Ameliorative role of Silicon on Osmoprotectants, Antioxidant Enzymes and Growth of Maize Grown Under Alkaline Stress

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Abstract

Purpose

Maize is grown under a wide spectrum of soil and climatic conditions. Maize is moderately sensitive to salt stress and response of maize under alkaline stress is scanty. Further, silicon is known to protect crops from abiotic stress. Hence, a pot experiment was conducted to study the mechanism on the effect of silicon on maize grown in alkaline stress

Methods

Maize CO 8 was grown in soil treated with four levels of alkaline stress (0, 25, 50, 75 mM) created through addition of sodium carbonate and three levels of silicon (0,100 and 150 kg ha^{-1}) applied to root through sodium meta silicate. The experiment was conducted in factorial CRD with three replications.

Results

Alkaline stress at graded levels caused reduction in growth (5 to 16%), dry weight (28 to 59%) and relative water content (5 to 23%). But electrolyte leakage (6 to 49%), proline (26 to 62%), phenol (8 to 44%), protein (6 to 19%), anti-oxidant systems viz., peroxidase (30 to 52%), SOD (4 to 16%) and catalase activities (32 to 127%) increased with increase in alkaline stress level. Soil application of silicon at different levels improved growth (5 to 10%) and dry weight (17 to 30%) of maize, relative water content (6 to 12%) and antioxidant enzymes (25 to 52%), water soluble protein (7 to10%), phenol (10 to 18%) while reduced electrolyte leakage(15 to 25%) and proline(17 to 29%).

Conclusions

It is evident from the study that root supplementation of silicon improved the growth and dry weight of maize crop grown in alkaline stress soil through its action on antioxidant systems and maximum effect was evident with 150 kg Si/ha

1. Introduction

In recent times, agricultural production in entire world has been confronted with change in climate and its variability [19]. The plant at every stage in its growth period is embraced by different abiotic and biotic stresses. Among stresses, salinization and alkalization are the major bottle necks to crop production.[23]. The major impacts that salt causes includes slowing down of physiological and metabolic processes, excess ions leading to toxicity, oxidative damage, hormonal and nutrient breach and osmotic stress [15&10]. The extreme salt stress can give rise to demolition of cell membrane, low nutrient absorption, generation of toxic substances and very low level of photosynthetic efficiency, all these culminating to reduced growth and finally abysmal crop productivity and sometimes even death [51 &5]. Alkaline stress employ same level of negative impacts as that of salinity, but greater repercussions is manifested when it is accompanied by high pH value[28]. The high pH value in soil alters the availability of nutrients in soil environment creating an imbalance among plant nutrients and thereby preventing the absorption of nutrients by roots. The erstwhile several studies have shown beyond doubt that alkaline stress has more deleterious effect on plants than salt stress. Alkaline stress accompanied by elevated pH value have choked to a greater extent on photosynthetic efficiency, carbohydrate and nitrogen metabolism, synthesis of amino acid and sugars in maize. The triggering action for the poor performance of crop and low yield in any stress environment has been the production of large quantity of reactive oxygen species (ROS) [20].The malfunctioning of electron transport and photorespiration pathways in chloroplast and mitochondria has been the root cause for excess production of ROS and it resulted in damage to chlorophyll and cell membrane [18]. Prevalence of osmotic balance is the key to stabilization of metabolic activity and cell turgor and in turn helped in growth and yield [46]. Maintenance of osmotic balance at cellular level is accomplished by production of number of osmolytes like proline, phenol, glycine, betaine and soluble sugar [43]. Proline production in the plant has been associated with plants stress tolerance and level of production of proline in plant is related to intensity of salt stress [37]. The proline acts ROS scavenger through antioxidant activity thereby it protects photosynthetic apparatus and proteins and thus ensures normal growth in saline soil [43]. The phenolic substance also exhibit antioxidant activity through non enzymatic action by preventing lipid peroxidation through trapping lipid alkoxyl and thus protect the plants from possible ROS damage [22]. Plants also protects itself from oxidative stress through production of antioxidant enzymes and they are produced in large quantities under stress condition. These enzymes include superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD). These enzymes exerts it influence under stress environment [68] by controlling ROS activity. The antioxidant enzymes help the plants to develop stress tolerance and avoid cell death [35]. The superoxide dismutase catalyzes the dismutation of superoxide O_2 to H_2O_2 and molecular oxygen. The plants with higher level of SOD activity in stress environment is a good indicator of stress tolerance [56]. Catalase contain heme group and all pervasive tetrameric enzyme which catalyze the breakdown of H_2O_2 to water and oxygen. Ascorbate peroxidase plays an important role of breakdown of H_2O_2 to water through utilization of two molecules of ascorbate producing monodehydroascorbate (MDHA). Water is critical to crop growth and in salt stress availability of water is limited causing reduced relative water content (RWC) hampering the growth of crop [59]. Salt stress also causes damage to cell membrane through electrolyte leakage. The membrane injury is attributed to decrease in membrane fluidity and loss of function due to lipid peroxidation [9].

It was observed that plant supplemented with silicon showed greater ability to protect itself from the negative impacts of salt stress. It was attributed to the involvement of silicon in numerous metabolic and physiological activities [6]. Silicon played a major role in the activities of antioxidant defense system in plant to protect from salt stress [38]. Silicon is the second most abundant element in lithosphere [16]. It is not considered as an essential element, although number of positive influence of silicon on agriculture is documented across the globe. At present it is considered as quassi and anti-stress element. Silicon excels its role under stress conditions and provide tolerance to plants [26].Silicon is a multifaceted element when it imparts salt tolerance to plants through enhanced growth and biomass, maintenance of nutrient balance, forming structural rigidity, escalate photosynthetic efficiency, sustaining ion homeostasis, triggering anti oxidative system in plants, promoting specific secondary metabolites that are related to stress tolerance. [34

& 54]. Number of early research workers have shown that silicon applied to root medium at various rates through different sources in different crops promoted antioxidant enzymes, various osmoprotectants and membrane stability [34 & 54]. Maize is a major cereal crop next to rice and wheat in the world. Maize is a major silicon accumulating crop and are grown in diversified environment. In order to assess the performance of maize when grown under alkaline stress, a pot experiment was initiated to unravel the mechanism of silicon nutrition in protecting maize crop grown in stress.

2. Materials & Methods

2.1 Experimental set up

The pot experiment was conducted in pot culture yard located in the vicinity of Department of Soil Science and Agriculture Chemistry, Faculty of Agriculture, Annamalai University. The experimental area is geographically situated at latitude 11•24'N and of longitude of 79•44'E and at an altitude of + 5.79 m above mean sea level. Bulk soil samples of 0–15 cm were collected from the university experimental farm. The experiment soil is clay loam in texture belong to Kondal series (Typic Haplusterts). The chemical characteristic of soil was pH- 8.3, EC- 0.67dSm^{-1}, SOC- 5.2gkg^{-1}, CEC- 32.5 Cmol (+) kg^{-1}, $KMnO_4$-N- 265 kg ha^{-1}, Olsen-P- 21.5 kg ha^{-1}, NH_4OAc-K- 196.5 kg ha^{-1} and available silicon- 37.9 mg kg^{-1}

2.2 Treatment and experimental design

There were three levels of silicon (0, 100 and 150 kg ha^{-1}) and four levels of alkaline stress (0, 25, 50 and 75 mM). There were 12 treatment combinations replicated thrice. The silicon source: sodium metasilicates ($Na_2O_3Si.5H_2O$) and alkaline stress source: sodium carbonate The 36(4 x 3 x 3) pots were arranged in completely randomized design in factorial arrangement.

2.3. Pot culture preparation and planting

Ten kg of processed bulk soil samples was transferred to thirty-six pots. Seeds of maize (*Zea mays* L. cv CO 8) were surface-sterilized with mercuric chloride (0.1%) for 5 min, and then rinsed three times with distilled water. Calculated quantity of silicon through sodium metasilicates as per the treatments were applied to the soil. The seeds were sown in pots (five seeds/pot). at the time of sowing, the seeds were irrigated at field capacity with various alkaline salt concentrations of 0 (control), 25, 50, and 75 mM Na_2CO_3 with each pot receiving 400 ml of a designated salt solution. The Na_2CO_3 concentrations used were equivalent to 0 (control), 0.528, 1.056, and 1.584 g Na_2CO_3 kg^{-1} soil, respectively. Leaching was avoided by maintaining soil water below field capacity at all times. The pots were then irrigated at field capacity with normal water through the whole experimental period. Thinning of maize plant was done to maintain two plants throughout the experiment. The duration of the trial was up to vegetative stage. Recommended dose of fertilizers (150:75:75 kgs of N, P_2O_5, K_2O /ha) through urea superphosphate and muriate of potash was applied uniformly to all the pots as solution culture.

2.4. Data collection and plant analysis

At the end of vegetative stage, height and dry weight of maize crop was recorded. Maize leaf was used to record relative water content, electrolyte leakage, proline, phenol, protein an anti-oxidant enzymes. The proline contents were determined by adopting the method of [12]. Soluble protein [39], SOD activity [13], POD activity [41], CAT activity [27], relative water content [31] and electrolyte leakage [40].

2.5 Statistical Analyses

The data was subjected to statistical analysis to get meaningful explanation for the variability obtained for various characters due to treatments following [24]. Regression analysis and correlation was worked out to find out the selective variation between variables

3. Results

3.1 Plant height and dry weight

Graded dose of silicon and alkaline stress levels ($P < 0.05$) had significant effect on plant height and dry weight of maize (Figs. 1 and 2). The maize crop experienced inimical effect on plant height as it experienced alkaline stress. The percent reduction in height ranged from 5.0 (25 mM) to 16.72 (75 mM). At all varying levels of alkaline stress, height of maize increased progressively with soil application of silicon and maximum height observed with 150 kg Si/ha in non-stressed soil (58.9 cm). The percent improvement ranged from 5 to 10%. Even in non -stressed soil, addition of graded concentration of silicon caused increase in shoot growth of maize and it was tune of 6.5 to 12.6% over control. The dry weight of maize showed progressively decline with increasing level of alkali stress. The percent reduction in dry weight ranged from 26.8 (25 mM) to 58.0 (75 mM). At all alkali stress levels, soil application of silicon significantly improved dry weight. The maximum shoot dry weight was associated with 150 kg Si/ha. The percent improvement in dry weight ranged from 16.6 to 29.9.

3.2. Relative water content

Main effects of alkaline stress and silicon dose and interplay between them had significant influence on relative water content (RWC) in maize leaves (Fig. 3). As expected, adverse effect occurred on relative water content in maize leaves when they were grown in alkali stress soil as obtained in non-stress soil (control). The relative water content showed linear drop when maize crop was grown in soil containing alkali stress ranging from 25 to 75mM. The percent reduction ranged from 5.0 to 23.1%. The relative water content in maize leaves improved when grown in silicon fed soil containing varying level of alkali stress. The percent improvement ranged from 6.9 to 12.12.

3.3. Electrolyte leakage

Interactive effects between alkaline stress and silicon dose were significant ($p < 0.05$) with regard to electrolyte leakage (EL) in maize leaves (Fig. 4). Generally, electrolyte leakage increased with alkali stress and decreased with Si levels. The percent increase in electrolyte leakage ranged from 6.3 (25 mM) to 49.1 (75mM). The least electrolyte leakage occurred under 0 mM alkali stress level and decreased significantly with increasing Si levels. The percent reduction in electrolyte leakage ranged from 14.8 to 25.0 with silicon levels.

3.4. Osmoregulators

Distinctive effects of silicon soil application and alkali stress was observed on proline, protein and phenol content in maize leaves compared to no silicon stress free soil (table1). Proline content in maize leaves increased linearly with concomitant increase in alkali stress level. It ranged from 16.9 (non-stress soil) to 27.4 μM/g tissue (stress soil). The percent increase in proline ranged from 26.6 to 62.1. At all alkali stress levels, proline accumulation decreased with silicon doses. It decreased from 26.4 to 18.9 μM/g tissue. The percent reduction in proline in maize leaves ranged from 18.9 to 28.4.

The phenol content showed an increasing trend as alkaline stress increased and ranged from 39.9 (non-stressed soil) to 57.5 μg/g (75 mM alkaline stress). The percent increase in phenol content ranged from 8.0 (25mM) to 44.1 (75 mM). At all stress levels, proline content made further upward progression with silicon levels. The phenol content varied from 43.1 to 50.9 μg/g. The percent increase in phenol content over control ranged from 10.4 to 18.1.

Alkali stress markedly improved on protein content in maize leaves over normal soil and non-silicon applied soil. In leaves of alkali stressed maize plants, protein content enhanced and it ranged from 26.3 to 31.5 mg/g DW. The percent improvement in protein content due to alkali stress ranged from 6.8 to 19.8. At all alkali stress levels, protein content increased with silicon levels. The value ranged from 27.5 to 30.2 mg/g DW. The impact was to the extent of 6.9 to 9.8 %.

3.5. Antioxidant enzyme activity

The ANOVA revealed a significant interaction ($P < 0.05$) between alkali stress and Si was observed on the activities of SOD, CAT, and POD in the leaves of maize (Fig. 7). SOD activity in maize leaves increased linearly with concomitant increase in alkali stress level. It ranged from 0.345 U mg^{-1} FW (non-stress soil) to 0.400 U mg^{-1} FW (stress soil). The percent increase in SOD activity ranged from 4.3 to 15.9. At all alkali levels, SOD activity increased with silicon concentration. It increased from 0.340 to 0.410 U mg^{-1} FW. The percent increase in SOD activity in maize leaves ranged from 8.8 to 20.6. Even in non-stressed soil, the SOD activity increased from 7.4 to 10.4% due to silicon fertilization over no silicon. As the alkali stress levels increased peroxidase activity in maize leaves increased. The peroxidase activity increased from 27.6 units' mg^{-1} protein. (Non-stress soil) to 42.0 units mg^{-1} protein (75 mM). The percent increase in peroxidase activity ranged from 30.1 (25 mM) to 52.2 (75mM). At all alkali stress levels, peroxidase activity increased from 32.9 (non-silicon) to 39.7 units mg^{-1} protein (150 kg Si/ha). The percent increase in peroxidase activity ranged from 10.3 to 20.7 with silicon levels.

Alkaline stress created by different concentration of sodium carbonate created an increase in catalase activity in maize leaf and it ranged from 6.3 to 14.3 U mg^{-1} FW. The per cent increase in catalase activity ranged from 31.7 to 126.9. At all alkali stress level, catalase activity improved further with silicon fertilization. The percent increase in catalase activity ranged from 24.7 to 49.4.

4. Discussion

As predictable, negative effects occurred for maize crop when grown in alkaline condition as compared to non- alkaline soil. The short stature of maize plant is associated with break in supply of nutrient and water for normal metabolic process on account of perturbation in stomatal functioning and root architecture [42]. The reduction in the growth of rapeseed cultivars grown in salt-stress conditions was associated with a reduction in the RWC and an increase in electrolyte leakage [30]. In the present study, it was observed reduction in RWC and increased EL in alkaline stress soil. There was negative correlation between electrolyte leakage with plant height (r= -0.897**). Decrease in plant height due to alkaline stress as perceived in the present study was in agreement with earlier workers [50]. Silicon interventions as soil application added to alkaline stress soil overcame the negative effect and it resulted in increase in plant height with silicon levels and maximum effect was noticed with 150 kg Si/ha. The positive effect of silicon could be due to increased cell division, cell elongation and also deposition of silicon in plant tissue causing erectness of leaf and stem. [21] observed increase in plant height of wheat with silicon under drought stress.

The reduced dry weight of maize with alkali stress levels is associated with water stress, ion toxicity, nutritional disorder, oxidation stress, membrane disorganization, reduction in cell division and expansion [42 & 47]. Decrease in plant biomass due to salt stress was reported [40]. This was confirmed by significant negative correlation between electrolyte leakage with dry weight (r = 0.830**). Addition of silicon to soil experiencing alkaline stress improved shoot dry weight. Improved relative water content, reduced electrolyte leakage, improved anti-oxidant systems on addition of silicon was noticed in the present study which would have increased shoot dry weight. Significant improvement in shoot dry weight of maize by silicon in salt stress was reported earlier by several workers [33].

Relative water content (RWC) in leaves is known as an alternative measure of plant water status reflecting the metabolic activity in tissues. The curtailment in relative water content under alkaline stress is mainly attributed to osmotic stress that is posed by high pH environment. This in turn generate accumulation of proline which causes water deficit condition, and as a consequence slows down water uptake. Strong linear regressive model of the increasing alkaline concentration (independently variable) and the decrease of RWC in leaf (r^2 = 0.9766) as dependent variable was noticed in the present study (Fig. 5). Exogenous silicon application triggered RWC recovery in alkaline stresses plant. [36] found that silicon promoted root growth and root hydraulic conductance thereby increasing root water uptake and further improving leaf water content by regulating the activity of aquaporins under salt stress. Improved RWC might be because of the deposition of silicon as silicate crystals in epidermis tissues as this constitutes an obstacle to water transpiration through stomata and cuticle helping to reduce alkalinity [32]. The present outcome was corroborated by earlier researchers [3]. This was confirmed by a strong linear relationship between silicon levels with RWC (r^2 = 0.9895**) (Fig. 5)

Electrolyte leakage is widely used as a test for the stress-induced injury of plant tissues and a measure of plant stress tolerance. Alkali stress at various concentration increased electrolyte leakage in maize leaf compared to non-stressed soil. It is mainly caused by the efflux of K^+ and so-called counter ions (Cl^-, HPO_4^{2-}, NO_3^-, citrate^{3-}, malate^{2-}) that move to balance the efflux of positively charged potassium ions [11]. Strong linear regressive model of the increasing alkaline concentration (independently variable) and the increase of EL in leaf (r^2 = 0.9546) as dependent variable was noticed in the present study (Fig. 5). Application of silicon reduced electrolyte leakage .This may be explained by the fact that Si has the ability to maintain cells by improving the permeability of their plasma membranes, which improves access into the cell by antioxidative enzymes [4]. This was supported by significant positive linear relationship observed between silicon levels and EL (r^2 = 0.9926**) (Fig. 5)

Amidst all stresses adaptation, osmotic adjustment is a part of salt stress forbearance mechanism to offset the loss of turgor by increasing and maintaining higher amount of intercellular compatible solutes in the cystol and vacuole [23]. Proline is one of the key osmolytes contributing to osmotic adjustment. Maize plant recorded a higher proline concentration when it was grown in alkali stress soil. In durum wheat seedlings proline can contribute for more than 39% of the osmotic adjustment in the cytoplasmic compartments of old leaves [17]. Production of proline in plant under stress environment is either associated with protein biosynthesis genes (P5CS, P5CR) or repression of the genes of its degradation pathway (PDH silencing) [44]. Addition of silicon reduced proline content in maize leaf grown in alkali stress soil. Si may provide a protective role helping to prevent lipid peroxidation induced by NaCl, because of this, proline content was significantly lower in the Si-treated maize seedlings under salt stress than those under salt stress without Si treatment [45]. [26] confirmed the present findings that addition of silica reduced the proline content and increases protein content in soybean to counter balance the salt stress.

Polyphenol cmpounds lend a hand in plant protection against ROS and it is produced in large quantity, whenever aerobic respiration or photosynthetic metabolism are disabled by environment stress [14]. The increase in phenolic contents in different plant tissues under increasing salinity has also been reported in a number of plants [49]. The present work also reports the increase in phenol content in maize plant grown in alkali stress soil. Silicon application improved phenol content in maize leaf grown in alkali stress. [29] reported 36% increase in phenol due to silicon in barley.

Maize leaf recorded more soluble protein grown in alkali stress compared to non –stressed soil Plant experiencing stress normally pile up small molecules mass protein which is used as storage nitrogen that could be mobilized after stress relief [58]. Additionally, these proteins could also have a role in osmotic adjustment [8]. [7] reported increase in soluble protein in maize genotypes grown in salt stress soil. The exogenous application of silicon improved soluble protein because silicon has pivotal role in binding amino acids to form specific proteins [53]. Silicon is actively engaged in the formation of DNA and functioning of mRNA [1]. [2] reported application of potassium silicate improved soluble protein. Furthermore, through the regression test, it showed that alkaline levels and silicon doses determined significantly the levels of proline (r^2 = 0.9791**, 1**), phenol (r^2 = 0.9502**, 0.9897**) and protein (r^2 = 0.9883**, 0.9983**), respectively (Fig. 6)

Antioxidant protective enzymes exist in higher plants under different growth conditions. In plants antioxidant enzymes mainly include catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) and its activity triggered by the increase in the production of ROS or the measured activity might be protective mechanism adopted by maize against oxidative damage [6] SOD, POD and CAT activities in maize leaves increased linearly with concomitant increase in alkali stress level. SOD is the probably the key enzyme to defend against toxic ROS [55]. POD catalysed the reaction between H_2O_2 and ROOH to H_2O and ROH ameliorated cell damage which can inhibit the Calvin Cycle [58]. Catalase is a principal enzyme that scavenges active oxygen species and prevents lipid peroxidation, cell membrane damage and chlorophyll degradation. CAT controls H_2O_2 level in plant cells and participates in the photosynthetic process. The significant positive correlation between the SOD and POD (r = 0.8736**), SOD and CAT (r = 0.8908**) and CAT and POD (r = 0.9446**) observed in this study suggested a synergistic effect of POD, CAT and SOD in resistance to alkali stress. This is similar to [57], who reported that the defence capability of antioxidant enzymes depended on cooperative actions of enzymes. Silicon interventions through soil application improved SOD, POD and CAT enzymes activities in maize leaf grown in alkali stress soil. [25] reported that Si application strengthens the antioxidant defence system and maintains normal physiological processes. [52] explained that Si enhanced the activity of antioxidative enzymes and reduced plasma membrane permeability. [48] observed that addition of silicon improved antioxidant enzymes in wheat seedling grown in salt stress.

5. Conclusion

It is concluded from the study that silicon has ameliorative role through antioxidant mechanism in supporting the maize crop growth grown in alkali stress soil and application of silicon at 150 kg ha^{-1} has been proven successful in mitigating the ill effects of alkali stress.

Declarations

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Conflicts of interest/Competing interests: There is no conflict of interest among the authors

Availability of data and material: The data generated are original and are available for access

Code availability: not applicable

Authors' contributions: Gokula Priya conducted the experiment and analysis, M.V.Sriramachandrasekharan developed the concept and wrote the first draft, Manivannan and Arumugam Shakila reworked on the manuscript and final manuscript prepared by all.

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Tables

Table 1. Effect of soil application of silicon and alkalinity levels on osmoregulators in maize leaf

Alkaline levels (mM)	Proline content (µM/g tissue)				Phenol content (µg/g)				Protein content (mg/g DW)			
	Silicon levels(kg/ha)				Silicon levels(kg/ha)				Silicon levels(kg/ha)			
	0	100	150	Mean	0	100	150	Mean	0	100	150	Mean
0	19.8	16.7	14.3	16.9	36.7	40.1	42.8	39.9	25.0	26.8	27.2	26.3
25	22.7	21.2	20.4	21.4	40.7	43.5	45.2	43.1	26.7	28.2	29.3	28.1
50	29.5	21.7	18.6	23.3	45.2	48.0	52.1	48.4	28.6	30.7	31.5	30.3
75	33.7	26.1	22.4	27.4	49.9	58.8	63.7	57.5	29.7	31.9	32.9	31.5
Mean	26.4	21.4	18.9		43.1	47.6	50.9		27.5	29.4	30.2	
	Si	Al	Si x Al		Si	Al	Si x Al		Si	Al	Si x Al	
SE_d	0.2	0.2	0.3		0.2	0.2	0.3		0.2	0.2	0.3	
CD@5%	0.3	0.4	0.7		0.4	0.4	0.7		0.3	0.4	0.7	

Figures

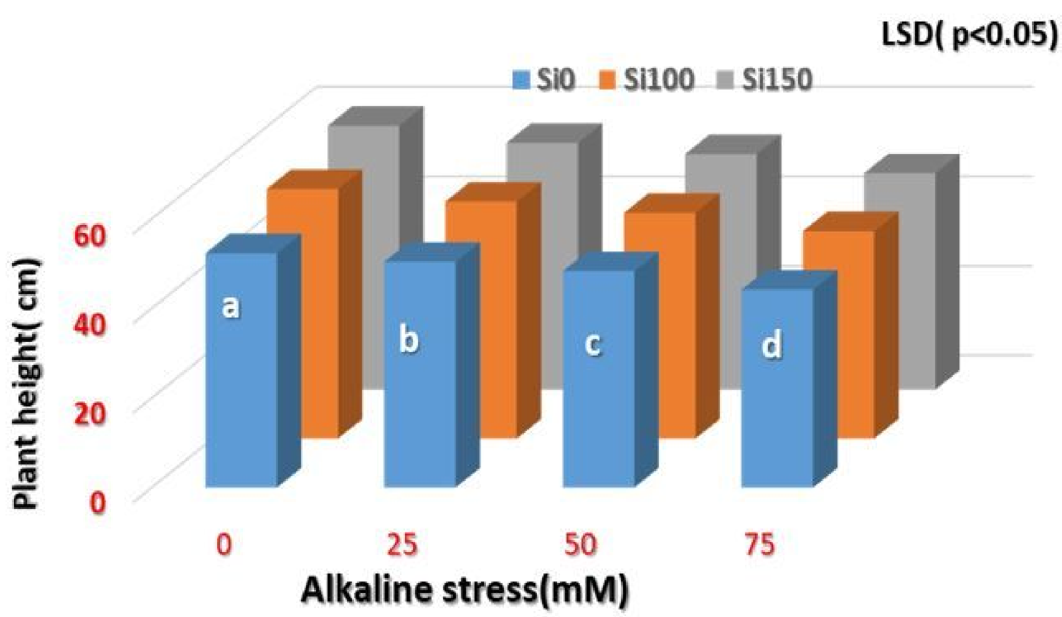

Figure 1

Effect of alkaline stress and silicon levels on plant height of maize values marked by different letter differ significantly

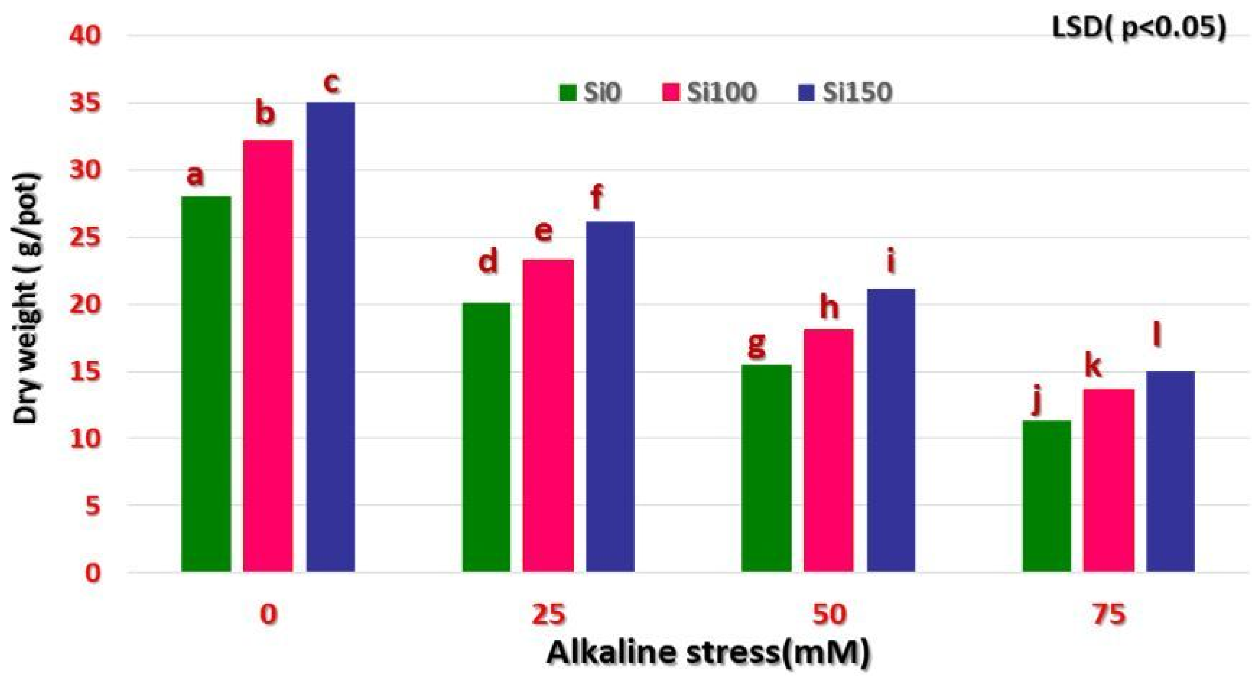

Figure 2

Effect of alkaline stress and silicon levels on dry weight of maize values marked by different letter differ significantly

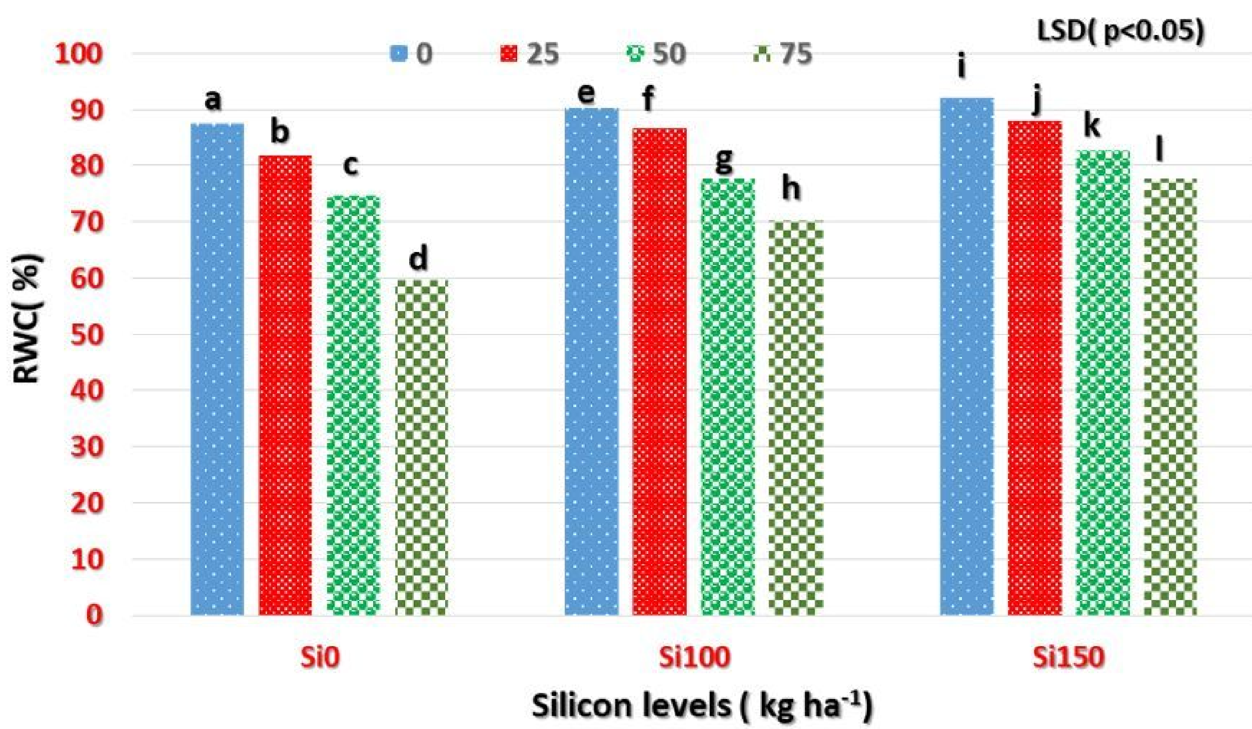

Figure 3

Effect of alkaline stress and silicon levels on RWC of maize values marked by different letter differ significantly

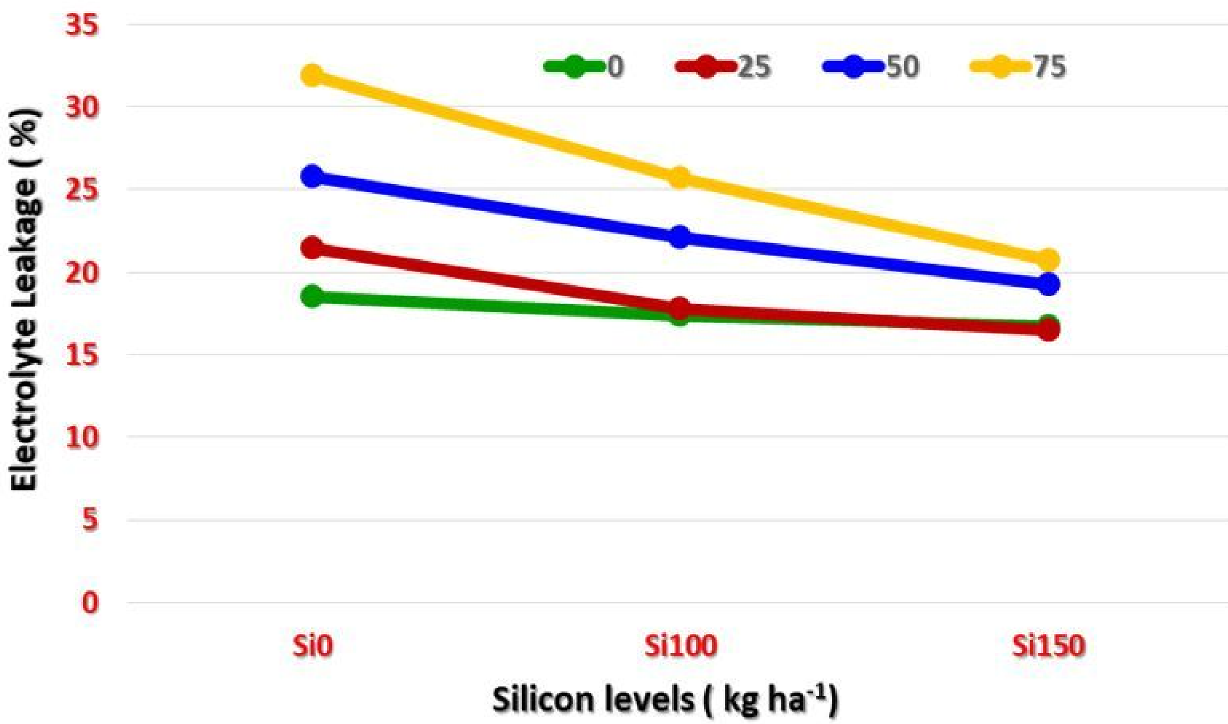

Figure 4

Effect of alkaline stress and silicon levels on electrolyte of maize leaf

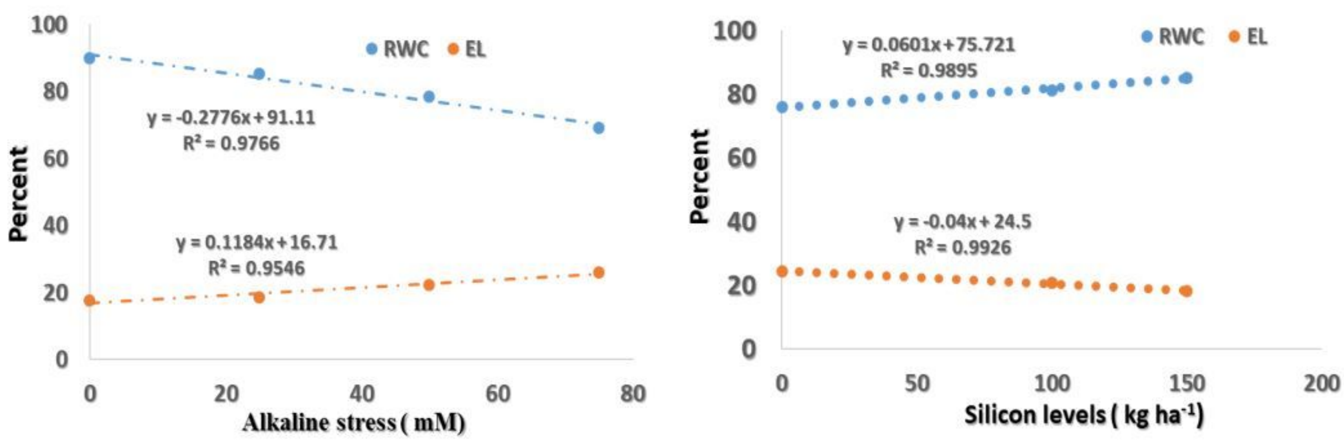

Figure 5

Linear regression relationship between RWC and EL with a) Alkaline stress b) Silicon levels (kg ha-1)

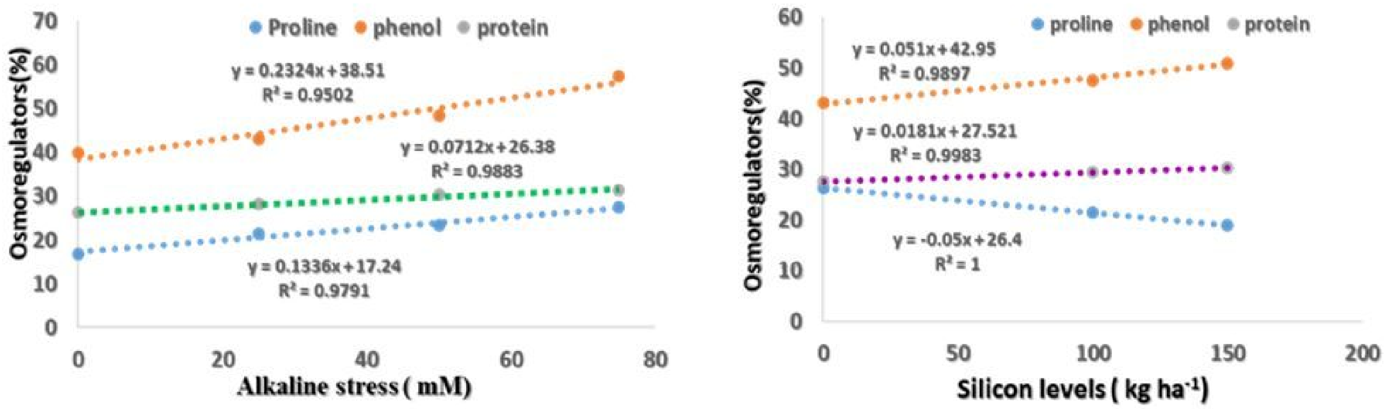

Figure 6

Interlinear relationship between osmoregulators with a) alkaline stress b) Silicon levels

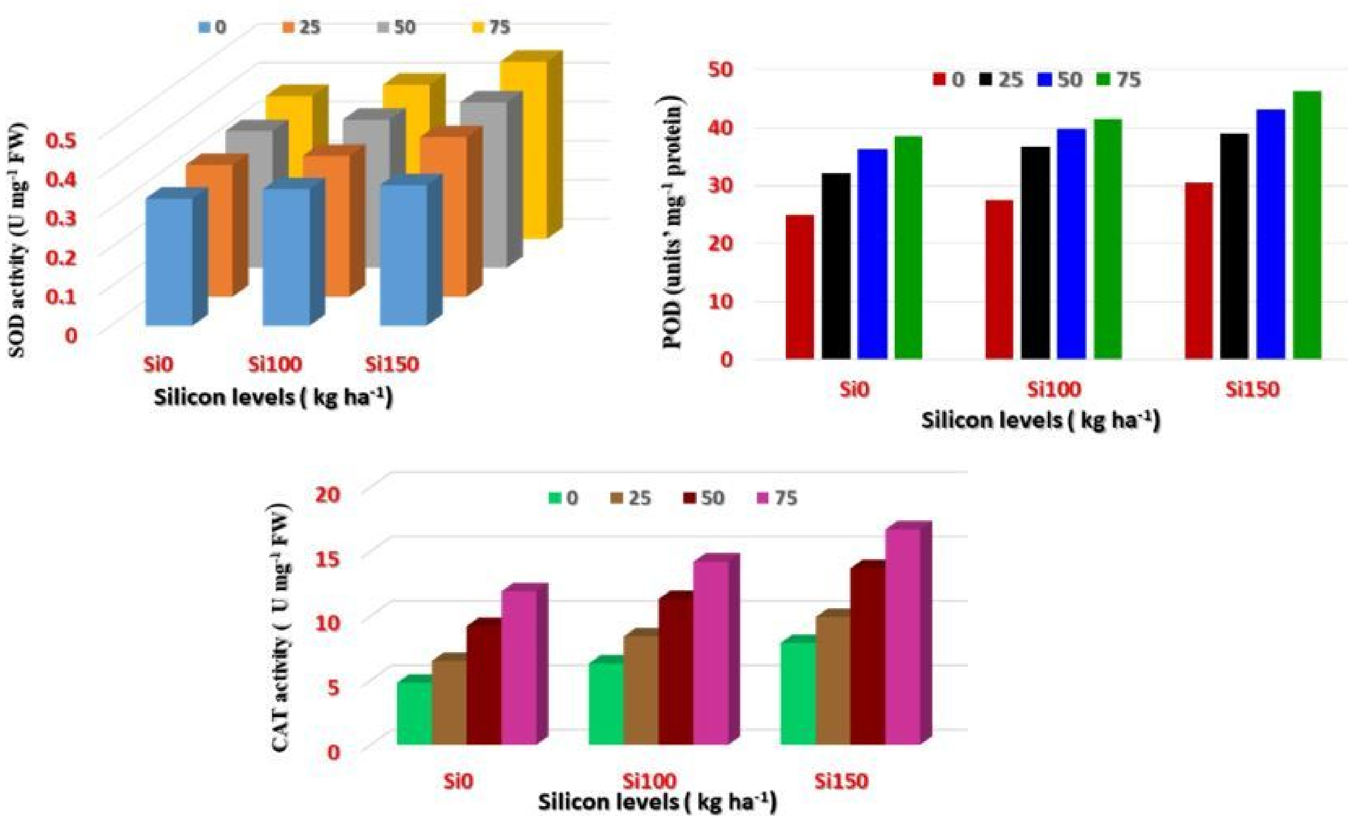

Figure 7

Effect of alkaline stress and silicon levels on antioxidant enzymes