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AMELIORATING DETRIMENTAL EFFECTS OF WATER DEFICIT STRESS IN MAIZE BY FOLIARALLY APPLIED SILICON AND CHITOSAN

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Abstract

Water deficit stress is the most devastating abiotic stress to limit crop productivity, particularly in regions of semiarid to arid climate. Different strategies have been used to improve plant resistance to water deficit stress, however, addition of organic and inorganic substances might be more effective and environment friendly. Present study investigated the protective role of silicon (Si) and chitosan against water deficit stress effects in maize (*Zea mays* L.). Experimental plan consisted of two soil moisture levels i.e. well-watered (100% field capacity, FC) and water deficit stress (50% FC), two levels of Si (0 and 2 mM) and two levels of chitosan (0 and 200 mg L^{-1}), and replicated thrice. Foliar application of Si and chitosan was made for three times i.e. 10, 20 and 30 days after germination. Water deficit stress reduced the shoot length by 30%, shoot fresh weight 46%, shoot dry weight 54%, and root length 40%, root fresh weight 54% and root dry weight 74% as compared to well-watered condition. Foliar applied Si and chitosan either alone or in combination stimulated the activities of antioxidant enzymes with a consequent improvement of 30% in chlorophyll-a and 40% in chlorophyll-b, 33% membrane stability index (MSI), 30% relative water content (RWC) and 46% soluble protein with Si+chitosan under water deficit stress compared to well-watered plants without Si+chitosan. In conclusion, foliar application of Si and chitosan was found to be effective for improving plant adaptation to water deficit stress, where maximum improvement was recorded with the integrated use of Si and chitosan.

Key words: Biochemical traits; Chitosan; Drought; Maize; Physiological processes; Silicon.

Introduction

Rapid increase in population growth, urbanization and recent climate variations are the big challenges and threating the world food security (Nadeem *et al.*, 2019). Water deficit stress is one of the most important stress factors to depress the productivity of agricultural system, particularly in regions with unreliable water resources. Daryanto *et al.*, (2016) reported more than 60% decline in crop yield due to water deficit depending upon many soil and plant factors. According to Lobell *et al.*, (2011), water deficit stress depresses the crop productivity by degrading photosynthetic pigments, photosystem structure and efficiency, nutrient metabolism, hormonal balance and enzyme activities. Water deficit stress drastically reduced the growth, turgor, water potential, adjustment of osmotic pressure, photosynthesis and stomatal conductance (Sheshbahreh *et al.*, 2019). Moreover, water deficit stress reduced the stability of cell membrane, chlorophyll contents, uptake of nutrients, while increase oxidative injuries in plants (Nadeem *et al.*, 2019).

In Pakistan, maize (*Zea mays* L.) ranks 3rd among cereals after wheat and rice. Globally, the total area under maize cultivation is approximated to 160 million hectares with 1147 million thousand tons production(Langner *et al.*, 2019). The maintenance of good maize productivity throughout the world, particularly in developing regions is severely threatened by various stress factors (Wang *et al.*, 2019). Water deficit stress is considered more important to affect maize productivity, probably it is mainly cultivated in semiarid areas like in Pakistan which have limited access to reliable water supply (Ali *et al.*, 2013). Major mechanisms responsible for a decline in maize growth and yield under water deficit stress might include reduction in root growth and leaf area, stomatal closure, damage of chlorophyll, photosynthetic efficiency, and cell metabolism (Sah *et al.*, 2020).

Plants adopt different strategies for improving water deficit stress resistance (Ullah *et al.*, 2016). In this respect, application of different organic and inorganic substances might have an important role to ameliorate water deficit stress effects in plants (Aziz *et al.*, 2018; Merwad *et al.*, 2018). Silicon (Si) and chitosan are known to regulate different plant growth, physiological and biochemical processes in stressed environment. These substances are very beneficial, easily available, easy to practice, environment friendly and deliver both economic and environmental efficacy (Farouk & EL-Metwally, 2019). The Si deposition in cuticle increases membrane rigidity which helps to maintain plant water contents by depressing transpirational losses, particularly under water deficit stress (Ashraf *et al.*, 2010). It has been found that sufficient amount of Si in plants can improve the mechanical strength, membrane stability, enzyme activities, water potential of leaf and photosynthesis, all these help to improve yield and yield related parameters in stressed environment (Kim *et al.*, 2017). Furthermore, Si can ameliorate the detrimental effects of oxidative damage in plants growing in stressed environment by improving antioxidant activities (Gong *et al.*, 2008). Gong & Chen (2012) reported that Si supplementation improved chlorophyll content and photosynthesis rate in wheat under water deficit, leading to improved plant productivity.

Chitosan, being having a role in plant physiology and biochemistry, can also help to improve plant resistance to various stress factors (Hidangmayum *et al.*, 2019). Farouk & Al-Sanoussi (2019) reported that chitosan might improve the plant morphological, physiological and biochemical parameters such as shoot length, biomass accumulation, leaf area, tiller productivity, chlorophyll and carotenoid content, photosynthesis, soluble carbohydrates, ascorbic acid and phenol contents, nitrogen, potassium and phosphorus accumulation in barley under water deficit stress. Although, past studies extensively investigated the contribution of Si to affect plant resistance in stress environment but information regarding the integrated use of Si and chitosan, particularly as foliar application for ameliorating water deficit stress effects in plants are lacking. In present study, Si and chitosan were applied foliarally to ameliorate the detrimental effects of water deficit stress on maize growth by stimulating antioxidant enzyme activities. The study was based on the hypothesis that integrated use of Si and chitosan would be more effective to improve maize growth than their individual application.

Materials and Methods

Experimental site was located between latitude 30°15'36" N; longitude 71°30'53" E and elevated 130 m from sea level. Under arid subtropical continental monsoon climate, the local area has a mean annual temperature of maximum 31.6°C, while minimum 16.9°C. The annual average rainfall recorded was 187 mm. The soil was collected from plough layer of a cultivated field under wheat-cotton cropping system. After air drying, soil was crushed, sieved using 2-mm sieve and analyzed for various characteristics according to standard procedures described by Richards (1954). Selected physico-chemical characteristics of experimental soil prior to experimentation are given in (Table 1).

The plastic pots having 30 cm internal diameter and 33 cm height were lined with polyethylene sheet and then filled with 12 kg soil pot^{-1}. In each pot, two healthy seeds of maize cultivar "Syngenta 8441" were sown but maintained one plant after germination. Recommended dose of fertilizers, nitrogen (250 kg N ha^{-1} as urea), phosphorus (125 kg P_2O_5 ha^{-1} as triple superphosphate) and potassium (125 kg K_2O ha^{-1} as potassium sulfate) were applied. Whole of P and K while ½ N fertilizers were applied at sowing, whereas the remaining ½ N 30 days after germination. To achieve 2 mM Si and 200 mg chitosan L^{-1}, the required amount of silicic acid and chitosan were dissolved in deionized water. Foliar spray of Si and chitosan was done for three times during the plant growth period i.e. 10, 20 and 30 days after germination. Experimental treatments including; (i) 100% FC, (ii) 100% FC+2 mM Si, (iii) 100% FC+200 mg chitosan L^{-1}, (iv) 100% FC+2 mM Si+200 mg chitosan L^{-1}, (v) 50% FC, (vi) 50% FC+2 mM Si, (vii) 50% FC+200 mg chitosan L^{-1}, and (viii) 50% FC+2 mM Si+200 mg chitosan L^{-1} were settled in accordance with completely randomized design (CRD) three factors factorial and replicated thrice.

Assay for chlorophyll content: Extraction method proposed by Strain & Svec (1966) was used for chlorophyll determination. Briefly, 1 g of fully fresh 3rd top most leaf (40-day old plants) was taken in 80% acetone for chlorophyll extraction. Chlorophyll "a" and "b" contents were determined at wavelength 663 and 644, respectively using spectrophotometer.

Relative water content: For the determination of relative water content (RWC), 3rd top most leaf from each pot was taken during 6th week after germination. After measuring fresh weight, the leaves were dipped in distilled water for four hours to measure turgid weight, and then oven dried at 80°C till constant weight. RWC were calculated according to following equation as described by Turner (1986);

$$\text{RWC}=\frac{\text{Fresh weight}-\text{dry weight}}{\text{Turgid weight}-\text{dry weight}}$$

Membrane stability index: Membrane stability index (MSI) was measured in accordance with the method described by Blum & Ebercon (1981). Fresh leaf samples of 0.2 g from 3rd top most leaf was taken during 6th week after germination in two sets of test tubes having deionized water. One test tubes set was placed in water bath for 30 minutes at 40°C, and other autoclaved at 120°C for 15 minutes. The electrical conductivity of 1st test tubes set was measured as C1 while 2nd as C2 using electrical conductivity meter. Following formula was used to measure MSI;

$$\text{MSI}=\frac{1-\text{C1}}{\text{C2}}\times 100$$

Table 1. Pre-sowing soil analysis of experimental site.

Soil properties	Units	Value	Reference
Textural class	--	Silt loam	Gee & Bauder (1986)
EC_c	dS m^{-1}	1.5	Bigham *et al*., (1996)
pH	--	7.16	Bigham *et al*., (1996)
Field capacity	%	24.5	Nachabe (1998)
Saturation percentage	%	29.85	Wilcox (1951)
SAR	(mmol L^{-1})$^{1/2}$	3.77	Richards (1954)
Organic matter	%	0.63	Nelson & Sommers (1982)
$CaCO_3$	%	13.4	Bouyoucos (1962)
CEC	Cmol (+) kg^{-1}	18.8	Thomas (1982)
Total N	%	0.19	Nelson & Sommers (1982)
Available P	mg kg^{-1}	5.14	Olsen *et al*., (1954)
Extractable K	mg kg^{-1}	113	Soltanpour & Workman (1979)

Assay for antioxidant activities: For the analysis of enzyme activities, plant tissues were extracted from 3rd top most leaf during 6th week after germination according to the method of Mukherjee & Choudhuri (1983). Fresh leaf sample of 0.5 g was frozen in liquid N and ground in 10 ml phosphate buffer (according to its pH). The mixture was centrifuged at 15,000 × g at 4°C for 10 minutes. CAT activity was measured in accordance with Aebi (1984), POD by Li (2000) and SOD by Zhang and Qu (2003). Total soluble protein contents were determined in accordance with Bradford (1976).

Element determination: Forty five day old plants were harvested. Growth attributes in term of shoot length, root length, shoot fresh weight and root fresh weight were recorded. After washing with distilled water, plant samples were dried in an oven at 70°C for 48 hours to record oven dry weight. For the determination of Si in plant, oven dried shoot samples was ground to 40 mesh, and digested by taking 0.1 g shoot sample in 50% NaOH and 50% H_2O_2 at 150°C for one hour (Elliott & Snyder, 1991). After developing color with acetic acid and ammonium molybdate, 2 ml oxalic acid was added, and finally sample was run on a spectrophotometer at 650 nm for determining Si concentration. For K and sodium (Na), plant samples were digested through wet digestion (Miller, 1998), and flamephotometer was used to measure the concentration.

Statistical analysis

The data were statistically analyzed using statistix 8.1, the analysis of variance test was performed according to experiment layout. Turkey-HSD test was used to differentiate between the significant treatments.

Results

Growth attributes: There was a significant ($p\leq0.05$) effect of water deficit stress, Si and chitosan on growth attributes of maize (Table 2). Water deficit stress (50% FC) reduced the shoot length by 30%, shoot fresh weight 46%, shoot dry weight 54%%, root length 40%%, root fresh weight 54% and root dry weight 74% as compared to well-watered condition (100% FC). Foliar applied Si and chitosan improved plant growth attributes at both soil moisture regimes, maximum improvement with the integrated use of Si and chitosan. It was found that integrated use of Si and chitosan improved shoot length by 21 and 30%, shoot fresh weight 36 and 69%, shoot dry weight 37 and 66%, root length 19 and 30%, root fresh weight 45 and 75% and root dry weight 49 and 65% at 100% FC and 50% FC, respectively compared to respective soil moisture regime without Si+chitosan.

Physiological characteristics: Water deficit stress decreased chlorophyll-a by 29%, chlorophyll-b 36%, MSI 29% and RWC 27% as compared to well-watered plants. Foliar applied Si and chitosan reversed the damaging effects of water deficit stress and improved the physiological characteristics, with maximum improvement by Si+chitosan (Table 3). It was found that foliar applied Si+chitosan improved chlorophyll-a by 11 and 30%, chlorophyll-b 18 and 40%, RWC 17 and 30% and MSI 17 and 33% at 100% FC and 50% FC, respectively as compared to respective soil moisture regime without Si+chitosan. Water deficit stress caused a reduction of 40% in soluble protein compared to well-watered plants. Foliar applied Si+chitosan improved soluble protein contents by 11 and 46% at 100% FC and 50% FC, respectively compared to respective soil moisture regime without Si+chitosan (Fig. 1).

Ionic concentration: Water deficit stress reduced the shoot concentration of K by 31% and Si 21% but increased Na by 41% compared to well-watered plants (Table 4). However, foliar applied Si and chitosan ameliorated the deleterious effects of water deficit stress on nutrient uptake, and improved K and Si while reduced Na concentration at both soil moisture regimes, with maximum change at 50% FC and by the integrated use of Si+chitosan. It was found that foliar applied Si+chitosan improved the concentration of K by 24 and 40% and Si 15 and 17% while reduced Na by 21 and 22% at 100% FC and 50% FC, respectively compared to respective soil moisture regime without Si+chitosan. There was a significant positive correlation of shoot K concentration with MSI (R^2 = 0.9277, Fig. 2) and RWC (R^2 = 0.8698, Fig. 3) when maize plants were grown under water deficit stress and provided with Si and chitosan.

Antioxidant enzyme activities: Water deficit stress caused a marked increase in SOD, POD and CAT activities as compared to well-watered plants. Foliar applied Si and chitosan further enhanced the activities of these antioxidant enzymes, maximum increment with Si+chitosan. SOD activity increased by 9%, POD 11% and CAT 10% under water deficit stress as compared to well-watered plants. Foliar applied Si+chitosan improved SOD activity by 37 and 43% (Fig. 4), POD 30 and 43% (Fig. 5) and CAT 42 and 47% (Fig. 6) at 100% FC and 50% FC, respectively as compared to respective soil moisture regime without Si+chitosan. Shoot Si concentration was positively correlated with SOD (R^2 = 0.8594, Fig. 7), POD (R^2 = 0.8638, Fig. 8) and CAT (R^2 = 0.7234, Fig. 9) activities in maize grown under water deficit stress in the presence of Si and chitosan.

Discussion

Marked decline in growth attributes of maize under water deficit stress was attributed to oxidative damage which disrupted the photosynthetic pigments, reduced photosynthesis, and resulted in lower biomass accumulation (Alghory & Yazar, 2019). According to Merwad *et al.*, (2018), water deficit stress adversely affected the morphological characteristic in term of leaf area, shoot fresh and dry weight, plant height and number of branches plant^{-1}. Water deficit stress also reduced the uptake of K while increased Na, leading to reduced plant growth characteristics. Showemimo & Olarewaju (2007) reported shedding of flowers and abortion of fruits in pepper under water deficit stress which subsequently reduced the plant growth and yield. According to Ullah *et al.*, (2016), drought stress badly affected the growth characteristics of tomato due to disturbance in water relations, photosynthesis and nutrient metabolism. Foliar applied Si and chitosan ameliorated the deleterious effects of water deficit stress on maize growth by maintaining plant water status, stabilizing membrane structure, improving the chlorophyll synthesis and stimulating antioxidant enzyme activities. Silicon and chitosan application improved protein synthesis which involved in osmotic adjustment, and thus improved plant water relations under water deficit stress. According to Gunes *et al.*, (2008), Si could improve plant resistance to water deficit stress by maintaining water and nutrient uptake which otherwise badly depressed. Dzung *et al.*, (2011) reported that chitosan supplementation under water deficit stress could increase the uptake of nutrients and synthesis of chlorophyll, improving plant growth characteristics of coffee.

Water deficit stress induced oxidative damage disrupted the chloroplast and disturbed the synthesis of chlorophyll. Water deficit stress also destabilized membrane structure because of increased oxidative damage as evidenced by reduced MSI under water deficit stress. Ahmadi *et al.*, (2010) reported that water deficit stress-induced oxidative damage could be the main reason of reduced chlorophyll content and MSI. Foliar application of Si and chitosan mitigated oxidative damage with the subsequent improvement in chlorophyll content, MSI, protein synthesis. Furthermore, Si and chitosan regulated stomatal movement, resulting in increased RWC in maize at 50% FC. Safoora *et al.*, (2018) also observed a marked increase in chlorophyll, MSI and RWC by Si and chitosan. Silicon-induced mechanisms for improving plant productivity under water deficit stress might include; increased RWC (Romero-Aranda *et al.*, 2006), increased MSI (Liang *et al.*, 2007), osmotic adjustment (Sonobe *et al.*, 2011), improved photosynthesis (Zuccarini, 2008), preservation of photosynthetic pigments and apparatus (Chutipaijit *et al.*, 2012), decreased transpiration rate (Gao *et al.*, 2006), greater K accumulation (Kaya *et al.*, 2006), and regulation the antioxidant system (Milne *et al.*, 2012). Hidangmayum *et al.*, (2019) reported that chitosan application could be a better option for improving plant growth in stressed environment, particularly under abiotic stresses.

Water deficit stress increased SOD, POD and CAT activities in maize but plants were still unable to manage the oxidative damage as evidenced by reduced MSI and increased chlorophyll damage. Foliar applied Si and chitosan enabled the plants to withstand water deficit stress by further increasing the activities of these antioxidant enzymes. Gong *et al.*, (2005) observed a marked increase in the activities of antioxidant enzymes in the wheat under drought stress. Farouk & Al-Sanoussi (2019) reported that application bio-stimulants could ameliorate oxidative stress in plants grown under drought stress by improving antioxidant activities. The higher protection against water deficit stress in case of integrated use of Si and chitosan indicated synergistic relationship between both substances for improving plant resistance to water deficit stress.

Table 2. Growth characteristics of maize grown at two soil moisture regimes by supplying silicon and chitosan through foliar application.

Treatment	Shoot length (cm)		Shoot fresh weight (g)		Shoot dry weight (g)	
	100% FC	50% FC	100% FC	50% FC	100% FC	50% FC
Control	41.9c	29.0f	16.4d	8.8h	6.5d	3.4h
Si	47.8b	37.9d	20.5b	12.7f	8.8b	4.7f
Chitosan	43.9c	34.0e	18.7c	10.8g	7.5c	3.9g
Si + Chitosan	51.0a	37.9d	22.5a	14.9e	10.4a	5.7e
	Root length (cm)		**Root fresh weight (g)**		**Root dry weight (g)**	
Control	19.4d	11.5h	8.9d	4.0h	1.5d	0.8f
Si	22.2b	14.0f	11.9b	5.9f	1.9b	1.2e
Chitosan	20.8c	13.1g	10.9c	4.9g	1.7c	1.1e
Si + Chitosan	23.1a	15.1e	13.0a	7.1e	2.2a	1.4d

Control: No Si or chitosan; Si: 2 mM Si; Chitosan: 200 mg chitosan L^{-1}; Si + Chitosan; 2 mM Si + 200 mg chitosan L^{-1}

Means with different letters are significantly different from each at 5% probability level

Table 3. Physiological characteristics of maize grown at two soil moisture regimes by supplying silicon and chitosan through foliar application.

Treatment	Chlorophyll-a (mg mL^{-1})		Chlorophyll-b (mg mL^{-1})		Membrane stability index (%)		Relative water content (%)	
	100% FC	50% FC	100% FC	50% FC	100% FC	50% FC	100% FC	50% FC
Control	1062c	743f	660.7c	422.3f	71.4c	50.5g	73.2c	52.8g
Si	1132ab	909d	730.3b	532.3e	77.5b	62.6e	79.7b	64.2e
Chitosan	1098bc	820e	692.6bc	481.6e	74.2bc	55.7f	77.3b	57.9f
Si + Chitosan	1188a	973d	783.3a	593.6d	83.9a	67.5d	86.1a	68.9

Control: No Si or chitosan; Si: 2 mM Si; Chitosan: 200 mg chitosan L^{-1}; Si + Chitosan; 2 mM Si + 200 mg chitosan L^{-1}

Means with different letters are significantly different from each at 5% probability level

Table 4. Shoot concentration of Na, K and Si in maize grown at two soil moisture regimes by supplying silicon and chitosan through foliar application.

Treatment	Na (mg g^{-1})		K (mg g^{-1})		Si (mg g^{-1})	
	100% FC	50% FC	100% FC	50% FC	100% FC	50% FC
Control	5.1e	7.2a	7.2c	4.9f	12.3b	9.7d
Si	4.2f	6.0c	8.4a	6.2d	13.7a	10.9c
Chitosan	4.8e	6.6b	7.9b	5.5e	12.8b	10.2d
Si + Chitosan	4.0f	5.6d	9.0a	7.0c	14.2a	11.4c

Control: No Si or chitosan; Si: 2 mM Si; Chitosan: 200 mg chitosan L^{-1}; Si + Chitosan; 2 mM Si + 200 mg chitosan L^{-1}
Means with different letters are significantly different from each at 5% probability level

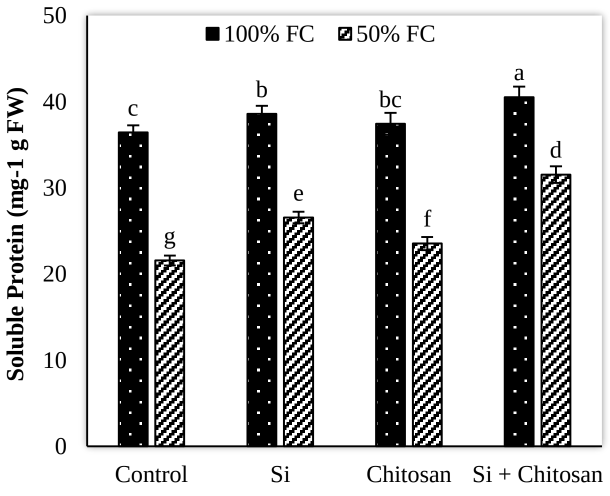

Fig. 1. Soluble protein contents in maize grown at two soil moisture regimes by supplying Si and chitosan through foliar application.

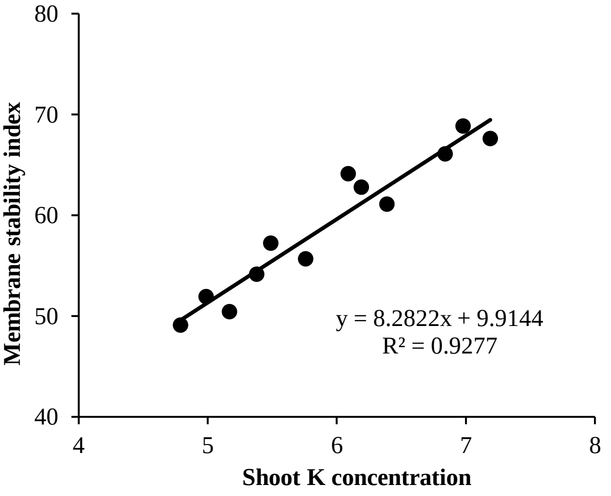

Fig. 2. Relationship of shoot K concentration with MSI in maize grown under water deficit stress by supplying Si and chitosan through foliar application.

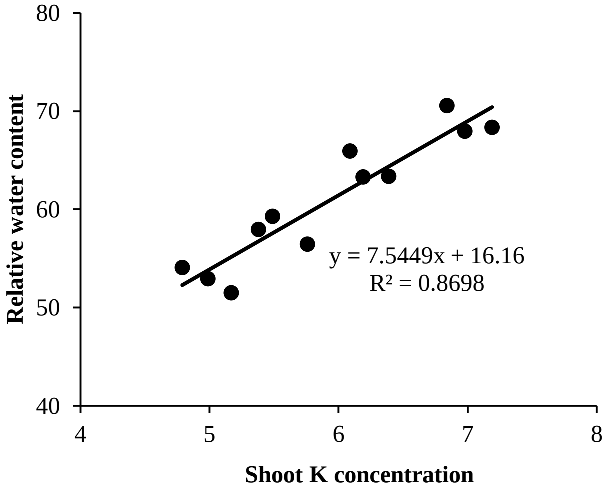

Fig. 3. Relationship of shoot K concentration with RWC in maize grown under water deficit stress by supplying Si and chitosan through foliar application.

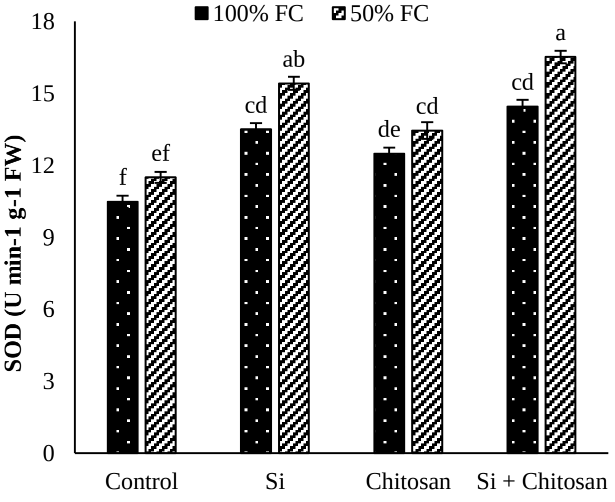

Fig. 4. SOD activity in maize grown at two soil moisture regimes by supplying Si and chitosan through foliar application.

Conclusions

Water deficit stress increased the oxidative damage which disrupted the membrane structure, chlorophyll and protein synthesis, leading to a marked decline in maize growth. Foliar applied Si and chitosan either alone or in combination improved antioxidant enzyme activities, chlorophyll and protein synthesis, RWC, MSI and K concentration, all these help to improve maize growth under water deficit stress, maximum improvement in case of Si+chitosan. The results suggested that integrated use of Si and chitosan could a promising approach for improving plant resistance to water deficit stress.

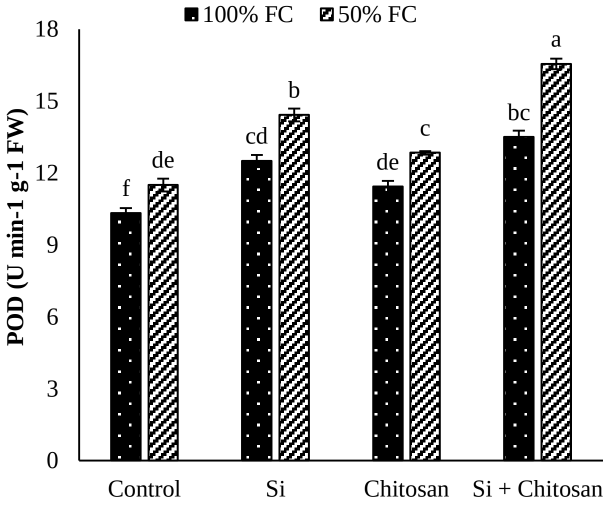

Fig. 5. POD activity in maize grown at two soil moisture regimes by supplying Si and chitosan through foliar application.

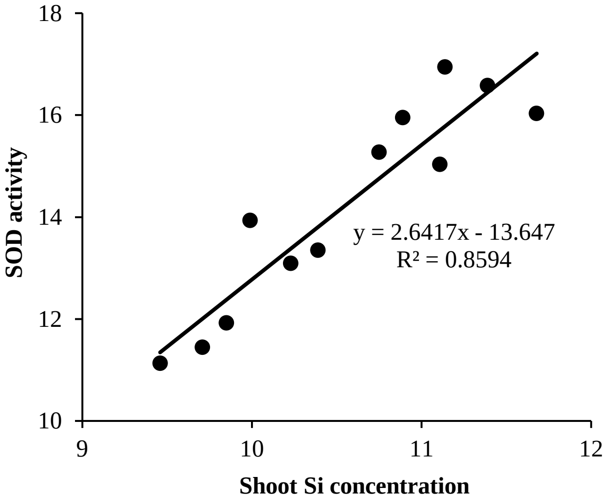

Fig. 7. Relationship of shoot Si concentration with SOD activity in maize grown under water deficit stress by supplying Si and chitosan through foliar application.

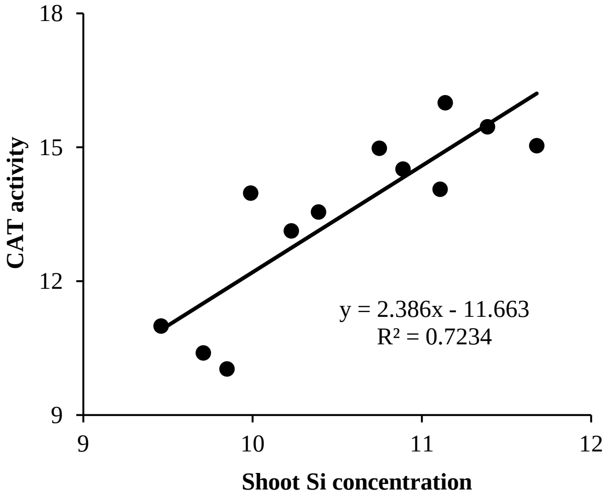

Fig. 9. Relationship of shoot Si concentration with CAT activity in maize grown under water deficit stress by supplying Si and chitosan through foliar application.

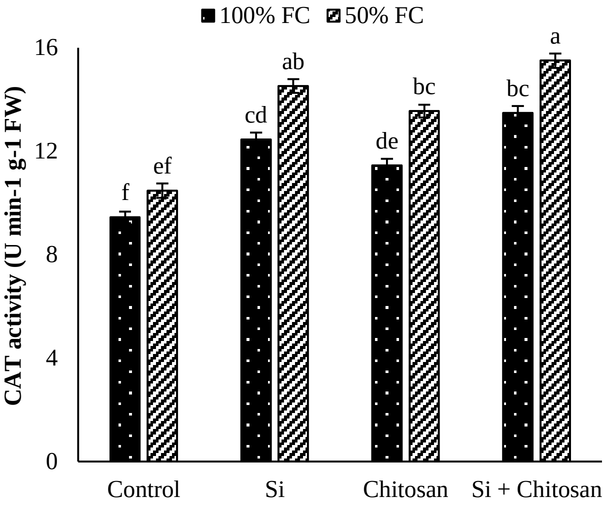

Fig. 6. CAT activity in maize grown at two soil moisture regimes by supplying Si and chitosan through foliar application.

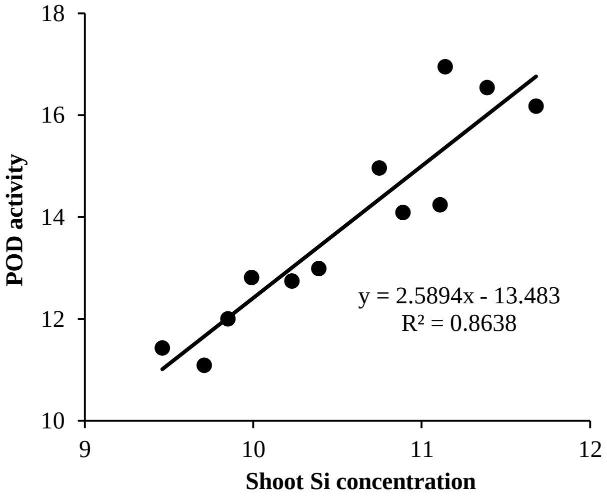

Fig. 8. Relationship of shoot Si concentration with POD activity in maize grown under water deficit stress by supplying Si and chitosan through foliar application.

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