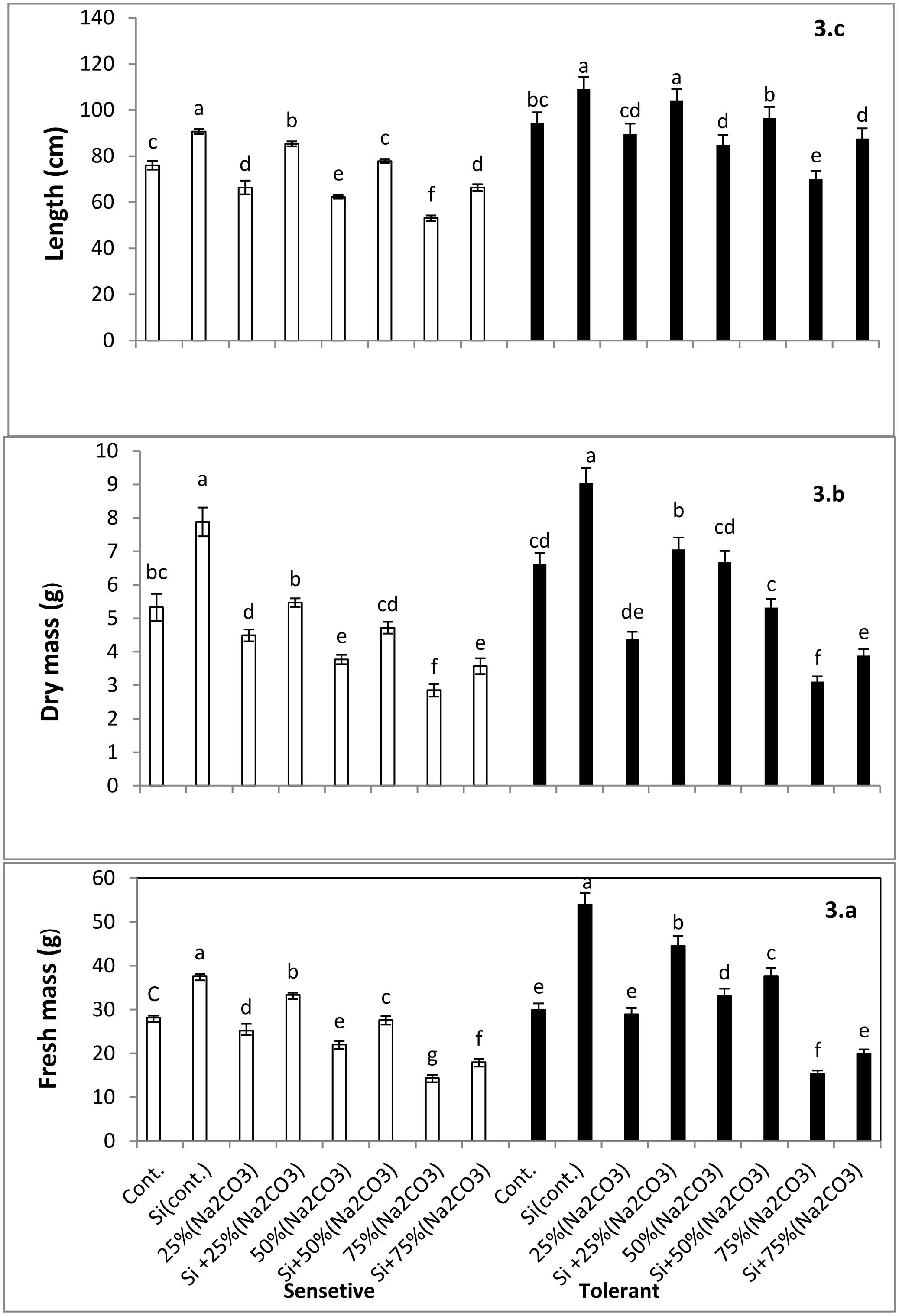


Figure 3: Effect of sodium meta-silicate on growth vigor of shoot [shoot fresh mass (g) **3.a.**, dry masses (g) **3.b.** as well as shoot length (cm) **3.c.**] of alkalinity stressed sorghum plants . Vertical bars represent standard error of the mean (n=10). Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311.

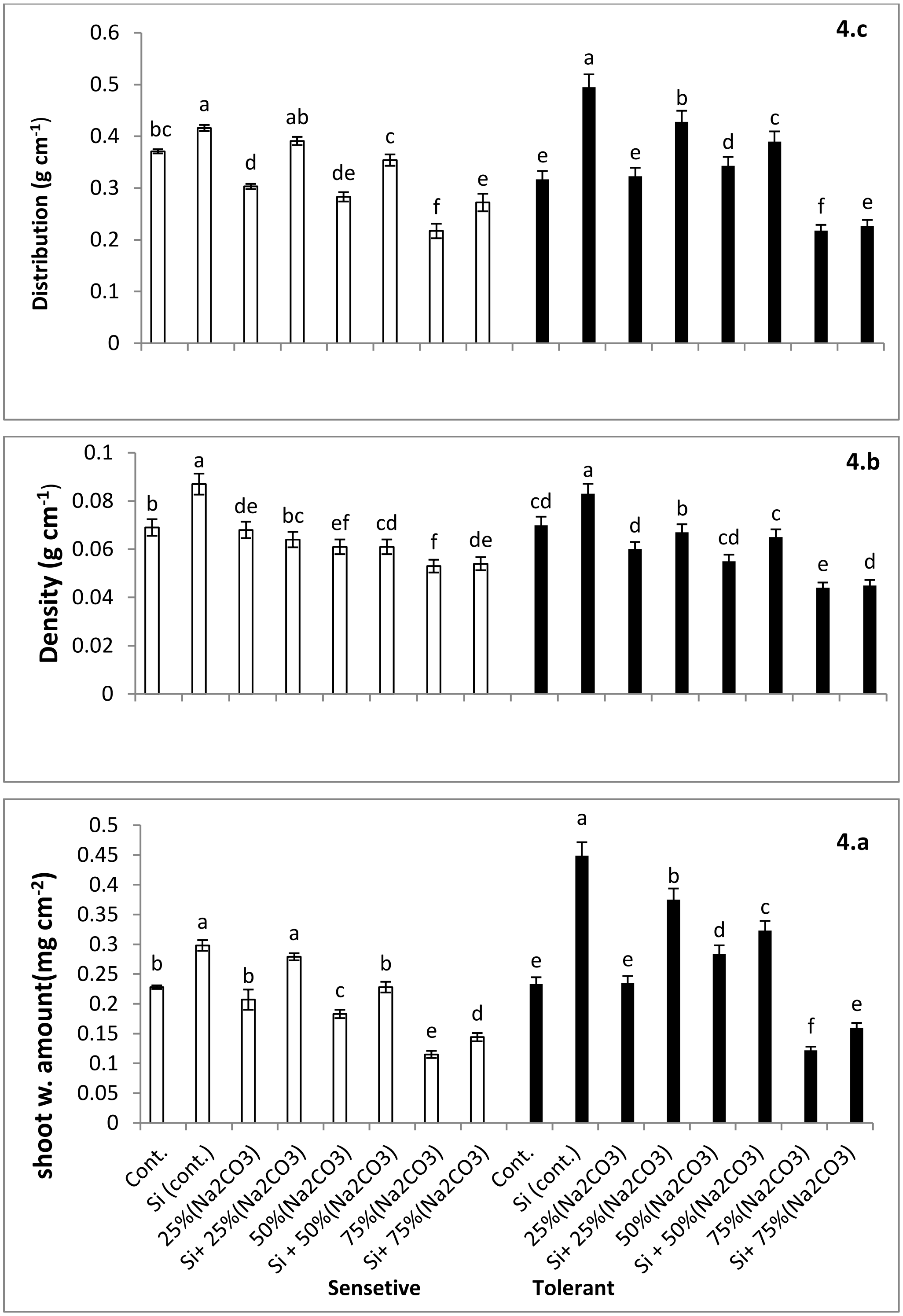


Figure 4: Effect of sodium meta-silicate on growth vigor of shoot (shoot water amount 4.a., shoot distribution 4.b. and shoot density 4.c.) of alkalinity stressed sorghum plants. Vertical bars represent standard error of the mean (n=10). Different letters indicate significant differences between treatments at p ≤ 0.05, according to CoHort/ CoStat software, Version 6.31.

Changes in flag leaf growth

Data in Figures 5a-5b and 6a-6c 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 mass as well as degree of succulence and the degree of leaf sclerophylly) as compared to the control plants. 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.

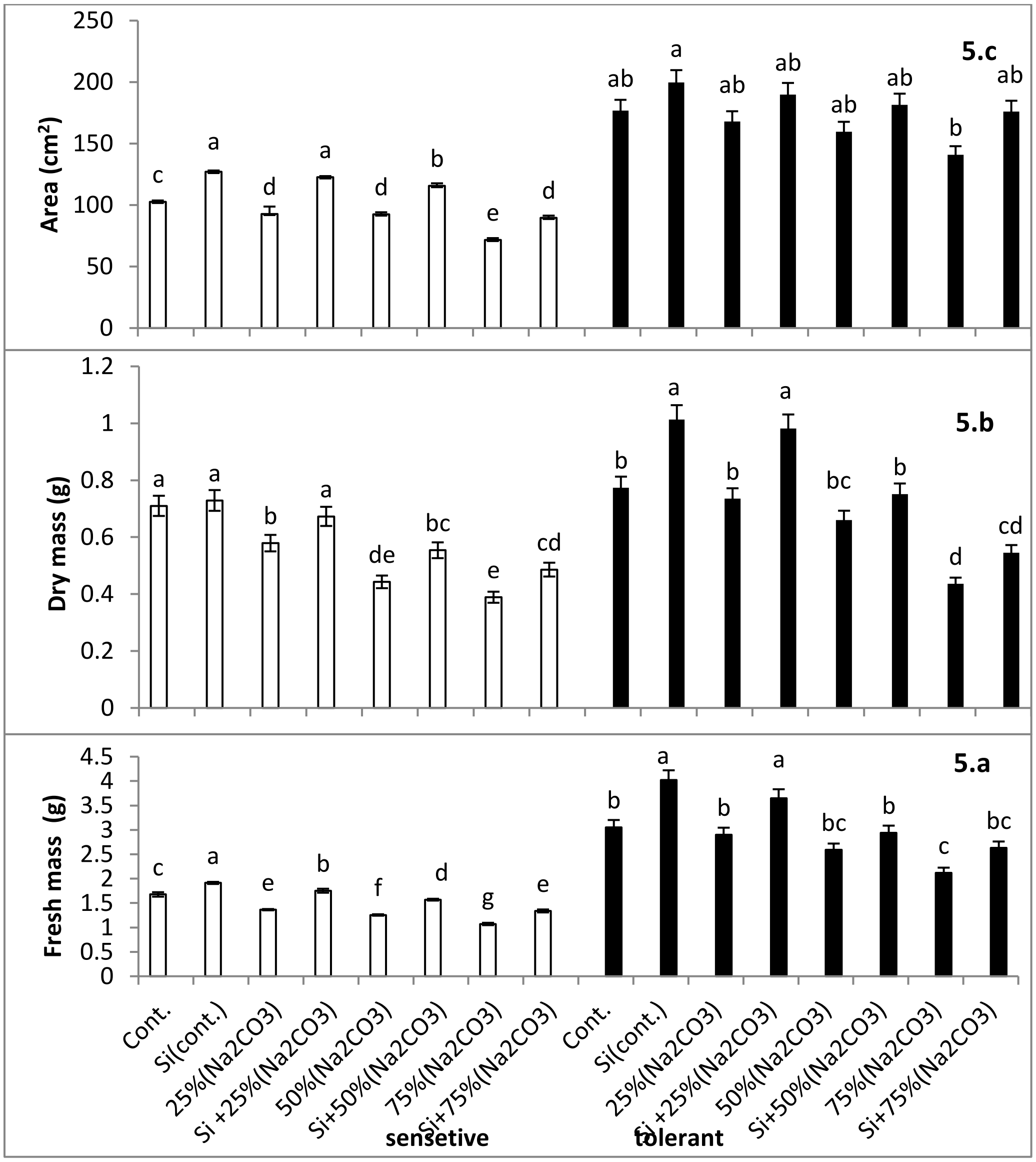


Figure 5: Effect of sodium meta-silicate on growth vigor of flag leaf [fresh mass (g) 5.a., dry mass (g) 5.b. and flag leaf area (cm^2) 5.c.] of alkalinity stressed sorghum plants. Vertical bars represent standard error of the mean (n=3). Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311.

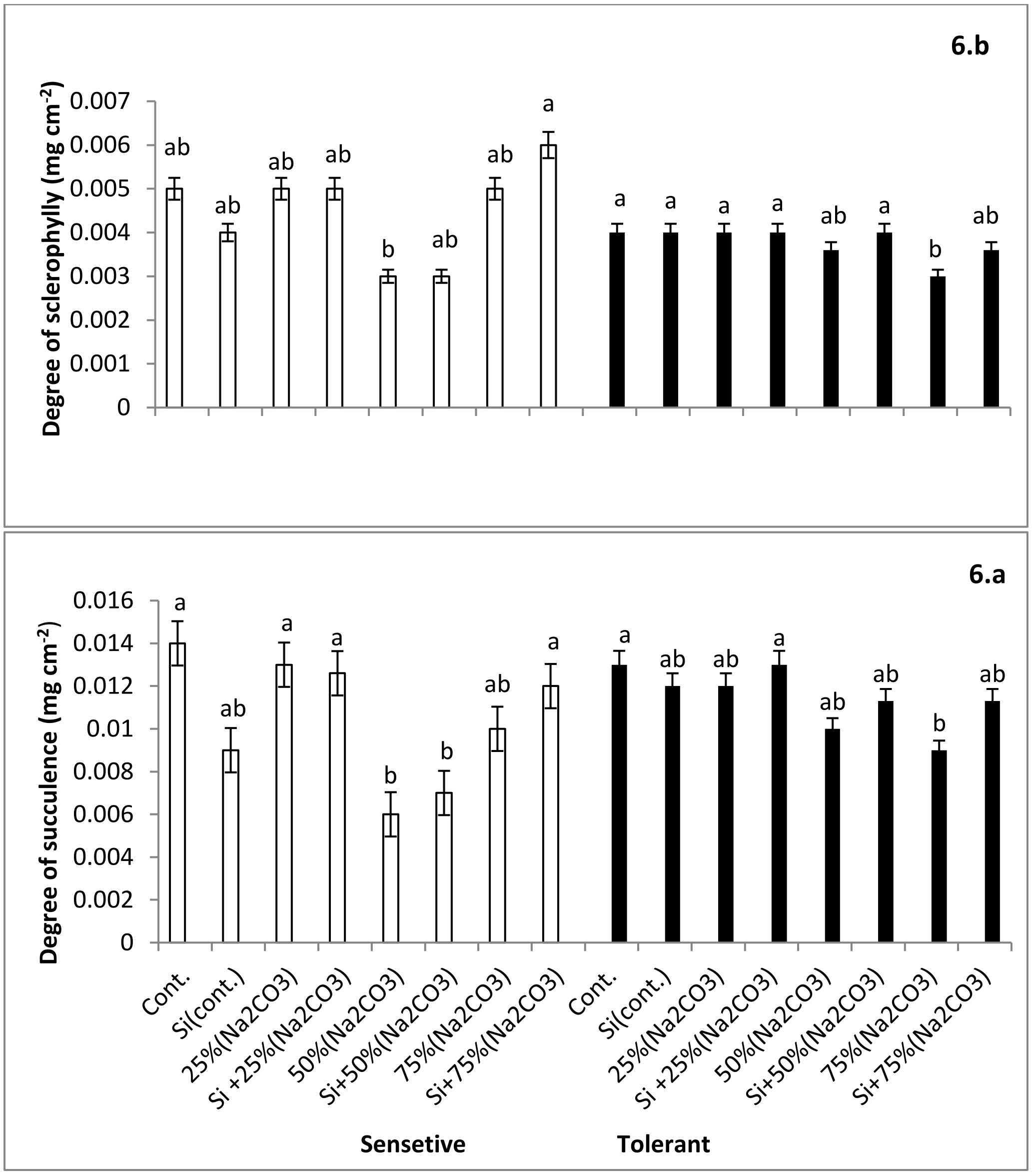


Figure 6: Effect of sodium meta-silicate on growth vigor of flag leaf [degree of succulence (mg cm^{-2}) 6.a. and degree of sclerophylly (mg cm^{-2}) 6.b.] of alkalinity stressed sorghum plants. Vertical bars represent standard error of the mean (n=3). Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311.

Anatomical features in flag leaf of the main shoot

The data in Figures from 7a to 9c as well as plates 1 and 2 revealed that alkalinity stress markedly affected the anatomical features in flag leaves of both sorghum cultivars. In general, the anatomical features in alkalinized plants were stimulated by silicon in both sorghum cultivars. Furthermore, the effect of silicon induced marked increase in conducted canals (xylem area and phloem tissue area), leaf thickness, and ground tissue thickness, number of vascular bundle tissues, proto-xylem vessel area as well as meta-xylem vessel area and tracheids area of both sorghum cultivars and vice versa with the higher concentration of high alkalinity (75%). Also, the tolerant cultivar recorded more significantly increase ($p \leq 0.05$) in the majority of cases.

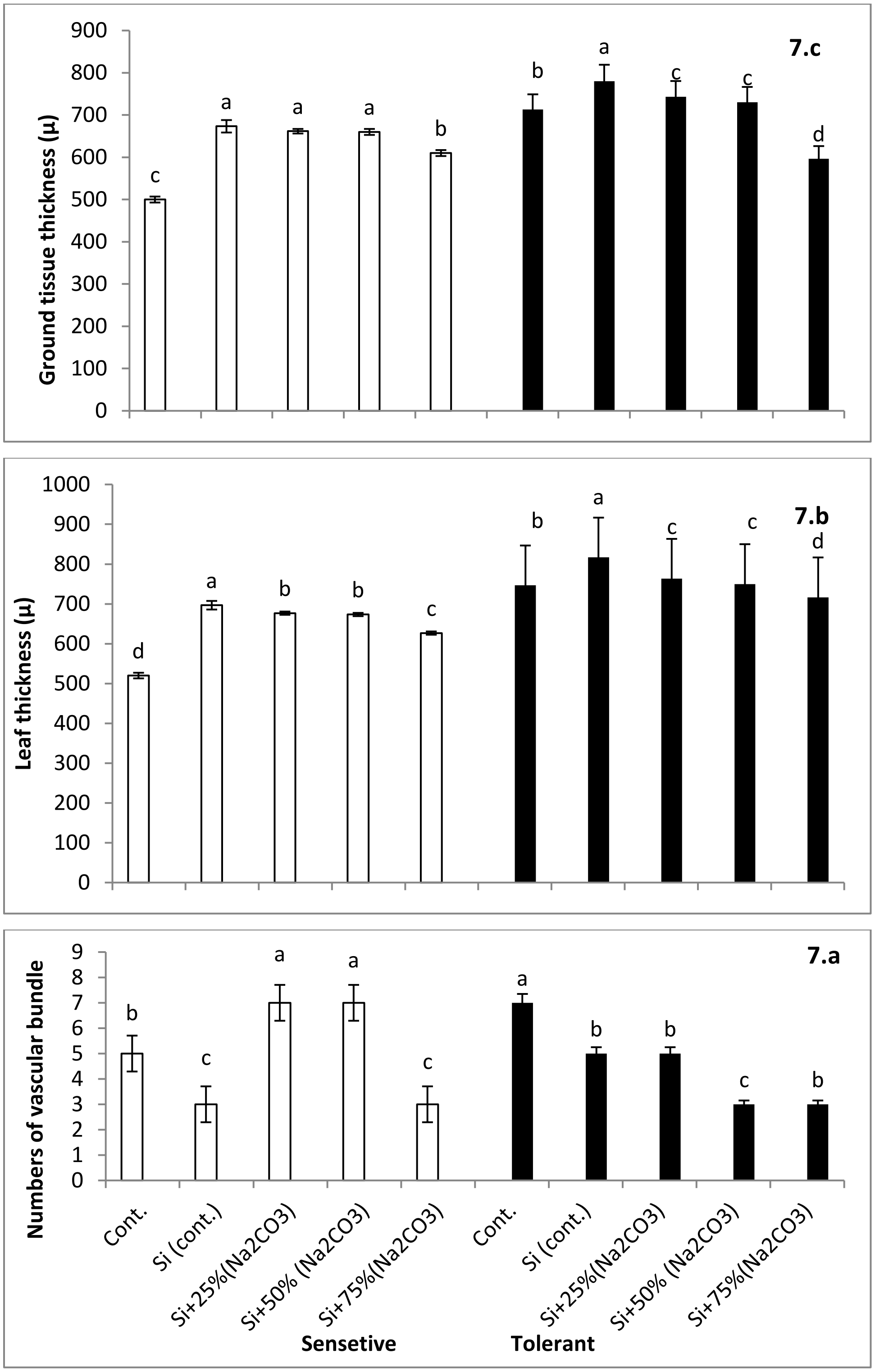


Figure 7: Effect of sodium meta-silicate on numbers of vascular bundle 7a, leaf thickness area 7b and ground tissue thickness 7c. in flag leaf of alkalinity stressed sorghum plants. Vertical bars represent standard error of the mean (n=10). Different letters indicate significant differences between treatments at p ≤ 0.05, according to CoHort/ CoStat software, Version 6.311.

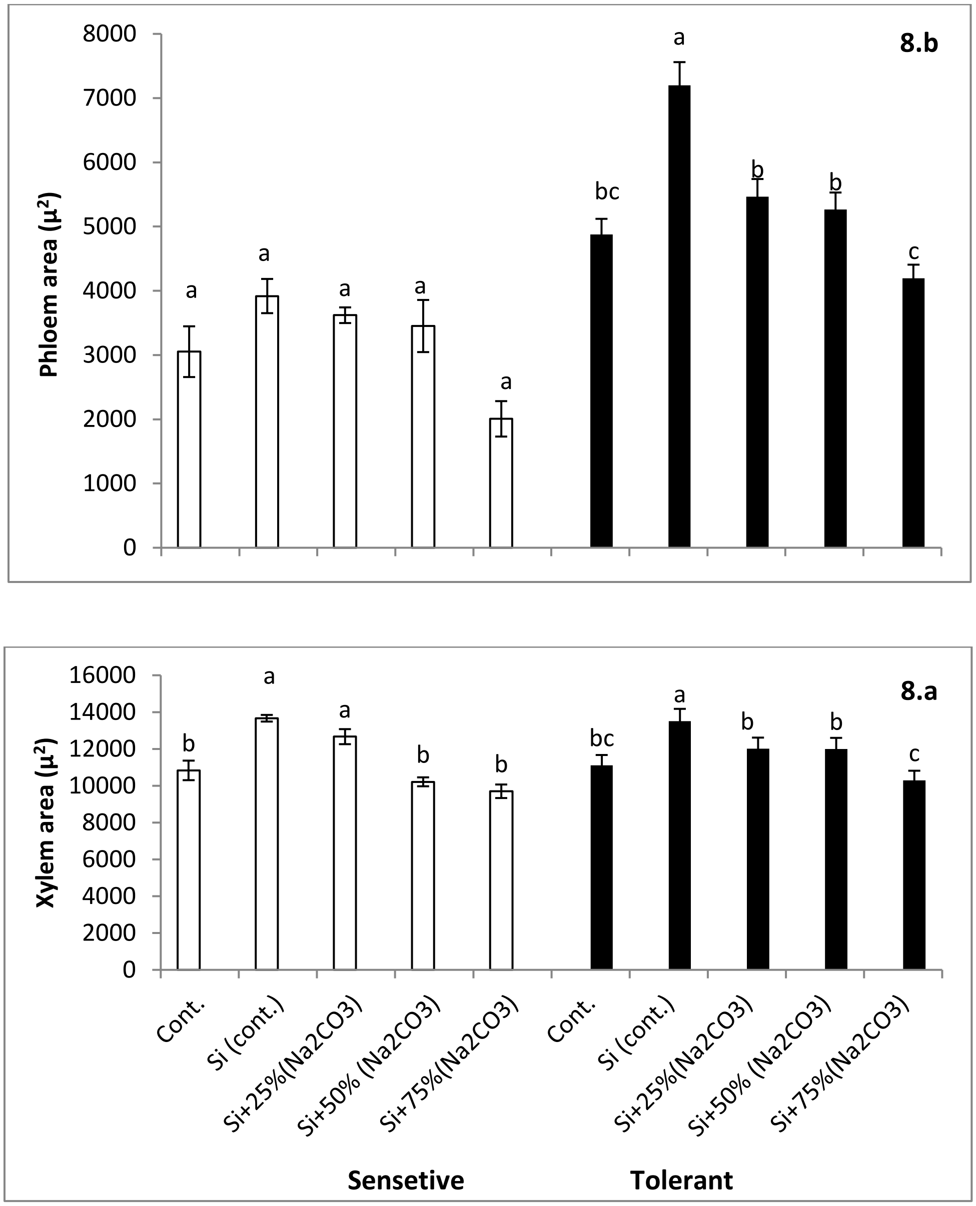


Figure 8: Effect of sodium meta-silicate on xylem area 8.a. and phloem area 8.b. in flag leaf of alkalinity stressed sorghum plants. Vertical bars represent standard error of the mean (n=10). Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311.

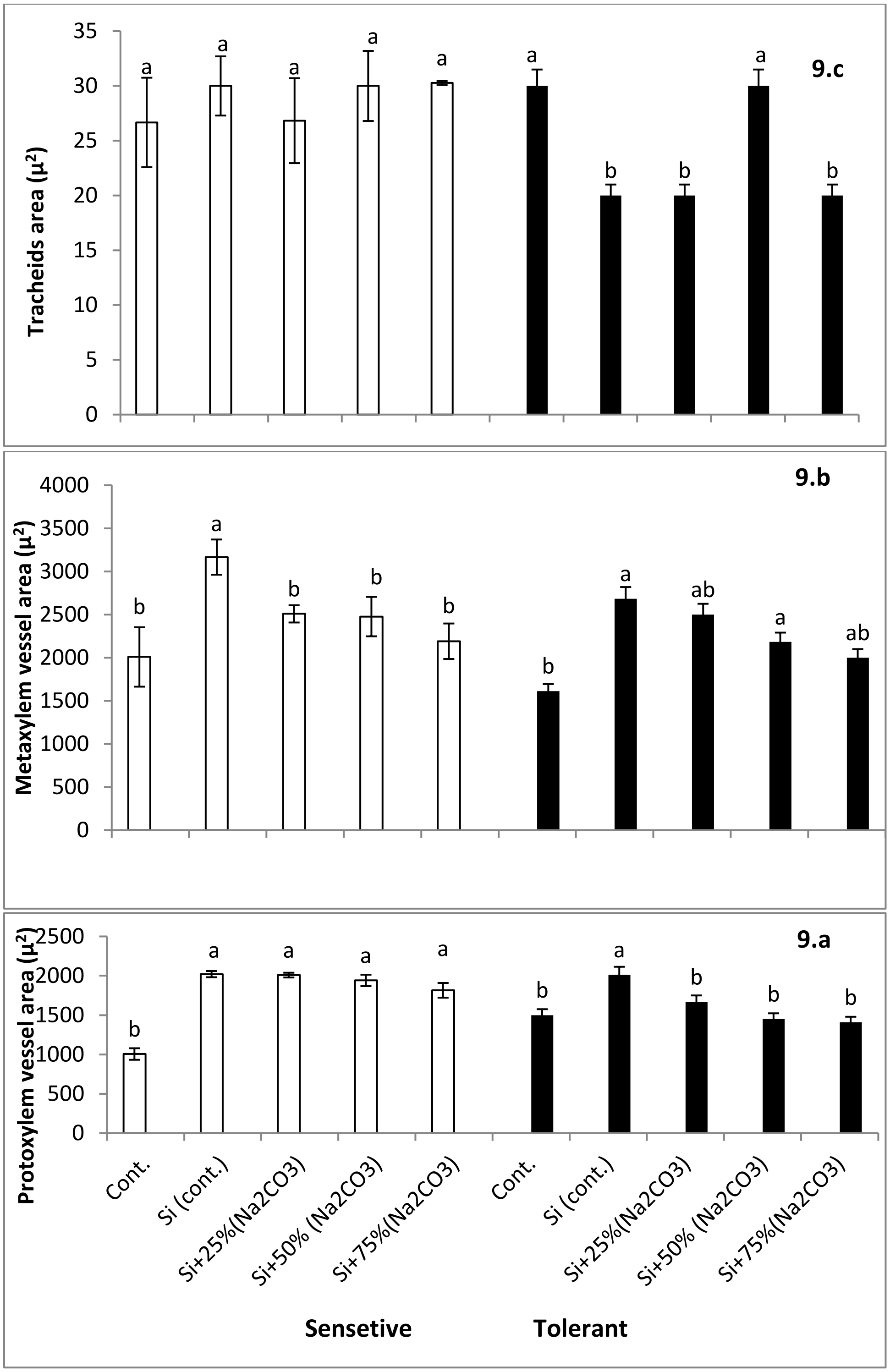
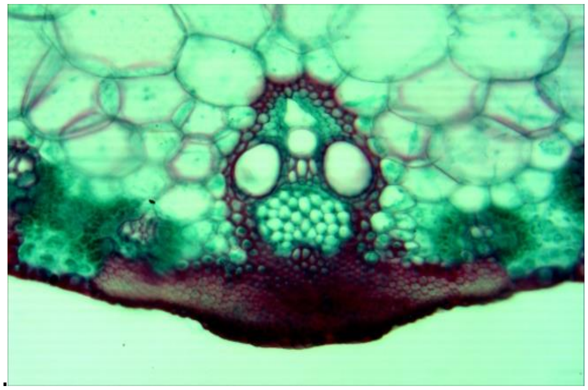
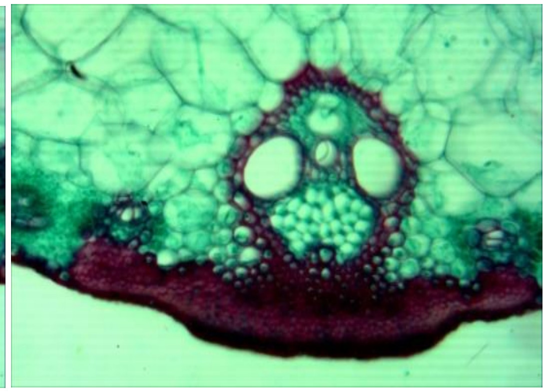


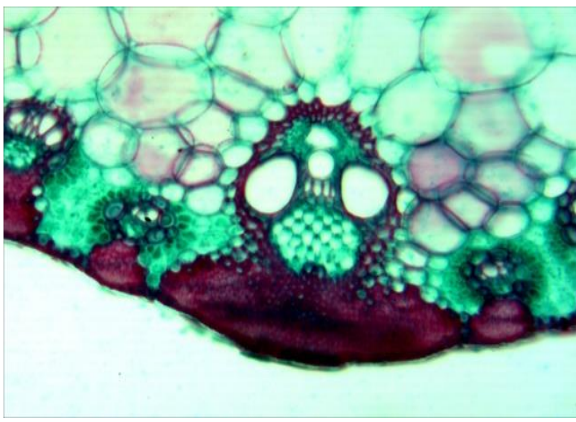
Figure 9: Effect of sodium meta-silicate protoxylem vessel area on 9.a. , metaxylem vessel area 9.b. and tracheids area 9.c. in flag leaf of alkalinity stressed sorghum plants. Vertical bars represent standard error of the mean (n=10). Different letters indicate significant differences between treatments at $p \leq 0.05$, according to CoHort/ CoStat software, Version 6.311.



1.a.

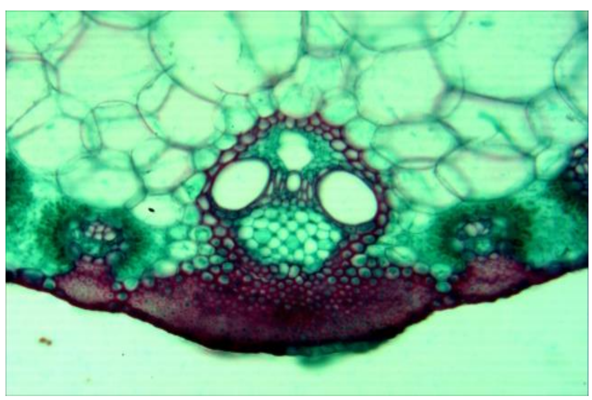


1.b.



1.c

1.d.



1.e.

Plate 1: Interactive effect of sodium meta-silicate and alkalinity on anatomical features of sorghum flag leaf of sensitive cultivar . The following plates showing the interactive effect of sodium meta-silicate and alkalinity 1.a. cont., 1.b. Si(cont.), 1.c. Si + 25% Na_2CO_3, 1.d. Si + 50% Na_2CO_3 and 1. e. Si + 75% Na_2CO_3.

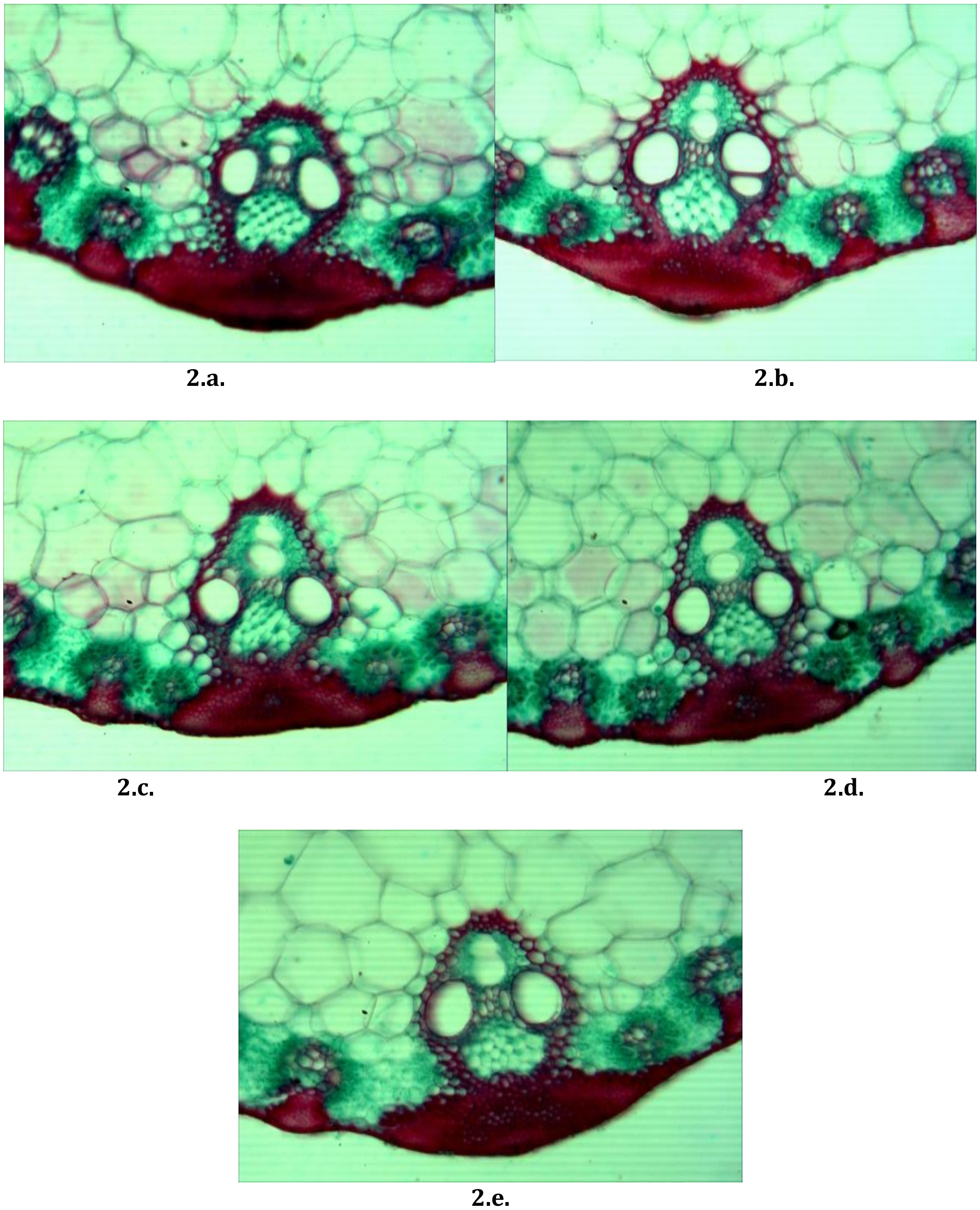


Plate 2: Interactive effect of sodium meta-silicate and alkalinity on anatomical features of sorghum flag leaf of tolerant cultivar . The following plates showing the interactive effect of sodium meta-silicate and alkalinity 2. a. cont., 2.b. Si(cont.), 2.c. Si + 25% Na_2CO_3, 2.d. Si + 50% Na_2CO_3 and 2.e. Si + 75% Na_2CO_3.

Discussion

The detrimental influences caused by alkaline 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 [28].

Plants are continuously challenged by stressful environmental conditions like water deficit leading to changes in biochemical and physiological processes. Consequently, plant growth and development are adversely affected. Plants respond in many ways to water-stress and at a number of levels. In order to define alkalinity tolerance or sensitivity of both cultivars, growth parameters like lengths, dry and fresh weights of roots and shoots as well as flag leaf were tested under the effect of alkalinity-stress during grain-filling. Plants experience various environmental stresses that could result in both general and specific effects on their growth and development. Morphological characteristics were shown to be an important factor in controlling the yield of crop plants.

In general, the obtained results showed that alkalinity stress caused a marked decrease in the growth vigor of root, shoot and flag leaf, pigment contents as well as total protein and nucleic acids content in flag leaf of both sorghum cultivars. Exogenous application of silicon alleviated the adverse effects of alkalinity stress by improving growth vigor of root, shoot and flag leaf. Among the cultivars, tolerant one showed a better performance and produced more biomass under alkalinity stress when compared with sensitive one. In agreement with these results, stressing mulberry plants generally decreased root growth parameters as revealed by [29].

The plant responses to alkaline stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of growth [30]. The response and adaptation of plants to such conditions are very complex and highly variable [31]. Exogenous application of Si is an effective method to alleviate the adverse effects of alkalinity stress on sorghum bicolor.

The results obtained in the present study, as shown in Figures (5&6), 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 sclerophylly. In agreement with our results [32] also reported that total leaf area as well as leaf fresh and dry mass in *Abelmoschus esculentus* plants were significantly reduced under alkalinity 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 [33]. The retardation of leaf growth in stressed plants could be attributed to decreased turgor that may diminish cell production within the leaves [34].

Stressing the studied sorghum cultivars 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). Hence [35] 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 alkalinity stress by reducing its leaf area in order to minimize the deleterious effects of stress.

In the present study, the cumulative degree of leaf succulence also decreased under stress conditions. These results agree with those obtained by [36,37] working with three varieties of sunflower plants. Therefore, they added that greater leaf succulence can be recorded as a means of increasing stress tolerance. 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. In accordance with these results, leaf sclerophylly was found to increase by stressing wheat plants [31].

The current work showed that the increase in leaf growth was more pronounced showing beneficial effects of Si on alkalinity stressed sorghum plants. (Figure 5) indicate that, 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 were supported by [38]. 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 [39].

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 [40] resulting in higher leaf area of maize plants as recorded in Si-primed plants under non-stressed and alkaline stress conditions. Furthermore, Si pre-treatment as seed-priming improved other growth parameters of alkaline- stressed maize seedlings. Thus, the results of this study and previously published reports collectively indicate the protecting role of Si against a wide range of environmental pressures [41].

Most plants have developed morphological and physiological mechanisms which allow them to adapt and survive under stress conditions [42]. These mechanisms mainly comprise a reduction of the leaf size, leaf rolling, dense leaf pubescence, deeply developing stomata, accumulation of mucilage and other secondary metabolites in the mesophyll cells, increase of mesophyll compactness [43].

The application of silicon induced some modifications in the anatomical features of the flag leaf which appeared to be an adaptive response to alkalinity stress. Silicon induced marked increase in conductive canals between flag leaf (i.e. source) and peduncle (i.e. sink) that help manipulation of photo-assimilates towards developing grains and consequently improved yield quality and quantity (Figures 7, 8 &9 and Plates 1, 2). Furthermore, silicon plays an important role in alleviating the ill effect of alkaline stress on anatomical features of flag leaf especially conductive canals [14].

The increase in the leaf thickness might be due to the increase in the mesophyll tissue. These tissues (mesophyll) are characterized by high concentration of chloroplast. As the leaf thickness could be considered as a good indicator to specific leaf weight which is a reliable index to photosynthetic efficiency. It could be concluded that, the resistant cultivar was more efficient in synthesizing metabolites than the sensitive one. The conductive canals between source and sink in wheat cultivars have been reported to play a prominent role in the translocation of photosynthates to the developing grains [44]. Si induced additional increases in the areas of conductive canals (xylem and phloem) in flag leaf of both cultivars. This furnishes better translocation of assimilates from flag leaf (as source) towards the developing grains (as sink) through the conductive canals.

Conclusion

In conclusion, from current study we could concluded that alkalinity generally reduced almost all growth criteria of root, shoot and flag leaf of both sorghum cultivars during grain-filling. Extent of reduction was more obvious particularly in sensitive cultivar. Moreover, plants supplementation with silicon can be a potential method for increasing their ability to overcome harmful effect of alkalinity stress since silicon increase tolerance of plants towards alkalinity through increasing their ability to sustain physiological drought and counteracting the negative effects of alkalinity stress on all growth criteria of root, shoot and leaf area of both sorghum cultivars as well as inducing modifications in the anatomical features of alkalinized flag leaf. Finally, Si, as a fertilizer, silicon plays an important role in alleviating the ill effect of alkaline stress on morphological characteristics as well as anatomical features of flag leaf of sorghum plants.

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