

# Augmentation of drought stress tolerance of two wheat cultivars through exogenous spraying of Silicon

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## ABSTRACT

Two wheat cultivars cv. Sohag 3 (durum wheat) and cv. Seds 12 (bread wheat) were tested to assess their physiological behavior under different water regimes (100%, 70%, 50% and 30% FC) and their responsiveness to foliar application of 8mM silicon. The data clearly indicated the hypersensitivity of cv. Sohag 3 to water deficit as compared to cv. Seds 12 where dramatic reduction of shoots, roots, spikes and grains dry weight. Cv. Sohag 3 reduced total proteins and great accumulated of soluble proteins at the expense of insoluble one as well as reduction of soluble, insoluble and total carbohydrates. On the other hand, cv. Seds 12 maintained the insoluble proteins and enhanced of the soluble one as well as its ability to boost soluble, insoluble and total carbohydrates. In addition, water stress stimulated high oxidative damage in terms of overproduction of hydrogen peroxide, lipid peroxidation, electrolyte leakage and lipoxygenase enzyme of cv. Sohag 3 compared to oxidative damage limiting capacity of cv. Seds 12. Silicon application greatly augmented the water scarcity and exhibited better growth under harsh conditions due to the up- regulation of C and N- metabolism of the studied cultivars and reduced membrane damage as indicated by reducing H2O2, MDA, electrolyte leakage and lipoxygenase. All of these criteria answered on the stimulatory effect of silicon on yield attributes (spikes and grains) of the

#### Keywords

Silicon, Wheat, growth, carbohydrates, proteins, hydrogen peroxide, lipid peroxidation, electrolyte leakage and lipoxygenase enzyme

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### **1. INTRODUCTION**

One of the greatest challenges that will face earth's population over the next few decades will be the need to satisfy the food requirements of an ever growing population, while the available water reserves are declining steadily [1]. Drought continues to be an important challenge to agricultural researchers and plant breeders and it is assumed that by the year of 2025, around 1.8 billion people will be facing absolute water shortage and 65% of the world's population will be living under water stressed environments [2]. The world's water deficit will grow to 200 billion cubic meters per year by 2050, this deficit will be a significant challenge, costing the world \$104 billion per year, while cost Egypt alone \$11 billion annually. Wheat is the major crop plant in the daily diet of 35% of world population [3] and almost 32 % of wheat crops face various types of drought stress during the growth season in developing countries [4].

Due to the fact that drought is a yield-decreasing factor even when its damages are not visible, it is crucially important to figure out methods for increasing crops yield under these conditions [5]. There are different approaches to mitigate the drought hazards, which include selection of stress resistant varieties along with using cheap and easy alternative way for overcoming the negative effects of drought such as using of metal elements like silicon. Silicon is the second most prevalent element in the soil and one of the most important macro- elements, performing an essential function in healing plants in response to environmental stresses. Plant growth improvement by Si application under drought conditions has been documented in monocot and dicot species [6, 7]. Silicon acts as a physical or mechanical barrier in plants and not only acts as cell wall incrustation, but is also actively involved in many metabolic and/or physiological processes [8]. Extensive studies have been performed in order to find possible mechanism(s) for silicon-enhanced tolerance of higher plants to abiotic stresses. However further studies must be done to illustrate the potential role of silicon potential role in plant life.

Accordingly the aim of the present investigation is to gain more information on the beneficial role of silicon foliar application in augmentation of drought tolerance of two wheat species in terms of growth, yield, some metabolic products,  $H_2O_2$ , lipid peroxidation, metabolites leakage and lipoxygenase enzyme.

#### 2. MATERIALS AND METHODS

A pot experiment was carried out in open air under natural conditions at the garden of Faculty of Science- Assiut University during winter season (2012-2013) from early December to early May. Durum wheat cultivar " cv. Sohag 3" obtained kindly from Agriculture Research Center of Shandwell and bread wheat cultivar "cv. Seds 12" were kindly brought about from breeding program directed by Faculty of Agriculture – Assiut University. The two cultivars were selected to be used in the current long duration experiment based on a previous experiment at vegetative stage occurred on six wheat cultivars where cv. Sohag 3 was the drought sensitive and cv. Seds 12 was drought tolerant. Ten wheat grains were sown in plastic pots containing 6.5 Kg clay soil. All pots were irrigated with tap water nearly around field capacity. After 25 days from sowing water deficit was imposed by decreasing the availability of water by about 70% (mild drought), 50% (moderate drought) and 30% FC (severe drought) respectively. After 30 days from drought imposition, the pots were divided into two groups:

- i. First group (Reference control): included 100, 70, 50 and 30% field capacity.
- ii. Second group: included the aforementioned water levels sprayed with 8 mM silicon which used as sodium metasilicate nonahydrate.

The concentrations of silicon was also chosen based on previous experiment from four concentrations where it was the most effective doses in alleviating drought stress imposed under vegetative stage [9]. The pots were weighed daily and watered to restore the appropriate moisture by adding the calculated amount of water. Plants left grown under the different treatments for three weeks later then plants were picked up for doing some measurements. The rest of pots were kept up to the middle April (nearly 22 weeks from sowing) irrigation stopped gradually, spikes and grains were collected and weighted.

Dry weight determination: Separated shoots and roots were oven dried at 105°C. Successive weighing was carried out until constant mass of each sample was reached.

Determination of soluble and total carbohydrates: The anthrone-sulphuric acid method [10, 11] was used for the determination of carbohydrates.

Soluble and total proteins were carried out using the alkaline reagent solution according to the method of [12].

Electrolyte leakage which reflects membrane damage was measured by an electrical conductivity method described by [13].



Lipid peroxidation was determined in leaves by measuring MDA formation using thiobarbituric acid reaction as described by [14].

Hydrogen peroxide content (H<sub>2</sub>O<sub>2</sub>) of leaf samples was spectrophotometrically measured as described by [15].

Lipoxygenase (LOXs, 1.13.11.12) activity was detected in leaves according to the method of [16].

Statistical analysis: The data were subjected to one-way ANOVA using SPSS 10.0 software program. Means were calculated for three replicate values. Means were compared by the Duncan's multiple range tests and statistical significance was determined at 5% level.

## 3. RESULTS

**3.1 Growth attributes**: The dynamics of shoots and roots dry weight of the two tested cultivars under different water regimes were registered in Figures 1a,b,c& d. As was expected, as the severity of drought stress intensified, the dry weight of shoots and roots declined significantly in both studied cultivars but more so in cv. Sohag 3 which maximally recorded at severe drought where the percent reduction in shoot + root was 72.24% and 51.34% relative to control for cv. Sohag 3 and cv. Seds 12, respectively indicating the susceptibility of cv. Sohag 3 compared to cv. Seds 12. The response of shoot and root of both cultivars to drought stress pointed out that both of them behaved more or less similarly at different water levels in cv. Seds 12, whilst shoot were more susceptible than roots in cv. Sohag 3. Supplementation of silicon to optimally irrigated plants on both cultivars induced highly significant accumulation of dry matter at control but more so in cv. Sohag 3. Also, silicon not only curtailed the reduction in dry matter at mild and moderate drought level but also promoted these values above the control. On the other hand, at severe drought, silicon application exhibited insignificant effect on dry matter acquisition of cv. Seds 12, whilst the total dry matter more than doubled in relation to the corresponding drought plants in cv. Sohag 3.

The susceptibility of cv. Sohag 3 and tolerance of cv. Seds 12 to drought stress greatly ascertained from spikes dry weight as depicted in Figure 1e&f that the two studied cultivars reacted to drought stress in similar manner as recorded for dry matter production. Spikes dry weight decreased in response to severe drought as much as 56.82% and 77.97% relative to control for cv. Seds 12 and cv. Sohag 3 respectively. With regard to grains dry weight (Figure 1g&h), the susceptibility of cv. Sohag 3 appeared earlier even at the mild drought level where the percent reduction of grains was 46.34% relative to control and this trend deepened at severe drought where cv. Sohag 3 produced grainless-filled spikes compared to cv. Seds 12 which succeeded to produce grain-filled spikes with percent reduction of 67.69% relative to control.

Silicon foliar application greatly triggered grains and spikes dry matter accumulation in well-watered conditions which was more pronounced for cv. Sohag 3 compared to cv. Seds 12 and greatly enhanced yield attributes of wheat cultivars under drought conditions. Si application at 70% and 50% FC succeeded in maintaining grains and spikes dry matter more or less around the control values in cv. Seds 12, but in cv. Sohag 3, Si extensively boosted highly significantly dry weight of grains and spikes over the control. Silicon foliar application did not prevent the decline in grains and spikes dry matter at 30% FC but vastly stimulated yield compared to control in cv. Seds 12. Interestingly, in cv. Sohag 3, the applicant motivated biosynthesis of grain- filled spikes at 30% FC which suppressed completely under drought conditions only.

#### 3.2 Metabolic products:-

3.2.1 Carbohydrates: In the current study there are differences in the accumulation and distribution of carbohydrates between the two studied cultivars, among the different organs as well as the water regime imposed are represented in Figures 2&3. Reduction in soluble, insoluble and total carbohydrates as consequence of drought imposition in cv. Sohag 3 in all of the tested organs and maximally at severe drought (Figure 2 a,b,c a,b,c,d,e,f,g,h&i). In cv. Seds 12, moisture stress induced stimulation rather than inhibition of the total carbohydrates and their fraction (soluble and insoluble) but the extent of fractionation of the total carbohydrates varied considerably from roots to shoots (Figure 3 a,b,c,d,e,f,g,h&i). Roots preferred higher percent accumulation of soluble carbohydrates compared to the insoluble one under water scarcity. Conversely, leaves and stems had directed the percent increase of total carbohydrates under drought stress towards the accumulation of insoluble one compared to the soluble carbohydrates. (i.e: at 30% FC which exhibited the highest accumulation trend of soluble, insoluble and total carbohydrates for all of the tested organs, the percent increase relative to control in roots was 41.49%, 16.50% and 19.25%, respectively. In stems 8.62%, 45.21% and 17.50%, respectively and that of leaves was 6.71%, 36.18% and 15.67%, respectively. Moreover, the absolute values of soluble and insoluble carbohydrates in cv. Seds 12 under control or even drought conditions showed two situations in roots and shoots. In roots, the soluble carbohydrate was vastly lower than that of insoluble carbohydrates. On the other hand, in shoots the soluble carbohydrate was progressively higher than that of insoluble carbohydrates. The interactive effect of different water regimes and foliar



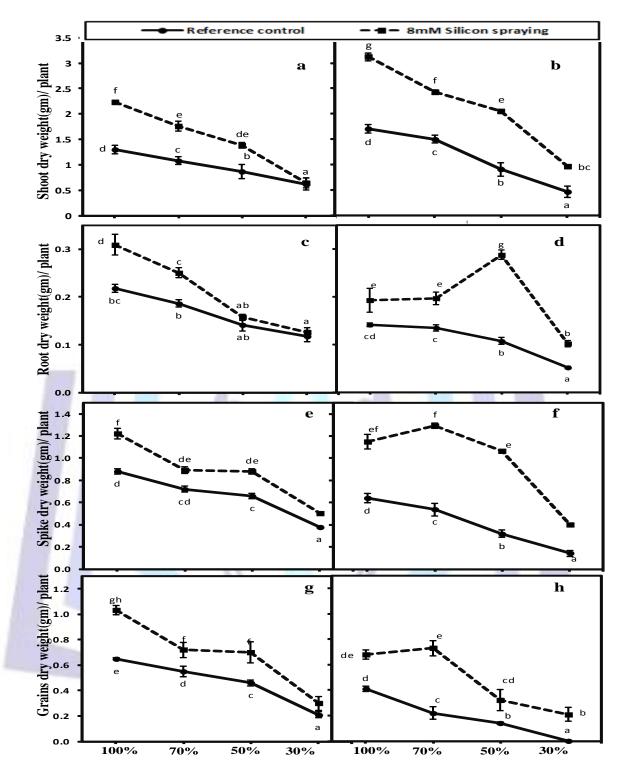


Figure 1 : Shoot, root, spikes and grains dry weight of wheat cultivars Ses 12 (Figure 1 a,c,e&g) and Sohag 3 (Figure 1 b,d,f&h) under 100%, 70%, 50% and 30% FC as affected by foliar spray with 8 mM silicon. The vertical bars indicate  $\pm$  SE. Bars carrying different litters are significantly different at *P*<0.05.





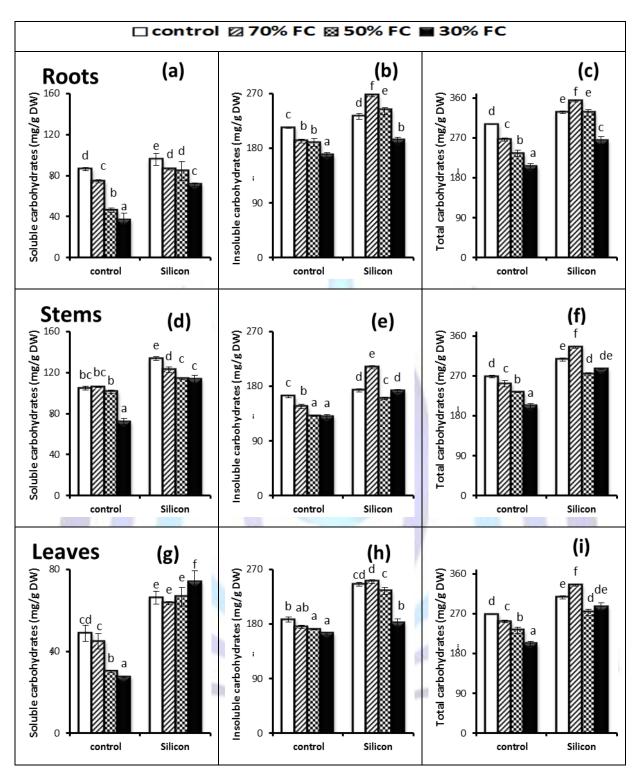


Figure 2: Soluble, insoluble and total carbohydrates, respectively of roots (a,b&c), stems (d,e&f) and leaves (g,h&i) of wheat cultivar Sohag 3 under 100%, 70%, 50% and 30% FC as affected by foliar spraying with 8 mM silicon. Each histogram represents a mean value of three replicates, and the vertical bars indicate  $\pm$  SE. Bars carrying different litters are significantly different at *P*<0.05.



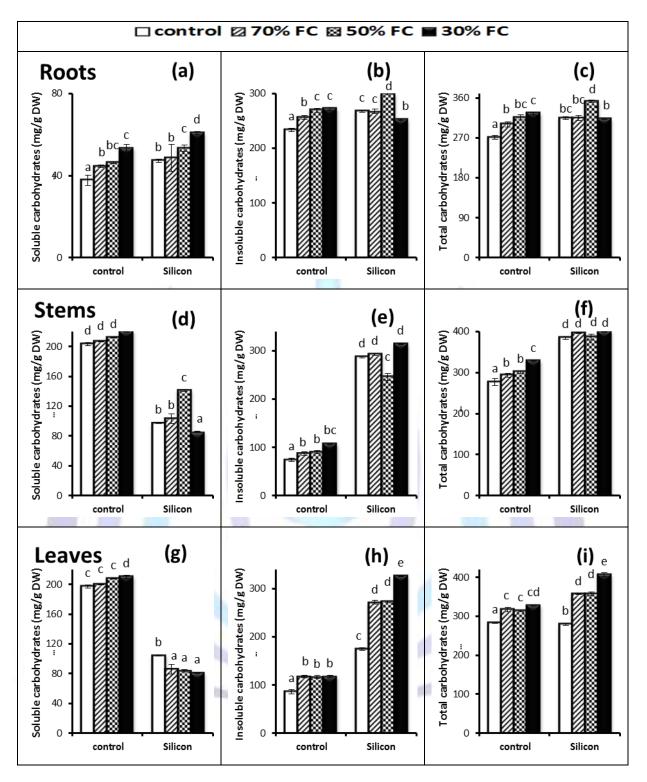


Figure 3 : Soluble, insoluble and total carbohydrates, respectively of roots (a,b&c), stems (d,e&f) and leaves (g,h&i) of wheat cultivar Seds 12 under 100%, 70%, 50% and 30% FC as affected by foliar spraying with 8 mM silicon. Each histogram represents a mean value of three replicates, and the vertical bars indicate  $\pm$  SE. Bars carrying different litters are significantly different at *P*<0.05.



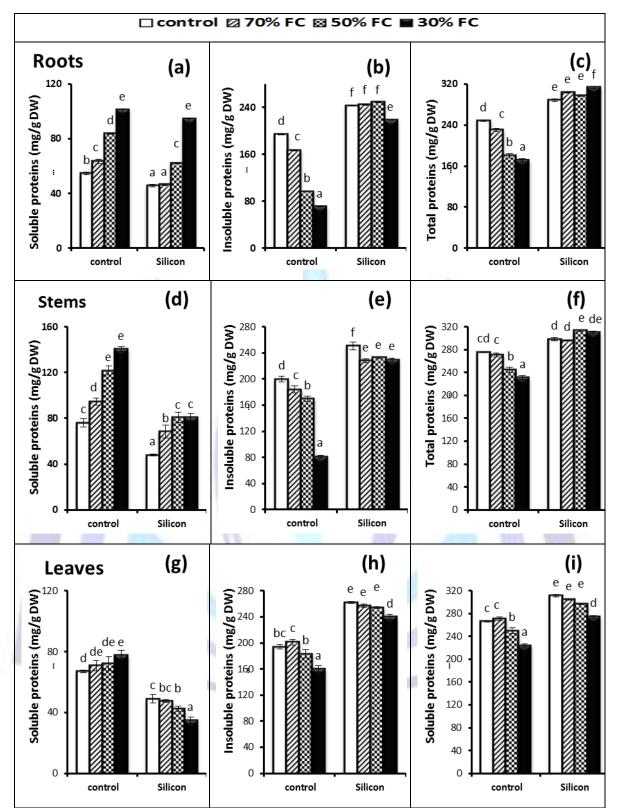


Figure 4 : Soluble, insoluble and total proteins, respectively of roots (a,b&c), stems (d,e&f) and leaves (g,h&i) of wheat cultivar Sohag 3 under 100%, 70%, 50% and 30% FC as affected by foliar spraying with 8 mM silicon. Each histogram represents a mean value of three replicates, and the vertical bars indicate  $\pm$  SE. Bars carrying different litters are significantly different at *P*<0.05.



□ control 2 70% FC 2 50% FC 3 30% FC **(c)** (b) **Roots** (a) Soluble proteins (mg/g DW)  $_{20}^{+1}$ Insoluble proteins (mg/g DW) 360 cd d f 400 Total proteins (mg/g DW) С 300 b аa а 300 cd d 240 200 180 120 100 60 0 0 0 control Silicon control Silicon Silicon control (f) Stems (e) (d) **Total proteins (mg/g DW)** 100 100 100 Insoluble proteins (mg/g DW) 280 e e g e e 240 200 bc ab b 160 а 120 80 40 0 0 0 control Silicon control Silicon control Silicon (**h)** d d (i) Leaves (g) сС Insoluble proteins (mg/g DW) 280 b b b b b 240 b 200 160 120 80 40 0 0 0

Figure 5 : Soluble, insoluble and total proteins, respectively of roots (a,b&c), stems (d,e&f) and leaves (g,h&i) of wheat cultivar Seds 12 under 100%, 70%, 50% and 30% FC as affected by foliar spraying with 8 mM silicon. Each histogram represents a mean value of three replicates, and the vertical bars indicate  $\pm$  SE. Bars carrying different litters are significantly different at *P*<0.05.

control

Silicon

Silicon

control

control

Silicon



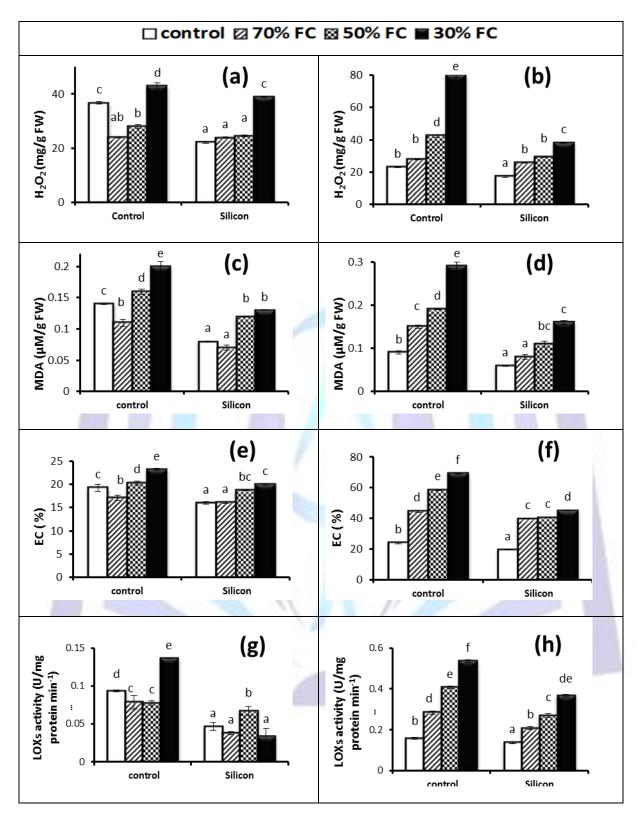


Figure 6: Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), lipid peroxidation (MDA), electrolyte leakage (EC%) and lipoxygenase enzyme of leaves of wheat cultivar Seds 12 (Fig. 6a,c, e&g) and cv. Sohag 3 (Fig. 6b,d, f& h) respectively under 100%, 70%, 50% and 30% FC plants as affected by foliar spray with 8 mM silicon. Each histogram represents a mean value of three replicates, and the vertical bars indicate  $\pm$  SE. Bars carrying different litters are significantly different at *P*<0.05.



application of Si manifested an abrupt increase in total carbohydrates and their fraction whatever the tested cultivar, organ or treatment used except for decline of soluble carbohydrates for stems and leaves in cv. Seds 12.

3.2.2 Proteins: - The studied plants cope with stresses by modifying metabolism resulting in distributing the ultrastructure components and alterations of macromolecular synthesis such as proteins which feasibly behaved differentially in the two studied cultivars as declared in Figures 4&5. In cv. Sohag 3, drought stress posed highly significant reduction of total proteins in all tested organs which was more pronounced for roots (i.e: the percent reduction of total proteins at severe drought relative to control was 30.88%, 16.55% and 15.80% for roots, stems and leaves respectively. The data of soluble and insoluble proteins (Figure 4 a,b,c,d,e,f,g,h&i) declared high depletion of insoluble proteins and accumulation of soluble proteins which was more pronounced at the level of 30% FC where the percent increase of soluble proteins was 85.18%, 84.65% and 15.86% over the control for roots, stems and leaves respectively and the percent reduction of insoluble proteins was 63.56%, 59.25% and 17.44% relative to control for roots, stems and leaves respectively. However, in cv. Seds 12, drought triggered stimulation rather than inhibition of the total proteins with percent increase of 16.43%, 46.39% and 38.66% over the control for roots, stems and leaves respectively (Figure 5 a,b,c,d,e,f,g,h&i). The direction of the accumulated total proteins fractioning between soluble and insoluble one under stress exhibited two situations: in stems and roots, water deficit triggered accumulation of soluble protein fraction and mainly maintained the insoluble one comparable to control (i.e: at 30% FC, the percent increase of soluble proteins was 80.88% and 165.33% over the control for roots and stems respectively). Conversely, leaves directed most of the accumulated total proteins under drought towards insoluble proteins and little was directed towards soluble proteins (i.e: at 30% FC, the percent increase of soluble proteins and insoluble proteins was 18.72% and 50.75% over the control respectively). Foliar application of silicon rendered both cultivars more tolerant to drought through promotion of soluble, insoluble and total proteins except for soluble protein of cv. Sohag3. In cv. Sohag 3, silicon treated plants reduced soluble proteins in relation to absolute control in the three tested organs

**3.3 Oxidative stress markers:** The dynamics of oxidative stress markers used in the current study, hydrogen peroxide, lipid peroxidation, metabolites damage and lipoxygenase enzyme, were illustrated in Figure 6 where notable gradual increasing trend as water shortage increased in the soil was displayed, but the magnitude of their overproduction varied considerably for both cultivars. Cv. Sohag 3 exhibited the highest damaging effect, while cv. Seds 12 showing the lowest impact which upmost recorded at 30% FC where the percent increase in comparison to control for cv. Seds 12 and cv. Sohag 3 of hydrogen peroxide was 16.91% and 244.30% (Figure 6 a,b), lipid peroxidation was 44.35% and 225.71% (Figure 6 c,d), metabolite leakage was 20.55% and 188.07% (Figure 6 e,f) and that of lipoxygenase enzyme was 46.15% and 239.24 (Figure 6 g,h), respectively. It is worth noting that a substantial decrease in  $H_2O_2$ , MDA and electrolyte damage was observed at mild drought and non- significant change of these parameters was denoted at moderate drought for cv. Seds 12. Silicon foliar application rendered the plant more drought tolerant through curtailing the production of  $H_2O_2$ , MDA, electrolyte leakage and lipoxygenase enzyme (Figure 6 a,b,c,d,e,f,g&h) which nearly maintained around the control values for cv. Seds 12 and greatly reduced compared to the corresponding drought treatments in cv. Sohag 3. Also, silicon sprayed well-watered plants exhibited lower values of these parameters than non-sprayed control plants.

### 4. DISCUSSION

Wheat cultivars respond differently to water stress in the form of physiological and biochemical changes indicating existence of genotypic variation [17]. Present water scarcity is an emerging issue and cause of deterioration in quality and productivity of crops to reduce crop yield all over the world. Silicon is known to be better against the deleterious effects of drought on plant growth and development [18]. The data provided in this work went parallel to the previous studies on the damaging effects of water- deficit on growth attributes in terms of shoots, roots, spikes and grains dry weight. There was variation in the morpho-physiological characteristics between the two cultivars in their response to drought that cv. Sohag 3 was more susceptible to drought stress than cv. Seds 12, but the extreme vulnerability of the former clearly manifested from its production of empty spikes at severe drought compared to low grain production of the latter at the same water regime. In this respect, one can say that production of spikes is an inherit character consequently under drought condition, the cultivars may be had the ability to produce spikes but the filling of spikes with grains is problematic. This may be related to the gain of dry matter under different water regimes and the quality of metabolites produced. As the cultivar able to keep highest dry matter especially under stress conditions, its ability to allocate the conserved dry matter to grains production increased implying that there threshold dry matter below which the production of grains suppressed. This interpretation is a reflection of the finding of Taheri et al., [19] who stated that more aboveground part results in higher photosynthetic rate, which leads to an increase in the ability of the plant for grain filling and also for forming more grains. As aforementioned, cv. Seds 12 had the highest dry matter yield compared to the cv. Sohag 3 at the similar level of drought stress. These results confirmed our previous findings during the vegetative stage of the hypersensitivity of cv. Sohag 3 to drought stress and tolerance of cv. Seds 12 was



also reported during vegetative growth in the preliminary experiment Dawood et al., [9] indicating that drought tolerance of each cultivar did not change over the plant life cycle. Foliar application of silicon was more effective in displaying substantially better growth under well-watered conditions and showed stronger anti-drought effects in terms of shoots, roots, spikes and grains dry matter accumulation with varying responsiveness based on the cultivars, water level imposed and even at the organization level. The outcomes of these results is that, silicon foliar application actuated higher responses in the aforementioned traits for cv. Sohag 3 compared to cv. Seds 12 which indicated the flexibility of cv. Sohag 3 to vastly change biochemical reaction under silicon application compared to stability of these reactions in cv. Seds 12. This appeared from high ability of cv. Sohag 3 to increase roots dry weight, thus produced vigor root which facilitate its penetration and distribution below ground surface to improve the water status of cultivar. The pivotal role of Si on plants was reported by Karmollachaab et al., [20] pointed out that silicon increases growth and yield of wheat grains through influencing some physiological activities of plant. Moreover, the positive effect of silicon on plant yield was more significant in stress conditions than that in non-stress conditions. Silicon increases the number and mass grain production of wheat by stimulating shoot and root biomasses [21]. Ali et al., [22] displayed that silicon application to wheat plants depicted marked enhancement in fresh and dry weights of root and shoot in comparison to plants grown without Si. They showed that about 2 times more effect of silicon in drought in comparison to the well-watered. Maghsoudi et al., [7] found that water deficit decreased shoot and root lengths, shoot dry weight and root dry weight. In contrast, foliar application of Si improved plant growth parameters and chlorophyll pigment concentration under water deficit; however, it did not significantly affect wheat growth under control conditions. So, for wheat under drought stress conditions, silicon is a necessary element and presence of sufficient amount of it guarantees plant survival. Also, since silicon is abundant and does not pollute environment, using this element in similar stressful conditions is recommended.

Adaptation of plant cells to water stress is associated with maintaining osmotic homeostasis by metabolic adjustments that lead to the accumulation of metabolically compatible compounds such as carbohydrates which is one of the main organic constituent of the dry matter derived from the current photosynthesis. The pattern of changes in carbohydrates reflected the status of every cultivar from sensitivity or tolerance to drought stress. The sensitivity of cv. Sohag 3 may be due to depletion of soluble carbohydrates which used in osmoregulation and insoluble carbohydrates which was important for plant body building. Thus the cultivar was poor in photoassimilates which played positive role in osmoregulation and translocated to grain filling, this might be answered the failure of the cultivar to produce grainfilled spikes at severe drought or may be related to the greater energetically cost of osmotic adjustment with sugars opposed to water stress. Similar reduction of carbohydrates under water stress was reported by [22, 23]. In most cases, the decreased carbohydrate content under stress conditions is related to the reduction of pigment and photosynthesis resulted from low expression of enzymes involved in photosynthesis under drought conditions [24]. Simultaneously, the yield losses often occurred due to a reduction in the starch production [25]. On the other hand, the tolerance of cv. Seds 12 was mirrored by accumulation of insoluble carbohydrates which conserved the ultrastructure of plant under stress as well as soluble carbohydrates accumulation to be used osmotically. All of this reflected on partial translocation of the conserved metabolites to grain filling to escape harsh conditions. In this respect, Westgate and Boyer, [26] found that the decrease in stored assimilates of maize during reproductive and flowering periods increased the susceptibility of grain formation to low leaf water potential. Also, they concluded that under low water potential, greater assimilate storage by itself was not enough for coping with current photosynthesis or reproductive storage deficiency, which led to the decrease in grain weight under drought stress. In confirmatory, Emam et al., [27] declared that drought stress reduced grain yield and harvest index (HI) of the two rice cultivars, parallel with reduction in total soluble carbohydrate, starch, protein, oil, phenols, and flavonoid contents. The increase in carbohydrates was reported by [28, 29]. Mafakheri et al., [30] stated that drought stress imposed during vegetative growth or anthesis in three chickpea varieties significantly increased water soluble carbohydrate concentration. The tolerant variety accumulated more soluble carbohydrate than the sensitive one in three chickpea (Cicer arietinum) cultivars. The promoting effect of silicon supplementation on growth may be due to further activation the tolerance mechanism reported under drought for cv. Seds 12 which lead to up-regulation of carbohydrates metabolism under well-watered and drought conditions for both cultivars which reflected on increasing dry matter production. Moreover, in cv. Seds 12; the carbohydrate fractions under the used applicant manifested two situations among organs: in roots; exacerbation of soluble and insoluble carbohydrates over that recorded for the stressed plants only. In leaves or stems; depression of soluble carbohydrates and abrupt accumulation of insoluble carbohydrates which reached in some cases 4 folds that of control. Silicon reversed the panorama of carbohydrates fractioning of leaves, stems and roots which originally used by silicon- deprived plants where the partitioning is reversed towards accumulation of structural (insoluble) carbohydrates rather than soluble one to build up the plant body especially above ground organs to gain more dry weight to be allocated to grains production. In cv. Sohag 3, silicon treatment enriched the cultivar with soluble sugars to be used osmotically and insoluble sugars to recover the damaging of drought on C- metabolism and regain sufficient dry matter above threshold to be translocated to grain production which enhanced greatly especially at severe drought. In line with the results of the present study, Crusciol



et al., [22] stated that there was a reduction in total soluble sugar concentrations in the treatment that received Si in potato tuber which was more marked in the presence of water deficit. They also highlighted that the yield of tuber benefit obtained under Si treatment resulted from enhanced tuber filling, probably as a consequence of greater production of photoassimilates, or due to changes in photoassimilates partitioning.

Plants also respond and adapt to water deficit at both cellular and molecular levels, for instance through accumulation of osmolytes and proteins specifically involved in stress tolerance [31]. With regard to protein fractions, one can say that insoluble proteins played an important role in differentiation the drought tolerance between the two studied cultivars. Because the drought tolerant cultivar Seds 12, not only maintained the insoluble proteins of stems and roots, but also accumulated that for leaves under drought stress, whilst the hypersensitive drought cultivar, cv. Sohag 3, inhibited the insoluble proteins in all of the tested organs and exhaust most of total proteins (inhibited dramatically under limited irrigation) towards soluble one (osmoregulation). With calculating soluble to insoluble proteins ratio, we found that this ratio folded multiple times in cv. Sohag 3 under drought stress as compared to control indicating disturbance of N- metabolism and high depletion of insoluble proteins in favors high increase in soluble one, consequently the increase soluble to insoluble proteins in cv. Sohag 3 is a state of sensitivity and down regulation of protein metabolism. This also may give an indication that production of soluble protein over the insoluble one could be at the expense of growth. This also gave another answer of the great question around the hypersensitivity that discriminated cv. Sohag 3. In this respect, Sultana et al., [32] reported that the production of soluble protein was at the expense of growth to be used osmotically. Interestingly, this interpretation went parallel to Pitman, [33] who declared that in some cases accumulation of solutes is so high that it goes beyond the limits of regulation of cytoplasmic content with associated impairment of growth. On the other hand, cv. Seds 12 limited the increase of soluble to insoluble proteins ratio compared to cv. Sohag 3 in stems and roots whilst leaves reduced this ratio. Such increase or decrease in soluble to insoluble proteins ratio of cv. Seds 12, did not result from triggering of any protein fractions at expense of the other but explained on basis of the ability of the cultivar to accumulate one fraction and maintenance the other. Thus the increase in soluble to insoluble proteins ratio in case of cv. Seds 12 is a state of great upregulation of proteins partitioning and tolerance mechanism. These results indicated that each organ of the same plant displayed distinct strategy in the soluble and insoluble protein fractions, different strategies among different plant organs could completed each other. Roots and stems accumulated the functional form of protein (soluble) to maintain osmoregulation, although leaves accumulated suitable amount of structural protein (insoluble one). Concomitantly, using soluble to insoluble proteins ratio as tolerance or sensitivity criterion is problematic and is not straight forward trait. The stable tolerance trait at least in our study is the stabilization of insoluble proteins (structural component). On regard to soluble protein fraction, one can say that it played an important role as osmoregulator substance especially for cv. Seds 12, but when the promotion of soluble proteins occurred at expense of insoluble one as in cv. Sohag 3 this is a stress marker and down-regulation of protein metabolism rather than tolerance mechanism. Thus insoluble proteins stabilization is a key factor enhancing the drought tolerance rather than soluble one. In this regard, Al-Jebory, [34] reported that plant growth under water deficit can be affected by changes in gene expression, leading to the synthesis and activation of novel proteins under water deficit conditions. It is believed that these stresses induced proteins allow plants to make biochemical and structural adjustments that enable plants to cope with the stress. Drought stress clearly increased the soluble proteins for various species as concurred with the studies of [30, 35, 36]. Silicon application increased total proteins and their fraction of both cultivars except for soluble proteins of cv. Sohag 3. The reduction of soluble proteins of cv. Sohag 3 greatly went parallel to the previous suggestion that the extraordinary elevated soluble proteins promotion for cv. Sohag 3 under Si- deprived condition was a stress marker, thus the used protectant mainly reduced them lower than control. Thus the machinery of turn over protein could be associated with cultivars and their plant organs on one hand and the mechanistic effect of exogenous application of Si from the other hand. Ahmad and Haddad, [37] indicated that Si partially offset the negative impacts of drought stress and increasing the tolerance of wheat by accumulation of protein content compared with the plants treated with drought. In consistency with reduction of soluble proteins of cv. Sohag 3 plants treated with silicon Crusciol et al., [22] observed that soluble protein concentrations were smaller in the treatment involving Si, especially under a water deficit condition. The reason for this reduction may be the breakdown of proteins to supply a carbon skeleton for proline synthesis.

As aforementioned, for osmotic adjustment plants for instance boosted soluble proteins and/or soluble carbohydrates under stress conditions based on the cultivar studied. In the current study, cv. Sohag 3 used soluble protein as the main metabolic product up- regulated osmotic adjustment under water scarcity. Otherwise, in silicon treated plants, osmotic adjustment was achieved mainly by soluble carbohydrates. Cv. Seds 12 used both soluble proteins and carbohydrates for accomplishing osmotic homeostats under Si- deprived plants and soluble proteins only for Si-treated plants

In addition to osmotic stress, one of the synchronous responses of plants to moisture stress is the generation of reactive oxygen species that induces oxidative stress and damages protein, membrane lipids, and other cellular



components and activated other biochemical reactions. In the current study, the data vastly declared the oxidative damage-limiting capacity of cv. Seds 12 under drought stress compared to cv. Sohag 3. In this respect, the production of H<sub>2</sub>O<sub>2</sub> is linear with its ability to attack membrane lipids and increases the content of MDA which has been considered as a suitable marker for membrane lipid peroxidation. All of this compromise cell membrane functions resulting in increased cell leakiness which found to be linked with the differences in drought tolerance between the two studied cultivars where H<sub>2</sub>O<sub>2</sub>, MDA and membrane leakage was more prominent for cv. Sohag 3 compared to cv. Seds 12. This was confirmed with the finding of Sharma et al., [35] who reported a decrease in membrane stability reflects the extent of lipid peroxidation caused by ROS. Alam et al., [38] reported that the lipid peroxidation levels under drought stress increased by 193, 189 and 31% and increased accumulation of H<sub>2</sub>O<sub>2</sub> by 57, 129 and 37% in Brassica napus, Brassica campestris and in Brassica juncea seedlings, respectively, compared to unstressed control seedlings. The significant increase of MDA and H<sub>2</sub>O<sub>2</sub> contents with drought stress progressed in the two wheat cultivars suggested drought stress caused oxidative damages in both two species, similarly as detected by [39, 40]. Abdul Razzaq et al., [17] stated that soil water deficit results in decrease in cell membrane stability index and high cell leakiness. Almeselmani et al., [4] showed that membrane integrity was conserved for tolerant compared to susceptible varieties. Siddiqui et al., [41] reported an increment in electrolyte leakage, MDA and H<sub>2</sub>O<sub>2</sub> with drought stress in faba bean plants and this increase was found to be dependent on the severity of drought stress. Behnamnia, [23] reported increase in electrolyte leakage in tomato.

The raised tolerance of cv. Seds 12 was furthermore ascertained from the ability to maintain cellular membrane integrity under mild and moderate drought levels suggesting that this cultivar may have an efficient free radical quenching system, which can maintain higher membrane stability and lower ROS under drought treatment. In the same line Zhang et al., [42] found that low MDA levels were associated with drought stress tolerance in wheat. Also, Simova-Stoilova et al., [43] reported a progressive decrease in  $H_2O_2$  content in four wheat varieties, when subjected to drought stress.

Another way to detect lipid peroxidation is determination of Lipoxygenase activity. It is worthy to mention that increased LOX activity is responsible for oxidation of polyunsaturated fatty acids and thus enhances lipid peroxidation under stress conditions [44]. Thus results of lipoxygenase activity and MDA levels provided evidence of higher lipid peroxidation of cv. Sohag 3 compared to cv. Seds 12. Concomitantly, the increase of lipoxygenase activity during the progression of water deficit suggested strict relationship of this enzyme with drought stress conditions and clearly used as stress marker to harsh conditions. Alam et al., [38] found differential accumulation of lipoxygenase activities in Brassica napus, Brassica campestris and Brassica juncea seedlings were increased by 217%, 51% and 135% respectively under drought stress, compared to non-stress control. Similar relationship of increased LOX activity and oxidative stress was observed in previous research findings [44, 39, 23]. Lipoxygenase catalyzes the dioxygenation of polyunsaturated fatty acids containing a cis, cis-1, 4-pentadiene backbone, producing hydroperoxy fatty acids, which are highly reactive compounds that are toxic to cells. These fatty acids are rapidly degraded into metabolites which lead to the production of jasmonates, conjugate dienoic acids and volatile aldehydes, such as malondialdehyde [45, 46]. This hypothesis was supported by MDA accumulation and high LOX activities as confirmed by Lima et al., [47] in Coffea canephora. They observed high degree of lipid peroxidation could produce lipid derivatives acting as secondary messengers capable to activate some drought-stress-associated genes by means of specific transcription factors, in such a way starting the response of plant to desiccation Shinozaki and Yamaguchi-Shinozaki, [48]. Jasmonic acid, another final product of hydro-peroxy fatty acids catabolism, can act as a regulatory molecule with growth-inhibitory properties, analogous to those of abscisic acid [45], and it may be responsible for reduced olive tree growth during drought-stress conditions [49]. These speculations greatly recommended with the data of drought plants under silicon nutrition where significant reduction of hydrogen peroxide, lipid peroxidation, electrolyte leakage and lipoxygenase activity which mirrored the powerful role of selenium and silicon in augmentation of drought stress of the studied cultivars under drought stress. Reduction of drought -induced oxidative damage due to application of silicon was reported by many researchers. Waraich et al., [50] reported an increase in the production of antioxidants and a decline of ROS generation mediated by silicon causes a reduction of photo-oxidative damage, maintenance of chloroplast membrane integrity and thus enhancement of plant drought tolerance. Gong et al., [51] showed that the intensity of oxidative destruction tested by the concentration of thiobarbituric acid reactive substances in the leaves of wheat was increased by drought, and there was a smaller increase upon application of silicon moreover, drought stress increased the LOX activity at both developmental stages and Si application decreased the LOX activity of drought stressed plants. Also, Gunes et al., [52] found that silicon applied to the soil reduced sunflower tissue H<sub>2</sub>O<sub>2</sub>. Kaya et al., [53] showed that 2 mM Na<sub>2</sub>SiO<sub>3</sub> decreased electrolyte leakage by 18.3% in 50% FC water-stressed corn. Liang et al., [54] concluded that using silicon leads to its deposition in cell membrane and certification and hardening, and significant reduction of electrolytic leakage. Also, Shen et al., [6] illustrated that silicon reduced osmolyte leakage and lipid peroxidation.



#### 5. CONCLUSION

According to our results we can conclude that the growth panorama at different stages ascertained the susceptibility of cv. Sohag 3 to water deficit and the tolerance of cv. Seds 12. Cv. Sohag 3 showed great disturbance in the Nmetabolism due to high reduction of total proteins and interconversion of total manufactured proteins towards soluble proteins at the expense of insoluble one especially for the water absorbing organ "roots" which need balance in their metabolites production to withstand harsh conditions. In addition to down regulation of C- metabolism as appeared from reduction of soluble, insoluble and total carbohydrates hence the osmotically active substances are limited to be used in osmoregulation or to be diverted towards grain filling especially at severe drought where the limited produced -photoassimilates directed only towards osmoregulation and failed completely to be translocated towards grains thus the cultivar produced spikes without grains. In many plants net accumulation of osmotically active solutes allows turgor dependent processes to continue to some extent under water stress conditions. Also, cell membranes of cv. Sohag 3 manifested high oxidative damage properties in terms of overproduction of H<sub>2</sub>O<sub>2</sub>, MDA, electrolyte leakage and lipoxygenase enzyme. On the other hand, cv. Seds 12 exhibited great up-regulation of metabolic processes that the cultivar not only maintained the structural proteins but also accumulated progressively the insoluble proteins over the control in the green factory of plants "leaves" to produce vigor leaves photosynthesize efficiently under stressful conditions. In addition, cv. Seds 12 produce efficient amount of photoassimilate (boost soluble, insoluble and total carbohydrates) that used in two ways in osmoregulation of plant under drought and the other way towards grain filling. Thus the cultivar copes with drought stress through over production of osmoprotectants. Cell membranes of cv. Seds 12 attenuated oxidative damage limiting capacity where cv. Seds 12 efficiently detoxified H<sub>2</sub>O<sub>2</sub> and limited electrolyte leakage, MDA and lipoxygenase enzyme production. Silicon greatly exhibited better growth under harsh conditions due to the ability to up- regulate C and N- metabolism of the studied cultivars. In addition to enhancing membrane integrity of the cultivars and reduced the membrane damage as measured on reducing H<sub>2</sub>O<sub>2</sub>, MDA, electrolyte leakage and lipoxygenase enzyme. Thus we recommended the application of silicon as potential protectant against drought stress.

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