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Article

Enhancing Drought Tolerance in Wheat through Improving Morpho-Physiological and Antioxidants Activities of Plants by the Supplementation of Foliar Silicon

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Abstract: The main objective of the research is to assess the role of foliar application of silicon (Si) for enhancing the survival ability of wheat under drought stress through improving its morphology, physicochemical and antioxidants activities. Treatments were five doses of Si at the rate of 2, 4, 6 and 8 mM and a control. After completion of seeds germination, pots were divided into four distinct groups at various field capacity (FC) levels, such as 100% FC (well-irrigated condition), 75% FC (slight water deficit), 50% FC (modest water deficit) and 25% FC (severe water deficit stress condition). Foliar application of Si at the rate of 2, 4, 6 and 8 mM and a control were given after 30 days of sowing at the tillering stage of wheat. Findings of the present investigation indicated that increasing the level of water deficit stress reduced the morphological parameters (such as root and shoot fresh and dry-biomass weight) and physico-biochemical events ((such as chlorophyll contents by estimating SPAD value), total free amino acid (TFAA), total soluble sugar (TSS), total soluble protein (TSP), total proline (TP), CAT (catalase), POD (peroxidase), SOD (superoxide dismutase) and APX (ascorbate peroxidase)) of wheat; while foliar application of Si at 6 mM at tillering stage enhanced the drought tolerance in wheat by increasing morphology and physiochemical characters under all levels of drought stress. Similarly, antioxidants activities in wheat also enhanced by the application of Si at 6 mM under normal as well as all drought stress levels. Therefore, it may be concluded that foliar application of Si at 6 mM at the tillering stage of wheat is an important indication for increasing the drought tolerance by improving the morphology, physico-biochemical and antioxidants activities in plants under deficit water (drought) conditions.

Keywords: Foliar silicon; drought tolerance; morphology; physiochemical; antioxidants; wheat

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Abbreviations

APX	ascorbate peroxidase
CAT	catalase
CRD	complete randomized block design
FC	field capacity
K	potassium
N	nitrogen
P	phosphorus
POD	peroxidase
Si	silicon
SPAD value	Leaf chlorophill contents
SOD	superoxide dismutase
TFAA	total free amino acid
TP	total proline
TSP	total soluble protein
TSS	total soluble sugar

1 Introduction

Bread wheat (*Triticum aestivum* L.) is considered as a main staple food for human consumption across the globe and also as a noble source of protein, dietary fiber, minerals, and vitamins B-complex, etc. than other cereals [1–4]. In all over the world, 21% of the food the people depend on cereal crops [5]. While similar to other cereals, the production of wheat in all over the world is not at a satisfactory level to encounter the food demand of growing population, due to the adverse effect of different stresses (i.e., biotic and abiotic) [6–10], it demand is growing day by day to meet the food security of increasing population [11,12].

Among them, water availability is a significant measurement for morphological, physiological and biochemical activities of plants, while the shortage of water, inhabit the cell expansion and ultimately reduced the total biomass finally reduced the yield of many plants including wheat [13]. EL Sabagh et al. [10] showed that drought-stress is one of the main threats that negatively affected the morphological, physiological and biochemical behaviors of plants than other abiotic stresses. Drought adversely deteriorated the plant metabolic process by affecting the photosynthesis, water relations of plant and also the uptake of nutrients [14]. Farooq et al. [15], revealed that water contents, as well as the water potential of leaf, are decreased when plants are facing water unavailability (drought stress).

There are two ways to mitigate the adverse effect of plants such as the development of stress-tolerant cultivars and to find out improved management strategies to mitigate the adverse effect of drought under changing climate. Among the most abundant trace elements available in the earth crust, Si is the second most important one [16], which plays a significant role in improving the survival capacity of plants under different environmental stress through alterations morphological, physio-biochemical activities (producing antioxidants) in plants [17,16]. Gong et al. [18] and Maghsoudi et al. [19], found that under drought stress the exogenous application of Si increased the drought tolerance in plants through enriching the leaf water contents in wheat as compared to the wheat plants those were grown in drought stress without foliar application of Si. Similarly, Chen et al. [20] found that under drought stress, the exogenous application of Si played a significant role for increasing the assimilation of higher photosynthesis. Lobato et al. [21], observed that foliar application of Si under drought stress increased different pigments contents in *Capsicum annuum* L. such as Chl. a, b and total carotenoids. The application of Si improves the structure of chloroplast and also enhanced the activities of different antioxidant enzymes such as CAT, POD, SOD and APX [22]. Hattori et al. [23] observed that when Si was applied exogenously in drought-affected plants, both water uptake capability and also antioxidants enzymatic and non-enzymatic defense activities were improved to increase the survival ability of plants under drought stress.

Drought (water deficit) is one the emerging threat worldwide and adversely affect the morpho-physiology and biochemical activity of plants finally lead to a decrease in the grain yield of wheat. In Pakistan, wheat is one of the major staple food but the average yield of wheat is not as reasonable as compared to major wheat growing countries in the world; it is due to wheat crops are facing the adverse effect from different abiotic stresses during the growth stage. Among them, drought stress is the most important one. Considering the above important circumstance and above-mentioned citation, it is the hypothesis that the foliar application of Si enhances the drought tolerance in wheat through improving the morphological physiological and biochemical activities of plants under drought conditions. Thus the foremost aim of the current investigation was to find out the role of foliar application of Si for enhancing the survival ability of plants under drought stress through improving its morphology, physicochemical and antioxidants activities.

2 Materials and Methods

A paper for publication can be subdivided into multiple sections: title, list of all the authors and their affiliations, a concise abstract, keywords, main text (including figures, equations, and tables), acknowledgement, references, and appendix.

2.1 Location of the Experiment

To find out the role of foliar application of Si for enhancing the survival ability of plants under drought stress a pot experiment was conducted in the Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur-Punjab-Pakistan.

2.2 Treatments, Design and Experimental Procedure

Treatments were five doses of Si at the rate of 2, 4, 6 and 8 mM and a control. After completion of germination, pots were divided into four distinct groups at various field capacity (FC) levels, such as 100% FC (well-irrigated condition), 75% FC (slight water deficit), 50% FC (modest water deficit) and 25% FC (severe water deficit stress condition). Foliar application of Si at the rate 2, 4, 6 and 8 mM and a control were given after 30 days of sowing. The latest wheat variety named 'Johar-2016' was used as experimental material and grown under the control condition. Before sowing, seeds of wheat variety 'Johar-2016' were sterilized then washed thrice with distilled water. Each pot was filled with 500 g sand, five seeds in each pot were sown. All sand-filled pots were organized in a complete randomized block design (CRD) in three replications. The pots having sand were saturated according to their FC after sowing of wheat seed. During seeds sowing, N (nitrogen), P (phosphorus) and K (potassium) based fertilizers were applied as recommended. The wheat variety was harvested after 40 days of germination period and use afterward for further analysis and also for the following data:

2.3 Data and Their Collection Procedure

2.3.1 Growth Characters

Morphological data i.e., root and shoot length were measured with measuring tape at the time of harvesting. At the same time root and shoot fresh and dry-biomass weight were also recorded with the help of an electric balance.

2.3.2 Measurement of Chlorophyll Contents (SPAD Value)

The total chlorophyll contents of the leaf were measured with the help of SPAD-502 meter as described details by [24].

2.3.3 Data on Biochemical Attributes and Their Analysis Procedure

Data on the total soluble protein was assessed according to the method as described by Bradford [25], while the total soluble sugars were determined as the method described by Irigoyen et al. [26]. The total free amino acid concentration in the leaf tissue was also measured according to the procedure of [27]. The method which is described by the Bates et al. [28] was followed for the measurement of proline from the plants' leaf.

2.3.4 Extraction of Antioxidant Enzymes

Concentrations of different antioxidants such as CAT, POD, SOD and APX were measured spectrophotometrically. A homogenized 1 g fresh leaf sample was taken. The sample was homogenized with 50 mM phosphate buffer with pH 7.0 and 1 mM DTT for the determination of antioxidants enzymes the method is described by Dixit et al. [29]. The CAT and POD were determined by using the way of Guardado Félix et al. [30], while the SOD and APX were determined by Cakmak [31] and Giannopolitis et al. [32].

2.4 Statistical Analysis

Data on morphological, physiological and biochemical were statistically analyzed using statistical package MSTAT-C [33]. Means of all parameters were separated by the LSD test (the least significant difference) at a 5% level of significance by using the Statistical Package R [34].

3 Results

3.1 Morphological Characters

Application of the growth stimulator *viz.*, Si supplementation significantly enhanced the shoot fresh weight (SFW) and dry weight (SDW) under water deficit conditions. The maximum SFW and SDW were obtained from the foliar application of Si at 6 mM in the wheat variety under all water deficit regimes (Fig. 1). Indicated that Si applied at 6 mM was more effective than other levels (Tab. 1).

Table 1: Analysis of variance table for the shoot-root fresh and dry weight of wheat in water deficit conditions with the supplementation of foliar silicon (Si)

Source of variation	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Treatments (T)	***	**	**	**
Stree level (S)	***	***	**	**
T × S	*	NS	NS	NS

*, **, *** = Significant at 0.05, 0.01 and 0.001 level respectively; NS = non significant.

However, the application of Si at 6 mM and 8 mM spray were increased in both root fresh (RFW) and dry weight (RDW) under different water deficit conditions. Maximum RFW was observed by the application of Si at 6 mM spray followed by Si applied at 8 mM. While minimum RFW and RDW were observed in non-sprayed (control) wheat genotype under all levels of water deficit conditions (Fig. 1).

3.2 Physio-Chemical Characters

Leaf chlorophyll contents (SPAD value) of wheat were significantly higher due to the application of different levels of Si at 2 mM, Si at 6 mM and Si at 8 mM (Tab. 2). The results also illustrated that the chlorophyll contents decreased under all water deficit regimes but after the foliar application of supplement of Si i.e., Si at 6 mM maintained their level in non-stress followed by mild, moderate and severe stress conditions.

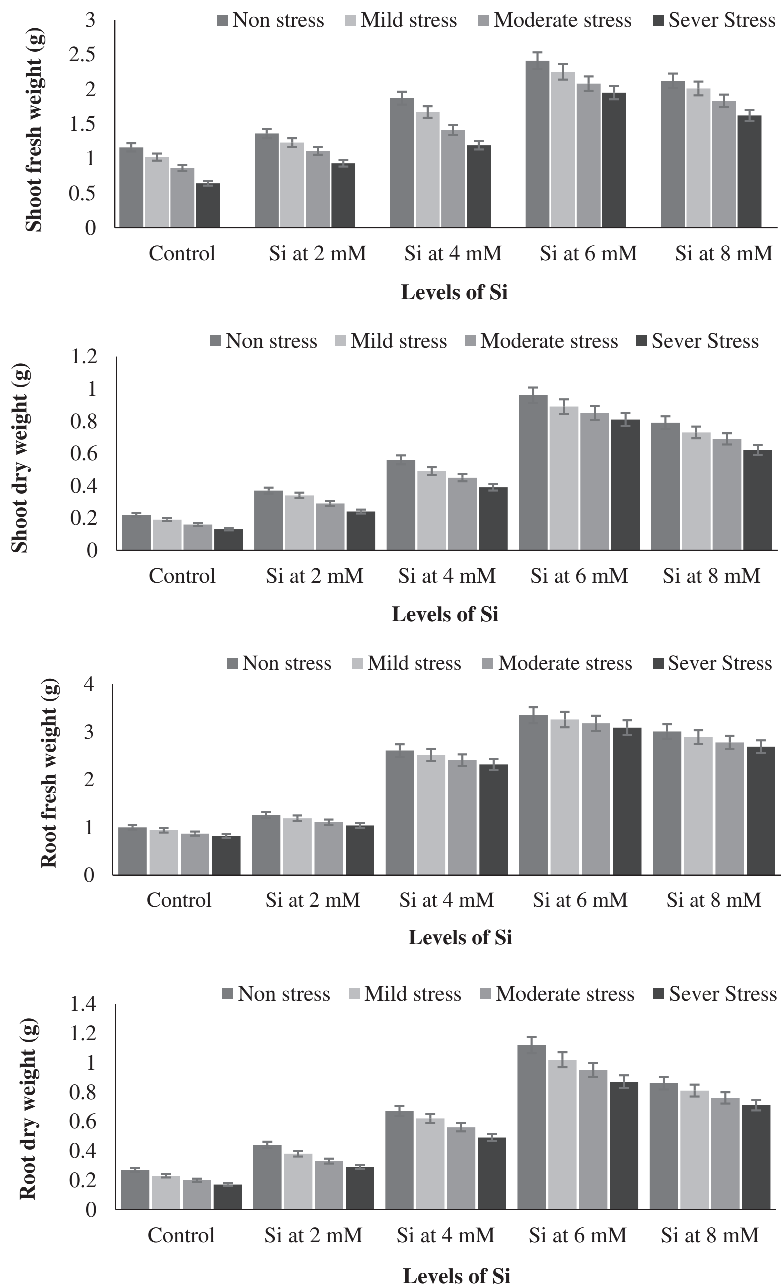

Figure 1: Supplementation of foliar Si effects on shoot-root fresh and dry weight of wheat in water deficit conditions (mean values ± SE)

Application Si at different levels increased the total free amino acid (TFAA) under severe water deficit conditions. When Si was applied at 6 mM, TFAA levels were increased under severe water deficit, moderate and mild water deficit condition.

Total proline (TP) contents were also significantly varied under different irrigation regimes and Si levels (Tab. 2). However, a reduced level of TP was observed under different water-deficit regimes, while the best collaborating effect between levels of water deficit (irrigation regimes) with exogenous application of Si was

Table 2: Analysis of variance table for total chlorophyll contents, total amino acids, proline, total proteins and total sugars of wheat water deficit conditions with the supplementation of foliar Si

Source of variation	Total chlorophyll content (SPAD value)	Total free aminoacids	Total proline	Total soluble proteins	Total soluble sugars
Treatments (T)	***	***	***	***	***
Stree level (S)	**	**	**	**	**
T × S	NS	NS	NS	NS	NS

*, **, *** = Significant at 0.05, 0.01 and 0.001 level respectively; NS = non significant.

observed when Si levels were increased (Data presented in Fig. 2). Among the levels of Si, the maximum TP contents were observed when Si was applied at 6 mM under control as well as mild water stress conditions.

In the case of total soluble protein (TSP), different Si levels and irrigation regimes were significantly exhibited (Tab. 2). However, under control irrigation and low level of applied Si showed the minimum production of TSP (Fig. 2), while the application of Si at 6 and 8 mM produced the maximum amount of total protein under control, mild, moderate and severe water deficit conditions.

Total soluble sugar (TSS) was found highly significant when different levels of Si supplement at the rate 2, 6 and 8 mM under different water deficit conditions. However, the maximum TSS was obtained when Si was applied at 6 mM under severe water deficit conditions as compared to other regimes (Fig. 2).

3.3 Antioxidant Activities

Data related to antioxidant activity in wheat genotype under water deficit conditions showed that different antioxidants were enhanced significantly. Among the different treatments, the application of Si at 6 and 8 mM influenced the maximum enzymatic activities i.e., APX, SOD, POD and CAT under control, mild, moderate and severe water deficit conditions (Fig. 3).

However, the interactive effect of both Si and water regimes exhibited significantly (Tab. 3). The maximum APX and SOD enzymes were produced when Si was applied at 6 mM under severe water deficit conditions. Similarly, POD and CAT enzymatic activities were also influenced due to the application of Si at 6 mM under all water deficit regimes (Fig. 3).

4 Discussion

The results of the current research indicated that drought stress caused the drastic effect on the growth of wheat plant due to their severe conditions while the foliar application of Si at 6 mM at vegetative stage increase the growth characters of wheat plant under all levels of drought stress such as mild stress, moderate stress, and severe stress conditions along with control. The present results supported by Ahmad et al. [13], who stated that the growth characters of plants under drought stress influenced positively when Si was applied exogenously. Similarly, Liang et al. [35] also confirmed that the growth and development of plants under drought stress improved significantly by the foliar application of Si. While Maghsoudi et al. [36] revealed that growth, as well as the chlorophyll contents in the leaf of the plants, were improved significantly through the foliar Si application which leads to enhancing the tolerance ability of plants under different stressful environments. Si induced the better growth and development of wheat (Gong et al. [18]) and rice (Chen et al. [20]) under drought stress that ultimately increased the yield of these crops.

The chlorophyll contents of the leaf of wheat decreased by increasing the water stress conditions, it was observed in the current observation, while Si applied as exogenously improved the chlorophyll contents in wheat under all water deficit conditions. Chlorophyll contents of the leaf are one of the main indicators in

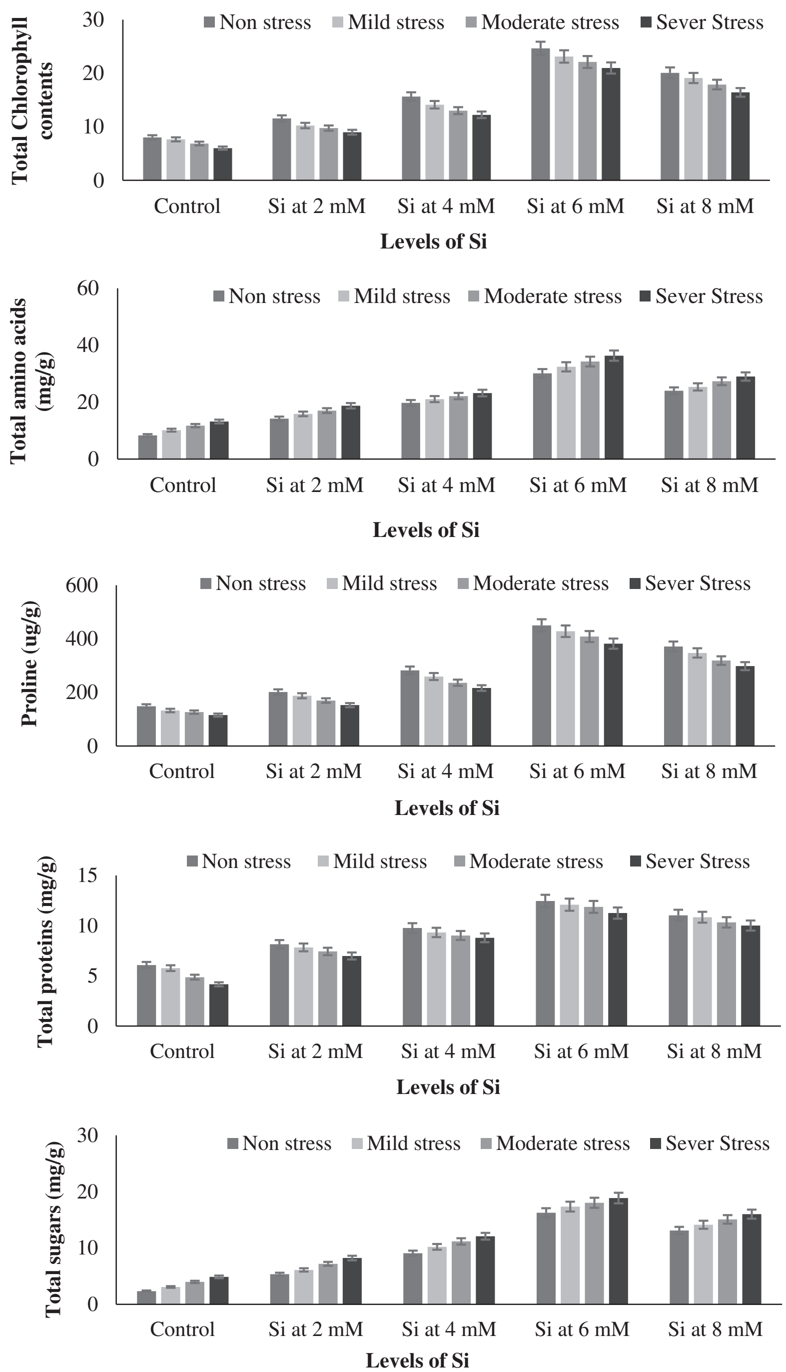

Figure 2: Supplementation of foliar Si effect on total chlorophyll contents, TFA, TP, TSP and TSS of wheat under water deficit conditions (mean values ± SE)

plants that are directly correlated with the photosynthesis rate of plants and also perform a significant role in the production of plant biomass [37]. Decreasing of chlorophyll contents in plant leaf in drought stress conditions as a result of the presence of enzymes that are responsible for degrading chlorophyll contents as well as the production of more chlorophyllase [37,38]. The Si application enhanced the chlorophyll contents of the leaf which are supported by Feng et al. [39], who stated that the chlorophyll contents of

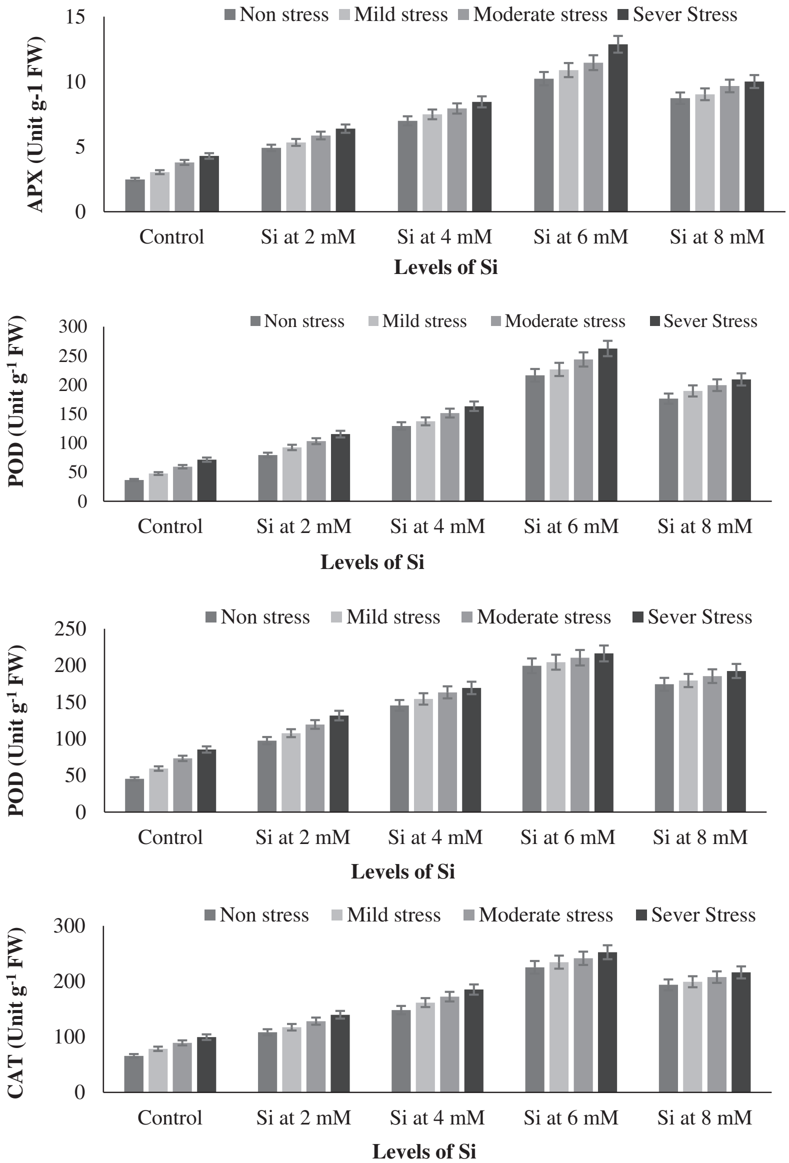

Figure 3: Supplementation of foliar Si effect on APX, SOD, POD and CAT of wheat under water deficit conditions (mean values ± SE)

Table 3: Analysis of variance table for APX, SOD, POD and CAT of wheat in water deficit conditions with the supplementation of foliar silicon (Si)

Source of variation	APX (Unit g^{-1} F.W)	SOD (Unit g^{-1} F.W)	POD (Unit g^{-1} F.W)	CAT (Unit g^{-1} F.W)
Treatments (T)	***	***	**	***
Stree level (S)	***	**	***	**
T × S	NS	NS	NS	NS

*, **, *** = Significant at 0.05, 0.01 and 0.001 level respectively; NS = non significant.

rice plants enhanced by the application of Si. The biochemical characters of the wheat plant at the tillering stage also boost down due to water deficit conditions while the foliar Si increases the biochemical characters such as TFAA, TSS, TSP and TP of wheat under all water stress condition which are presented in the current investigation. Similar results were also found in sunflower and chickpea, the results indicated that the foliar application Si enhanced the antioxidant activity such as proline under drought stress [40]. Proline plays an active for the adjustment of osmotic in plants under drought stress conditions. The TFAA, TSS and TSP concentration was also enhanced when Si was applied exogenously under drought. The physiological and biochemical characters of wheat were reduced under drought stress conditions [41].

Antioxidants enzymes viz., APX, SOD, POD, and CAT are produced under different environmental stresses (such as drought, salt etc.) for scavenging the activity of ROS in plants [11,42]. Similarly, the findings of the current research also showed that the foliar application of Si improved the activity of antioxidant enzymes under different water deficit conditions, while their concentration was decreased under well-watered condition.

5 Conclusion

Results of the current observation revealed that increasing level of drought stress reduced the morphology and physio-biochemical activities of wheat; while foliar application of Si at 6 mM at tillering stage enhanced the drought tolerance in wheat by increasing morphological (i.e., root and shoot fresh and dry-biomass weight) and physio-biochemical characters (such as SPAD value, TFAA, TSS, TSP, TP, CAT, POD, SOD and APX) under all levels of drought stress. Similarly, antioxidants activities in wheat also enhanced by the application of Si at 6 mM under normal as well as all drought stress levels. Therefore, it may be recommended that foliar application of Si at 6 mM at the tillering stage of wheat is an important indication for increasing the drought tolerance by improving the morphology, physicochemical and antioxidants activities in plants under drought stress.

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