



## Impact of Silicon Seed Priming on Osmoregulators, Antioxidants, and Seedling Growth of Maize Grown under Chemo-Stress

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### ABSTRACT

Seed priming with silicon is an effective approach in combating stress conditions and pave way for good growth. A pot experiment was conducted with treatment schedule viz., Silicon seed priming @ 0, 1, and 2mM through silicon metasilicates and alkalinity stress levels viz., 0,25,50 and 75 mM produced by totaling with sodium carbonate. The trial was taken up through FCRD and replicated thrice with maize var CO8. The crop was grown up to the vegetative stage. The outcome of the experiment was that osmoregulators and anti-oxidants were higher in maize plants that did not receive silicon. The phenol and soluble protein contents increased by silicon priming under normal and alkali stress soil, while proline content was reduced. At all-silicon levels, osmoprotectants showed a positive trend with an increase in stress levels and maximum increase in phenol content (60.4µg/g) and soluble protein (30.2 mg/g DW) was seen with 75 mM alkaline stress in the presence of 2 mM silicon. Anti-oxidant enzymes like SOD, peroxidase, and catalase activities increased with stress levels and increased further in the presence of silicon priming. At all-silicon levels, peroxidase, SOD, and catalase activities in maize increased with stress levels and the highest value of 48.1 units mg<sup>-1</sup> protein, 0.412 U mg<sup>-1</sup> and 17.2 U mg<sup>-1</sup>, respectively was noticed with 75 mM in the presence of 2mM Si. Maize seedling growth and dry weight showed a negative trend under alkali stress and turn over effect was observed with seed priming of maize seeds with silicon.

**Keywords:** Alkali stress, Anti-oxidants, Maize, Osmoregulators, Silicon

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### INTRODUCTION

Plant growth and development limited by salt stress is widespread environmental stress compared to other common stressors. Here the survival of plants will be a difficult exercise. Salt stress is caused by the presence of neutral salts and alkali stress by alkaline salts (Shi & Sheng 2005). The current status of the world's salt-affected area is 1,128 million ha (Mandal *et al.*, 2018). Nearly 5% of the net cultivated area in India has salt stress i.e. 6.7 million ha, of which 45 % is saline and 56.7% is sodic (Arora *et al.*, 2016). The alkalinity of soils (high pH) inhibits the ionic absorption of roots, modifies the nutrient availability in soil, and thus an imbalance in plant ions and mineral nutrients. Extreme alkalinity can bring several harmful effects in plants at the cellular level i.e. accumulation of high Na, increased ionic stress, osmotic stress by stirring up the water deficit, and eventually ensuing elevated assembly of reactive oxygen species (ROS) and oxidative (Kaiwen *et al.*, 2020). To handle the alkali stress-induced oxidative damage, plant cells trigger endogenous antioxidant protection mechanisms like enzymatic antioxidants (ascorbate peroxidase, catalase, peroxidase, polyphenol oxidase, superoxide dismutase) and non-enzymatic antioxidant metabolites (Mansoor *et al.*, 2022) to counteract the adverse salt-alkaline conditions (Guo *et al.*, 2015). Metabolic disorders caused by accumulated ROS in cells

that disturb normal physiological functions. The crops exposed to alkali stress are affected primarily through poor growth as roots perceive first stress information followed by other parts of the plant.

Chemically Silicon (Si) is a metalloid element capable to alleviate alkali stress. Silicon fortification influences oxidation of cell membranes and grounds protection of diverse functions in plant organs impinge by alkali stress. Oxidative damage can be abridged by silicon through the escalation of anti-oxidative enzymes under stress. Physiological parameters relating to varied stress can mitigate by Si supplementation, either totally or partly reinstated to the stage like unstressed plants (Frew *et al.*, 2018). Further, Si reduces oxidative damage to a certain level of ~30% by increasing antioxidant enzyme activity under saline stress. Various studies in different crops observed reduction oxidative stress by enhancing the enzyme activities which proved that there is a link between ROS and Si, and this link helps enhanced-expression of oxidative stress genes by silicon addition (Ma *et al.*, 2016). A controlled hydration process and re-drying of seeds (seed priming) realistic loom to work against the salinity effects in many crops due to ease, less cost, and efficiency, and use of salts in priming solution or addition of signaling molecule like H<sub>2</sub>O<sub>2</sub> can be used as an adaptation strategy to improve salt tolerance in crops (Afzal *et al.*, 2008). Silicon as a biostimulant plays the role of plant defensive element, thereby enhancing plant growth and yield of crops especially under stress (Sawas & Ntatsi, 2015). In this context, an experiment was taken to determine the seed's

priming with silicon in maneuvering salt stress through osmoregulants and antioxidant enzymes.

## MATERIALS AND METHODS

The present study was conducted at the Soil Science and Agriculture Chemistry Department, of the Agriculture Faculty in the Annamalai University. It is geographically located at 11° 24'N and 79°44'E with an MSL of +5.79 m. The university experimental farm is where samples of 0-15 cm bulk soil were collected from. The soil used for the experiment has an 8.3-pH, OC- 5.2gkg<sup>-1</sup>, CEC- 32.5 cmol (+) kg<sup>-1</sup>, EC- 0.67dSm<sup>-1</sup>, KMnO<sub>4</sub>-N - 265 kg ha<sup>-1</sup>, NH<sub>4</sub>OAc-K- 196.5 kg ha<sup>-1</sup>, Olsen-P- 21.5 kg ha<sup>-1</sup>, and clay loam in texture (Typic Hapluster) and available silicon- 37.9 mg kg<sup>-1</sup>. Three levels of silicon (source: sodium meta silicates) concentration (0, 1 & 2 mM) and alkaline in four levels (source: sodium carbonate) stress (0, 25, 50, and 75 mM) coupled as the combination of 12 treatments and three times replicated, imposed in FCRD. Maize seeds (*Zea mays* L. cv CO 8) were sterilized with mercuric chloride (0.1%) for 5 minutes before a three-time rinse with distilled water and then filled in pots with 10 kg of soil. They were then divided into two batches where the first batch was distilled water primed, and the second batch primed with 1.0 and 2.0 mM of Si (sodium meta silicate) solution that was freshly prepared, for half a day and air-dried. Both batches of air-dried seeds were sown and during the process, the pots were, at field capacity, irrigated with various concentrations of alkaline salts such as 0 (control experiment), 25, 50, and 75 mM of Na<sub>2</sub>CO<sub>3</sub> by adding a designated saline solution whose capacity was 400ml. Sodium carbonate was used at different concentrations for the experiment and they included: 0 (control), 0.528, 1.056, and 1.584 g kg<sup>-1</sup> soil. Natural conditions of temperature, light, and humidity were used to grow the plants and 2 plants were maintained in each treatment up to the vegetative stage where the trial was closed. The osmoregulants viz., proline (Bates *et al.*, 1973), phenol (Hoff & Singelton, 1977) and soluble protein (Lowry *et al.*, 1951) and antioxidant enzymes viz., SOD (Beauchamp & Fridovich, 1971), POD (Maehly & Chance 1954) and CAT (Havir & Mchale, 1987) were estimated in maize leaf at vegetative stage. Maize growth and dry weight were recorded treatment-wise at the vegetative stage. The data were statistically analyzed using the SPSS package. All the parameters were analyzed by one-way ANOVA. The interpretation of treatments effects was made based on the critical difference at a 5 % probability level.

## RESULTS AND DISCUSSION

### Maize seedling growth

Graded levels of alkaline stress caused a significant reduction in shoot length (-5.3 to -25.9 %) and root length (-9.52 to -23.8 %) of maize (Figure 1). While primed maize seeds with a graded concentration of silicon increased the shoot and root length both under normal and alkaline stress soil. In normal soil, the addition of silicon at 1 and 2 mM as seed primer improved shoot length (6.5 % and 18.7%) and root length (16.5% and 18.7%), respectively over control. In alkaline stress soil, silicon addition at 1 and 2 mM as seed primer enhanced shoot length from 6.0 to 18.6 percent and root length from 3.6 to 13.3 percent, respectively. Similarly, shoot dry

weight (-21.4 % to -63.3%) and root dry weight (-10.0% to -62.0%) were drastically reduced grown in different alkaline stress levels (Figure 2). Shoot and root dry weight improved both in normal and alkali stress soil on the addition of silicon at various concentrations as seed primer. In normal soil, the addition of silicon at 1 and 2 mM caused a 25.7% and 37.6% increase in shoot dry weight and 7.8 and 13.4 % in root dry weight over control, respectively. However, at various levels of alkaline stress, silicon seed priming at both 1 and 2 mM improved the shoot dry weight by (8.5 to 58.4%).

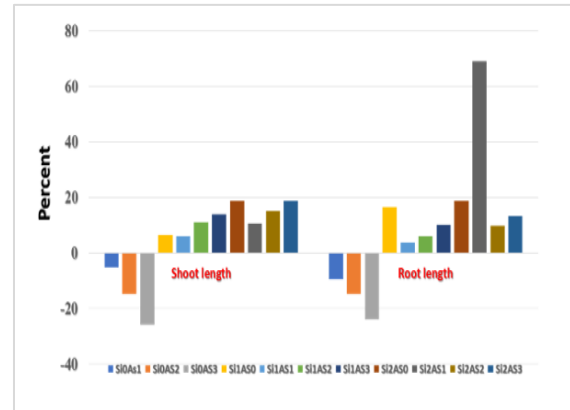


Figure 1. Percent reduction due to alkaline stress and stimulatory effect of silicon seed priming on shoot and root length of maize

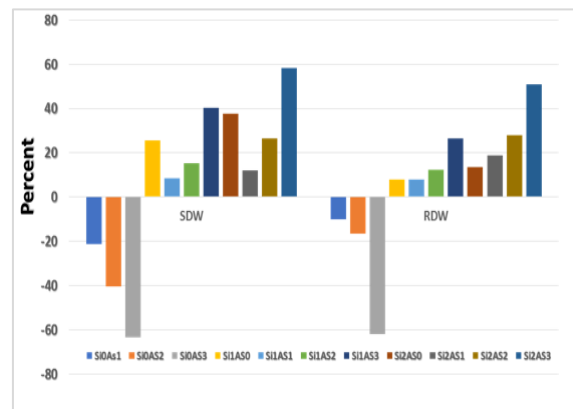


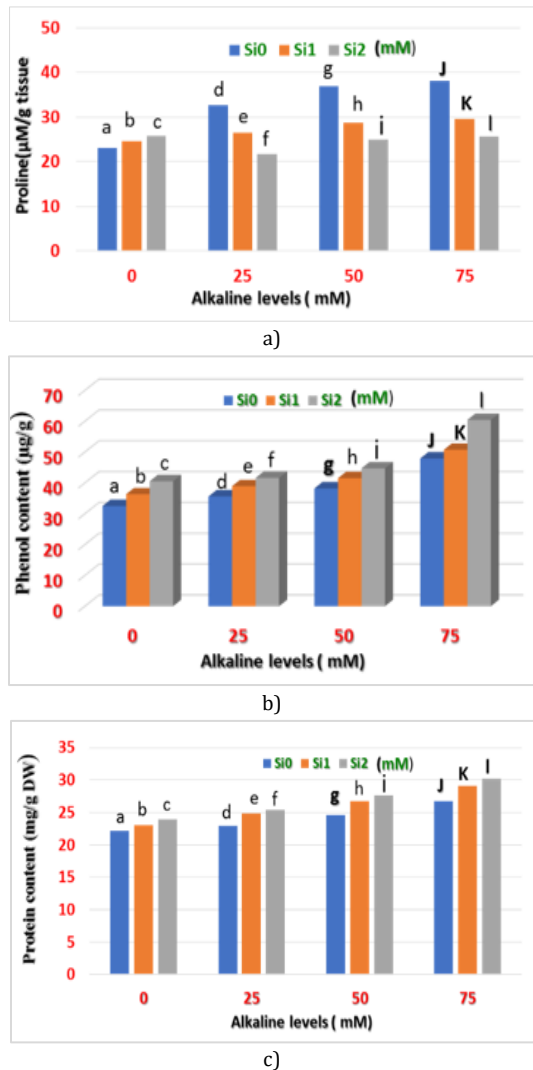
Figure 2. Percent reduction due to alkaline stress and stimulatory effect of silicon seed priming on dry weight of shoot and root of maize

### Osmoregulants

#### Proline

Silicon seed priming (SSP) and alkaline stress (AS) effects were observed on proline content in leaves compared to non-primed and non-stress soil (Figure 3). Proline content increased linearly with the level of alkaline stress and it ranged from 24.4 to 31.1 μM/g tissue of nonstress and stress soil, respectively (i.e. approximately 10.2 to 27.5 % increase). With an indirect relation, the increase in the concentration of silicon priming proline content in maize leaves reflected a decreasing trend and it decreased from 32.6 to 24.4 μM/g tissue. The interplay between silicon priming and alkali stress caused a significant

effect on proline content in maize leaves. With silicon concentration, the proline content declined at all alkaline stress. The reduction percentage in proline stretched between 23.5 to 50.2 (25 mM), 28.7 to 47.8 (50 mM), and 28.7 to 48.8 (75 mM).



**Figure 3.** Effect of silicon seed priming under alkali stress on osmoregulants viz., a) Proline b) Phenol c) Protein.

Values followed by different letters (a, b and c) in the same column are significantly different at P=0,05

#### Phenol

Phenol content significantly increased with alkali stress levels and seed priming at various silicon concentrations (Figure 3). The phenol content in maize leaves showed an upward trend with seeds treated with silicon compared to unprimed seeds. The phenol content was assorted from 38.6 to 46.8 µg/g and showed an escalating trend as alkaline stress intensified. Phenol content percentage increase ranged from 6.31 (25mM) to 45.6 (75 mM). A positive trend with an increase in stress levels, where the maximum increase was seen at 75 mM alkaline stress (60.4 µg/g) in the presence of 2 mM silicon was shown by phenol content at all-silicon levels. Moreover, phenol's content percentage progress with silicon levels protracted between 9.3 to 16.9 (25mM), 8.6 to 17.0 (50 mM), and 5.8 to 26.1(75 mM).

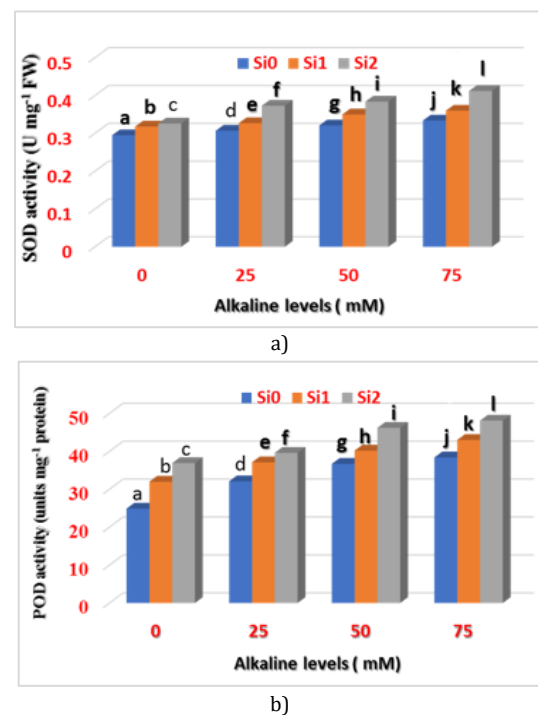
#### Protein

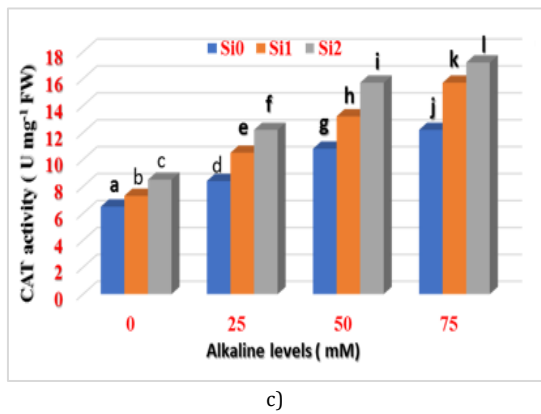
A significant improvement in measured protein content was observed in maize leaves exposed to alkali stress and as well with silicon seed priming compared to normal soil and non-primed seeds (Figure 3). Due to alkali stress, the percentage augmentation of protein content varied from 5.65 to 24.32. Silicon priming of maize seeds with different concentrations increased protein content to the extent of 7.91 to 11.25 %. At all-silicon levels, protein content increased with stress intensity, and a maximum value (30.23 mg/g DW) was observed in maize leaves which received 2mM Si and 75 mM sodium carbonate concentration. Considering the silicon levels, the percentage of protein content fluctuated from 8.29 to 10.9 (25 mM), 8.53 to 12.1 (50 mM), and 8.98 to 13.1 (75 mM).

#### Antioxidants activity

##### Superoxide dismutase

Silicon priming and alkali stress caused a marked influence on superoxide activity over non-primed stress-free soil (Figure 4). The rise in alkaline stress level led to a linear amplification of Superoxidase dismutase (SOD) activity in leaves (fresh weight-FW) with a stretch between 0.313 U mg<sup>-1</sup> (non-stress soil) to 0.368 U mg<sup>-1</sup> (stress soil). The percentage inflation in SOD activity stretched between 7.0 to 17.6. SOD activity in maize leaves reflected an increasing trend, that too from 0.314 to 0.373 U mg<sup>-1</sup> FW with inflation in the concentration of silicon priming. The interaction between silicon priming and alkali stress caused a noteworthy effect on SOD activity. The maximum amassment of SOD activity in maize leaves was established at 75 mM sodium carbonate concentration (0.412U mg<sup>-1</sup> FW) with 2mM Si. Similarly, at all alkalinity stages, the activities of SOD upsurge with silicon concentration and they hiked from 4.12 to 13.2 (25 mM), 7.3 to 22.0 (50 mM), and 10.5 to 39.7 (75 mM), respectively. Further, seed priming improves the antioxidant defense mechanism via moderating the antioxidant enzymes (Saha *et al.*, 2022).





**Figure 4.** Effect of silicon seed priming under alkaline levels on antioxidant enzymes, a) SOD b) POD c) CAT.

Values followed by different letters (a, b and c) in the same column are significantly different at  $P \leq 0.05$

#### Peroxidase activity

Significant influence of alkali stress levels and silicon levels on peroxidase activity over non-stressed soil and non-primed (Figure 4). The build-up of peroxidase activity in maize leaves amplifies significantly with the build-up in alkalinity and for that reason, the peroxidase activity was boosted from 31.32 units  $\text{mg}^{-1}$  protein. (Non-stress soil) to 43.22 units  $\text{mg}^{-1}$  protein (75 mM). A positive trend in peroxidase activity in maize leaves was observed due to the average effect of silicon priming. Peroxidase activity percent increased from 15.5 to 29.4 with silicon levels. At all-silicon levels, peroxidase activity in maize increased with stress levels and the highest value of 48.1 units of  $\text{mg}^{-1}$  protein was noticed with 75 mM in the presence of 2mM Si. The percent increase in peroxidase activity at different silicon concentrations under various alkali stress levels ranged from 28.9 to 54.4 (25 mM), 28.5 to 72.7 (50 mM), and 48.2 to 93.2 (75 mM).

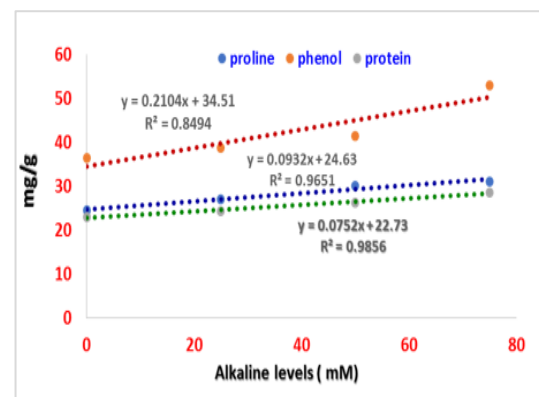
#### Catalase activity

Catalase a key antioxidant enzyme was significantly influenced by the action of silicon seed priming under different alkali stress levels in soil compared to non-stress soil without silicon priming (Figure 4). Different concentrations of alkaline stress recorded an upsurge in catalase activity in maize leaves and it extended between 7.4 to 15.0  $\text{U mg}^{-1}\text{FW}$ . Priming of maize seeds with silicon @ 1mM and 2mM concentration increased the catalase activity over non-primed seeds. The value varied from 9.5 to 13.4  $\text{U mg}^{-1}\text{FW}$ . The enhancement percentage in the catalase activity values drifted from 23.2 to 41.12. Catalase activity increased with levels of alkali stress, and the highest value was recorded with 2mM Si priming in 75mM alkali stress ( $17.2 \text{ U mg}^{-1} \text{ FW}$ ) at all-silicon levels. The percent increase in catalase activity due to silicon priming at various alkali stress concentrations ranged from 29.2 to 87.7 (25 mM), 12.3 to 141.5(50 mM), and 30.8 to 164.6 (75 mM).

Plant growth is affected by salinity/alkalinity by disrupting the ionic balance and looming osmotic stress (Zhu, 2002). Salt stress accounts for reduced cell expansion and division leading to a slow rate of physiological and biochemical processes causing poor photosynthetic efficiency and reduction in growth and DMP of maize (Zhang *et al.*, 2015). The present study showed a peculiar decrease in the seedling progress

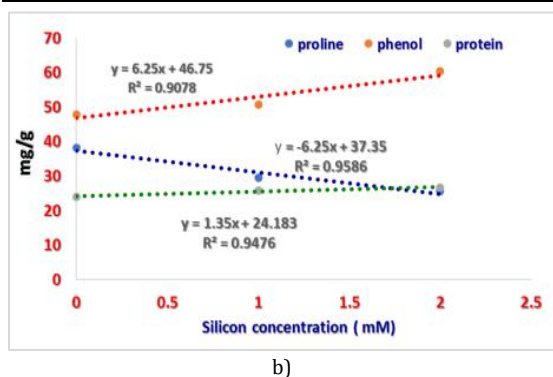
signifying the adverse effect of alkali stress on maize. In tune with the present result, the negative effect of alkali stress was reported earlier by El-Serafy *et al.* (2021). In contrast, seed priming with silicon distinctly improved maize seedling growth and dry weight under normal and alkali stress soil. Priming practices trigger the germination process with the production of biochemical in the seed, contributing to enzyme production and expansion of the cell wall, leading to the breaking of dormancy. Silicon might have a direct link to the physiological process of seed germination and an identified forerunner for the synthesis of lignin hence its taking part in seed coat resistance (Toledo *et al.* 2011). Further, enhancing the walls deposit (epidermis and vascular tissues of the stem, leaf sheath, and hull) and moderating certain physical and biochemical functions in plants reported by Siddiqui and Al-Whaib (2014).

Osmotic regulation is an integral of the salt-stress dodging technique to counteract the loss of turgor by rising and upholding an elevated sum of intercellular attuned solutes in the cytosol and vacuole have been found to be significant amongst all stress adaption mechanisms in plants (Cushman, 2001; Alzahrani *et al.*, 2018). Proline (a key osmolyte) makes certainty to osmotic adjustment (Khadri *et al.*, 2006). The build-up of proline in plants under stress may be caused by the stimulation of expression of proline biosynthesis genes or by the repression of the genes of its degradation pathway (Marco *et al.*, 2015). As here in the study, a strong linear relationship existed between alkali stress and proline concentration ( $R^2=0.9651^{**}$ ) (Figure 5a) was found. It may be a sign of stress alleviation and stress injury mitigation when proline escalation declines under alkali stress after Si priming. Silicon might have provided a protective role in serving to put off lipid peroxidation induced by sodium chloride. Since proline was considerably lesser in the silicon treated seedlings under salt stress when compared to non-treated. In addition, silicon concentration determined the level of proline ( $R^2= 0.9586^{**}$ ) (Figure 5b) was confirmed with regression significantly. This finding was in concordant with those of Lee *et al.* (2010) i.e. proline content in soybean under salt stress reduced with Si priming and Parveen *et al.* (2019) reported that Si priming of 6 mM appreciably condensed proline content in maize leaves in drought.



a)





**Figure 5.** Linear relationship between osmoregulators with a) Alkaline levels, b) Silicon concentrations

Polyphenolic compounds contribute to plant defense against reactive oxygen species (ROS), which are certainly formed when aerobic respiration / photosynthetic metabolisms get weakened by environmental stresses (Bettaieb *et al.*, 2011). Further, phenolic compounds display antioxidant activity by disabling the lipid-free radicals or putting off the decay of hydroperoxides into free radicals (Pokorny *et al.*, 2001). The present study results also displayed an increasing tendency in phenol content of maize plants grown under alkaline stress and the percent increment in phenol content stretched between 6.34 to 45.6. Also, a significant relationship was exhibited between alkali stress and phenol concentration ( $R^2=0.8494^{**}$ ) (Figure 5a). Similarly, Hussain *et al.* (2019) reported an approximately 36 % raise in phenol due to silicon in barley; Raza *et al.* (2019), and Khan *et al.*, (2018) reported an increase in the phenolic load due to silicon application in different crops. It was also confirmed with regression test that silicon concentration remarkably regulated the level of phenol ( $R^2=0.9078^{**}$ ) (Figure 5b).

The protein content in maize leaves showed advancement in protein content with alkali stress. As plants are under stress, they may result in proteins with tiny molecular mass and used as a source of nitrogen storage that assembles after being let off from stress (Zhang *et al.*, 2014). Additionally, the osmotic adjustment, also influenced by these proteins as mentioned by Ashraf and Harris (2004) and an increase in soluble protein in maize genotypes grown in salt stress was observed (Andre *et al.*, 2009). A strong relationship has been noticed in the present study between alkali stress and protein content ( $R^2=0.9856^{**}$ ) (Figure 5a). Silicon applied as seed priming increased the soluble protein. An increase in soluble protein is because silicon might have played a key role in adhering amino acids to form distinct proteins (Soundararajan *et al.*, 2014) and Abbas *et al.* (2015). Through regression test, it was concluded that silicon concentration greatly influenced the level of protein ( $R^2=0.9476^{**}$ ) (Figure 5b). In addition to that silicon nutrition proved as an eliminator of polyamine synthesis inhibitors in cucumber (Yin *et al.*, 2019).

Plants encompass refined anti-oxidative protection mechanism to clear ROS generated in the course of stress. Plants may make a huge quantity of reactive oxygen products, ensuing in waning of the plant membrane lipid peroxidation under alkaline stress conditions and certain protective enzymes helps in removing membrane lipid peroxidation (Tuna *et al.*, 2008). A symmetry was brought between the production and detoxification of

ROS by silicon priming, duly by enhanced activities of antioxidant enzymes, thus shielding the oxidative damage (Figure 4). Superoxidase dismutase (SOD), a key protective enzyme in removing reactive oxygen molecules, which can putrefy reactive oxygen into  $H_2O_2$  and  $O_2$ , then CAT and POD turn  $H_2O_2$  into  $H_2O$  and  $O_2$ . Further, silicon foliar spray retract the sodium toxicity in sunflower and sorghum via enhanced antioxidant activities (Hurtado *et al.*, 2020).

Removal of ROS by every antioxidant activated by seed priming combinedly worked out well and it is vital for enhancing the stress tolerance of plants significantly. The activities of antioxidant enzymes in cucumber were strengthened by silicon application which enhanced the drought tolerance (Farouk *et al.*, 2020) and Sayed and Gadallah (2014) observed strengthened the antioxidative defense system and decreased lipid peroxidation, and oxidative damage in maize under drought stress with silicon application. From study results, it was evident that silicon interference through seed priming improved SOD, POD, and CAT enzymes activities in maize leaf grown in soil with alkaline stress. Seed priming with silicon increased SOD (7.8 to 39.7%), POD (28.5 to 93.2%), and CAT (12.3 to 164.6%) over non-stressed soil (Figure 5). Seed priming might have activated the antioxidant defense system in the plant tissues under stress (Espanany *et al.*, 2016). Abdel Latef and Tran (2016), and Liu *et al.*, (2018) reported a significant increase in SOD, POD, and CAT activities in alkali soil due to silicon priming. Thus it is no surprise that reduction in ROS with silicon provision under stress conditions resulted in downstream changes in plants in terms of productivity as similar findings were observed by (Markovich *et al.*, 2017; Gaur *et al.*, 2020).

## CONCLUSION

From the study results, it can be concluded that osmo regulators and antioxidant enzymes are strongly influenced by silicon (Si) treatments under normal and alkali stress soil. Application of silicon @2mM as seed primer recorded a significantly higher osmoregulators and antioxidants. Maize seedling growth had a negative relation under alkali stress and turn over effect was observed with seed priming of maize seeds with silicon significantly. And this experiment may be of particular interest to maize growth and development under salt stress especially in coastal areas through seed priming with silicon.

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