Full Length Article

Silicon Attenuates Acidic and Alkaline Stress in Wheat Plant by Improving Nutrient Availability, Membrane Stability Index and Antioxidant Defense System

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Abstract

The defending role of silicon in relation to growth, leaf area, plant total biomass, photosynthetic pigments, lipid peroxidation, membrane characteristics and stability index (%), membrane leakage, macro and microelement availability, as well as enzymatic and non-enzymatic antioxidants contents, were assessed under acidic and alkaline stresses in wheat plants grown hydroponically. For this purpose, we used 9 treatments with three levels of Si (0, 1, and 3 mmol L^{-1}) in the form of Na$_2$SiO$_3$ against three levels of pH (5, 7 and 9). Results showed that acidic, as well as alkaline stresses significantly reduced plant physiological traits and photosynthetic rates by creating deficiencies accompanied by toxicities of various macro and microelements. Moreover, acidic and alkaline stresses reduced membrane stability through lipid peroxidation measured as malondialdehyde contents, enhanced membrane injury and induced oxidative stress through H$_2$O$_2$ contents, and triggers enzymatic and non-enzymatic antioxidative defense system through oxidative burst comparing with control (pH; 7). While the addition of Si at the concentrations of 1 and 3 mmol L^{-1} significantly encountered adverse effects of acidic and alkaline pH through further strengthening the antioxidant defense system by enhancing the activities of enzymatic and non-enzymatic antioxidants and suppressing reactive oxygen species (ROS) contents. In conclusion, Si application, as a non-corrosive beneficial element, enhanced wheat tolerance in acidic and alkaline induced oxidative stress by regulating the membrane characteristics and by improving the antioxidant defense system. © 2020 Friends Science Publishers

Introduction

Wheat (*Triticum aestivum* L.) is a significant crop widely cultivated for its grain as a worldwide staple food after rice (*Oryza sativa* L.), and completes 20% daily protein needs of 4.5 billion people all over the world (Flister and Galushko 2016). It is well acclimatized to a broad range of environmental and soil conditions, however, a major loss in biomass production and yield is being reported as a result of various abiotic stresses (Rady and Hemida 2015; Hussain *et al.* 2016). Among various abiotic stresses, soil pH is one of the most critical factors influencing wheat growth and development (Baquy *et al.* 2017). Soil pH can be assumed as a crucial variable due to its central role in many soil processes and properties, which may affect plant growth, microbial diversity, and solubility and availability of various nutrients (Gentili *et al.* 2018).

The pH range in most agricultural soils is from 4 to 9 (Fageria and Zimmermann 1998). In most cases, neutral or near to neutral pH is beneficial to crop production (Fageria and Zimmermann 1998). The pH below 7 causes soil acidity, which is a severe problem in many regions of the world for crop production and development (Sumner and Noble 2003). Acidic pH showed a direct effect on plant growth by acidic reactions and shows indirect effects on plant growth through nutrient unavailability (Lollato *et al.* 2013). It has been reported that acidic pH enhances the concentrations of cations like aluminum (Al) and manganese (Mn) to a toxic

level, while reduces the concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and molybdenum (Mo) to deficient level (Baquy *et al.* 2017). For this unequal distribution of nutrients, the majority of crop produces stunt growth and reduces the remarked yield potential. Additionally, in acidic pH plants suffered from severe cell membrane damage, and faced adverse effects on antioxidant defense system, photosynthesis, and respiration (Odiyi and Bamidele 2014; Wyrwicka and Skłodowska 2014).

Alternatively, pH above 7 caused soil alkalinity, which produces a massive influx of sodium (Na), a decrease in inorganic negative charge, and resulting ionic imbalance leads to a sequence of stress metabolic responses (Guo *et al.* 2017). It has been reported by Aldesuquy *et al.* (2018) that alkaline stress overproduces reactive oxygen species (ROS). Alkaline stress causes severe damage to the photosynthetic system by reducing high stomatal conductance and net photosynthetic rate in wheat crops (Yang *et al.* 2008). Additionally, alkaline stress significantly reduced amino acids and sugars along with a decrease of 5 and 6 metabolites involved in the Tricarboxylic acid cycle (TCA) and glycolysis in wheat plants (Guo *et al.* 2017). Alkaline pH not only negatively affects the metabolic processes but also hindered the translation between N and C, resulting in nutrient deficiency and ultimately reduces plant growth and development, especially in wheat (Yang *et al.* 2008; Guo *et al.* 2015). To guarantee sustainable agriculture, therefore, reducing acidic and alkaline stresses in the wheat plant is urgently required.

To encounter pH stress in crop plants, various strategies such as liming to alleviate acidic stress and acidifying organic materials including sphagnum peat moss or peat to reduce alkaline stress are used (Goulding 2016; You *et al.* 2016). However, these techniques may not be effective in many cases. Alternative to or along with these techniques, the application of silicon (Si) as fertigation could be a viable approach to minimize or alleviate acidic and alkaline stress in crop plants.

Silicon (Si), as the second most abundant metal on the earth's crust, still not classified as an essential nutrient for higher plants, and up to date considered as a beneficial element for various cereal crops (Ma *et al.* 2006; Sattar *et al.* 2016; Howladar *et al.* 2018). The beneficial effects of Si application on plant growth and development under various abiotic stresses are well documented (Sattar *et al.* 2017a, b; Zhu *et al.* 2019), where Si application demonstrates its real potential (Keeping and Reynolds 2009). Si application limited the adverse effects of different stresses like; heavy metal stress (Farooq *et al.* 2013), drought stress (Gong *et al.* 2005), and freezing and temperature stress (Kim *et al.* 2014). Numerous possible mechanisms have been reported where Si increases plant tolerance against saline stress by increasing plant water status (Romero-Aranda *et al.* 2006) and scavenging ROS (Zhu *et al.* 2004). Several beneficial effects of Si under abiotic stress have been reported by Ma

(2004), including modifying nutrient imbalance, minimizing mineral toxicity, ensuring membrane stability, and improving photosynthetic activity to enhance abiotic tolerance. Taking account of all these abiotic stresses, the most urgent is to address Si beneficial effects on acidic and alkaline stresses, which cause a severe reduction in plant growth and development, especially in wheat crops.

To acknowledge, there is insufficient work performed to address Si effect on growth-related attributes and its defensive role in alleviating acidic and alkaline stress in wheat plants. Therefore, the objective of the present study was to explore the beneficial role of Si on wheat plants in terms of total biomass production, photosynthetic rate, nutrient availability, membrane stability and anti-oxidative defense system under acidity and alkalinity stress in hydroponic conditions. Moreover, it can be assessed the optimum pH level for Si uptake, accumulation, and translocation in wheat plants which might be helpful for the coming Si-researchers.

Materials and Methods

Plant culture and experimental design

The experiment was conducted at the experimental site of the Farm Land Irrigation Research Institute, Chinese Academy of Agricultural Sciences in Xinxiang City, China. Healthy seeds of winter wheat genotype Xin Mai 23 were immersed overnight in deionized water and sown in sterilized quartz sand trays with the sand layer of 4 inches in width. The sand trays put in a growth chamber with a photoperiod of 16h light/8h dark with a light intensity of 375 μmole m^{-2} S^{-1}. The temperature of the growth chamber was set at 28°C to 30°C with a relative humidity of 85%. After two weeks of sowing, the five uniform seedlings were wrapped with foam at a root-shoot junction and transplanted in each hole (15 in. ×17 in. in size) of plastic sheets floating on 10 L capacity of plastic containers. These containers filled with 8 L modified Hoagland's solution (Hoagland and Arnon 1950). The nutrient solution replaced every three days.

The one-week-old seedlings were transplanted in three levels of pH for 90 days. After 20 days of transplantation, silicon (Si; Na$_2$SiO$_3$) with the concentration of 0, 1 and 3 mmol L^{-1} was introduced in nutrient solutions of three different pH (5, 7 and 9) for 21 days. Three levels of pH (5; acidic, 7; control, and 9; alkaline) were obtained by the addition of 0.1 *M* KOH to rise, 0.1 *M* HCl to lower the pH. The pH was modified three times every day with 8 h interval of time. The pots were arranged with complete randomized design (CRD) with three replications. The experiment was carried out under natural conditions with an air temperature of 22 to 30°C during the day and 15–20°C during the night. All plants were sampled after 90 days of transplantation. Leave and root samples were frozen immediately in liquid nitrogen and stored at -80°C for enzyme assays extraction.

Determination of plant growth and biomass

The 90 days old plants were sampled for the assessment of growth parameters like the fresh and dry weight of roots and shoots. Two plants from each replication were sampled and stored at -80°C in the freezer (Thermo Fisher Scientific, U.S.A. 702) for enzymatic analysis. Remaining plants were separated into the root and shoots and were measured for their fresh weights (kept at 70°C in the oven till constant dry weight), which were subsequently measured for N, P, K, Ca, Mg, Zn, and Cd contents.

Measurements of photosynthetic pigments

Photosynthetic pigments (Chlorophyll a, b, total chlorophyll, and carotenoids) were measured with an ultraviolet-visible spectrophotometer (TU-1810) by using the spectrophotometric method (Metzner *et al.* 1965).

Biochemical analysis

Anti-oxidative enzymes like superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (POD), of fully expanded leaves and roots were analyzed with an ultraviolet-visible spectrophotometer (TU-1810) by using the kits of Beijing Solarbio Science & Technology Co., Ltd. (http://www.solarbio.com). Briefly, 0.5 g fresh samples of leaves were milled with the help of a motor and pestle and standardized in 0.05 M phosphate buffer with pH 7.8 under chilled condition. The standardized mixture was centrifuged (TGL-18M) at 12,000 rpm for 10 min at 4°C after sieving through four layers of muslin cloth. The following formula assessed the activity of CAT:

$$CAT \left(\frac{u}{m.g.prot} \right) = (ODControl - ODTest) \times \frac{271}{60} \times \frac{1}{SQ} \times \frac{1}{Protein\,conc.} \quad (1)$$

SQ = Sample Quantity
$OD_{control}$ = absorption of light in control
OD_{test} = absorption of light in test samples

After mixing all reagents in the standardized mixture, the supernatant was again centrifuged at 3500 rpm for 10 min. The light diameter of 1 cm was adjusted to zero by double streaming water. OD was measured at 420 nm wavelength. The activity of POD was measured by the following equation:

$$POD \left(\frac{u}{m.g.prot} \right) = (ODTest - ODControl) \times \frac{12}{1\,cm} \times \frac{Vt}{SQ \times RT \times Protein\,conc.} \times 1000 \quad (2)$$

V_t = Total volume of the reaction liquid
SQ = Sample Quantity
RT = Reaction time
$OD_{control}$ = absorption of light in control
OD_{test} = absorption of light in test samples

After mixing all reagents in a standardized mixture, the supernatant was placed at room temperature for 10 min. SOD was measured at 550 nm wavelength. The activity of SOD was measured by the following equation:

$$SOD \left(\frac{u}{m.g.prot} \right) = \left(\frac{ODcontrol - ODtest}{ODControl} \right) \times \frac{1}{50} \times \frac{Vt}{SQ \times Protein\,conc.} \quad (3)$$

V_t = Total volume of the reaction liquid
SQ = Sample Quantity
$OD_{control}$ = absorption of light in control
OD_{test} = absorption of light in test samples

The level of lipid peroxidation in the leaf tissue was assessed by measuring the contents of malondialdehyde (MDA, a by-product of lipid peroxidation. Briefly, 0.2–0.5 g weighted fresh samples of leaves were milled with the help of a motor and added 2 mL 10% TCA and a small amount of quartz sand, ground to homogenate, add 3 mL TCA, further ground. The homogenized sample was centrifuged at 12000 rpm for 10 min. Took 2 mL supernatant, added 0.67% TBA, mixed and boiled for 15 min in 100ºC water bath. Cooled the sample at room temperature and centrifuged again. Absorption values of samples were measured at 532 nm, 600 nm, and 450nm respectively. The activity of MDA was measured by the following formula:

$$CMDA = 6.45(A532 - A600) - 0.56 \times A450 \quad (4)$$

$$MDA \left(\frac{\mu mol}{g} \right) = CMDA \times \left(\frac{Vt}{SQ \times 1000} \right) \quad (5)$$

V_t = Total volume of the reaction liquid
SQ = Sample Quantity
Proline was also assessed by using the kit of Beijing Solarbio Science & Technology Co., Ltd. Following formula was used to measure the proline contents:

$$Proline \left(\frac{\mu g}{g} \right) = \left(\frac{ODsample - ODblank}{ODst - ODblank} \right) \times Cst \frac{5\mu g}{ml} \times \frac{Vreagent}{Mtissue} \times COD \quad (6)$$

CoD= the coefficient of dilution in the pre-treatment process
C_{st} = Concentration of standard
Hydrogen peroxide contents in leaves and roots of wheat seedlings were also assessed by using the kit of Beijing Solarbio Science & Technology Co., Ltd. The following formula was used to measure hydrogen peroxides:

$$H_2O_2 \, (\mu M) = \left(\frac{ODsample - ODblank}{ODst - ODblank} \right) \times Cst\,163\mu M \times COD \quad (7)$$

CoD= the coefficient of dilution in the pre-treatment process.
C_{st} = Concentration of standard
OD_{st} = Absorption of standard sample

Electrolyte leakage

Electrolyte leakage we measured by the method of Dionisio-Sese and Tobita (1998). After harvesting of the wheat crop, 1 g of fresh leaves were cut into small parts of 2–3 mm length and put in test tubes containing 8 mL deionized distilled water. The test tubes were placed for 2 h in a water bath (HWS-28) at 37ºC and assessed initial electrical conductivity (EC_1) of the medium by using a conductivity meter (DDB-303A). Subsequently, samples were autoclaved by using Vertical Heating Pressure Steam

Sterilizer (LDZM-40KCS-III) for 20 min at 121ºC to eject all electrolytes. Samples were placed at room temperature at 25ºC and second electrical conductivity (EC_2) was measured. Total electrolyte leakage was calculated by using the following formula:

$$EC = \left(\frac{EC1}{EC2}\right) \times 100 \tag{8}$$

Determination of nutrient elements in plant tissues

The N, P, K^+, Ca, Mg, Zn, and Cd contents in the plants were analysed by inductively coupled plasma mass spectroscopy (ICP-MS, Agilent, and 7700 X, USA) after being oven-dried by following our previous study method (Fırat *et al.* 2017).

Statistical analysis

The data were processed and analyzed using the SPSS 21.0 (SPSS, Chicago, I.L.), and all the graphs were made using the Sigmaplot 12.5 software packages. The means of the three replicates were subjected to analysis of variance (ANOVA), and multiple comparisons were performed using Duncan's multiple range test (DMR) at $P < 0.05$.

Results

Biomass production

The growth of wheat was significantly inhibited by both acidic pH (high H^+ activity), as well as alkaline pH (high OH^- activity) in the growth medium (Fig. 1). Both low and high pH levels significantly reduced shoot fresh and dry weight, root fresh and dry weight, and leaf area of wheat plants as compared with the control (pH 7). The total dry biomass of what plants were 34 and 18% less in acidic and alkaline pH, respectively compared with control (pH 7). Similarly, leaf area of wheat plants was 16 and 9% less in acidic and alkaline pH, respectively compared with control. The addition of Si at the levels of 1 and 3 mmol L^{-1} significantly alleviated the adverse effects of both acidic as well as alkaline pH by increasing wheat growth parameters to normal pH (Fig. 3). In an acidic environment, Si application with 1 and 3 mmol L^{-1} increased shoot dry weight by 40 and 71%, root dry weight by 51, and 137%, and leaf area by 54 and 64%, respectively. Additionally, in the highly alkaline environment, Si application with 1 and 3 mmol L^{-1} increased SDW by 18 and 13%, RDW by 7 and 20%, and leaf area by 14 and 20%, respectively. Si application in a neutral pH solution showed a non-significant effect on wheat growth parameters, which reflected the non-stressful behavior of a neutral environment for wheat growth. Results showed that both levels of Si effectively alleviated acidic and alkaline pH stresses, but more significant results were recorded at 3 mmol L^{-1} Si at acidic pH (Fig. 1).

Photosynthetic pigments

The photosynthetic pigments were significantly reduced by both acidic pH (high H^+ activity) and alkaline pH (high OH^- activity) in the nutrient solutions as compared with control (pH 7; $P < 0.05$) (Fig. 2). Total chlorophyll and carotenoids contents were 45 and 27% less in low pH, while 27 and 11% less in high pH, respectively than control (pH 7.) The addition of Si with 1 and 3 mmol L^{-1} significantly alleviated the adverse effects of acidic and alkaline pH (5 and 9) on total chlorophyll and carotenoids contents of wheat plants (Fig. 2). Si with 1 and 3 mmol L^{-1} concentrations in acidic solution increased total chlorophyll and carotenoids contents by 104 and 175% respectively than alone acidic pH (5). Similarly, Si with 1 and 3 mmol L^{-1} concentrations in alkaline solution increased total chlorophyll and carotenoids contents by 46 and 61%, respectively compared with alone alkaline pH (9). While Si application in a neutral pH solution showed a non-significant effect on chlorophyll and carotenoid contents, which reflected the non-limiting behavior of neutral environment for wheat growth. Both levels of Si significantly increased photosynthetic pigments of wheat plants under unfavorable pH, but the optimum increase was recorded at Si 3 mmol L^{-1} in an acidic environment (Fig. 2).

Antioxidant enzymes

The activities of enzymatic (catalase; CAT, superoxide dismutase; SOD, and peroxidase; POD) and non-enzymatic antioxidants (proline) in roots and leaves were significantly increased by both acidic pH (high H^+ activity) and alkaline pH (high OH^- activity) as compared to control (pH 7; $P < 0.05$) (Table 1). The concentration of CAT in leaves was 38 and 48% higher, SOD in leaves was 69 and 32% higher, POD in leaves was 46 and 27%, higher and proline in leaves was 85 and 56% higher for acidic (5) and alkaline (9) pH respectively, as compared with neutral pH (7). The same trend was recorded in the concentrations of these antioxidants in the roots of wheat plants (Table 1). The addition of Si with the concentration of 1 and 3 mmol L^{-1} in acidic and alkaline nutrient solutions further elevated the concentrations of enzymatic and non-enzymatic antioxidants in both roots and shoots of wheat plants (Table 1). Si concentration with 1 and 3 mmol L^{-1} in acidic solution increased CAT contents in leaves by 27 and 56%, SOD contents in leaves by 36 and 61%, POD contents in leaves by 26 and 50%, and proline contents in leaves by 35 and 112%, respectively compared with alone acidic pH. Similarly, Si concentration with 1 and 3 mmol L^{-1} in highly alkaline pH 9 increased CAT contents in leaves by 15 and 20%, SOD contents in leaves by 24 and 43%, POD contents in leaves by 21 and 37%, and proline contents in leaves by 9 and 47% respectively as compared with alone alkaline pH. The non-significant effect of Si application on antioxidants was recorded at neutral pH, which showed non-limiting behavior of neutral pH for wheat plants (Table 1).

Table 1: Effect of silicon application on of the activities of CAT, SOD, and POD in root and shoot of wheat sown under varying pH levels

Treatments	Leaves			Roots		
	CAT (unit mg^{-1} protein)	SOD (unit mg^{-1} protein)	POD (unit mg^{-1} protein)	CAT (unit mg^{-1} protein)	SOD (unit mg^{-1} protein)	POD (unit mg^{-1} protein)
pH 5 + Si0	4.31 ± 0.14cd	33.21 ± 0.65d	34.5 ± 0.52c	0.72 ± 0.02de	16.39 ± 0.24c	47.01 ± 1.53cd
pH 5 + Si1	5.46 ± 0.07b	45.28 ± 1.31b	43.51 ± 1.76b	0.96 ± 0.02b	18.63 ± 0.13b	54.28 ± 1.77b
pH 5 + Si3	6.71 ± 0.39a	53.56 ± 0.91a	51.82 ± 1.05a	1.42 ± 0.01a	27.87 ± 0.72a	63.32 ± 1.82a
pH 7 + Si0	3.12 ± 0.10e	19.67 ± 0.33f	23.57 ± 1.69e	0.44 ± 0.02g	11.34 ± 0.34g	28.68 ± 0.88g
pH 7 + Si1	3.78 ± 0.19d	26.39 ± 0.67e	25.82 ± 0.14e	0.45 ± 0.02g	12.09 ± 0.13fg	38.84 ± 0.63ef
pH 7 + Si3	4.03 ± 0.07d	30.87 ± 0.09d	26.94 ± 0.55e	0.53 ± 0.01f	13.00 ± 0.19ef	37.05 ± 1.33f
pH 9 + Si0	4.62 ± 0.18c	26.04 ± 1.85e	29.87 ± 0.09d	0.67 ± 0.01e	13.85 ± 0.45e	40.28 ± 0.32ef
pH 9 + Si1	5.31 ± 0.04b	32.38 ± 0.47d	36.38 ± 0.39c	0.73 ± 0.02d	14.89 ± 0.07d	43.53 ± 1.10de
pH 9 + Si3	5.57 ± 0.05b	37.31 ± 1.45c	41.08 ± 0.56b	0.87 ± 0.01c	17.44 ± 0.22c	48.17 ± 1.74c

Means ±SD (n=9) with different letters in the column indicate significant ($P \leq 0.05$) differences between treatments
Where CAT, POD, and SOD stand for catalase, peroxidase, and superoxide dismutase, respectively
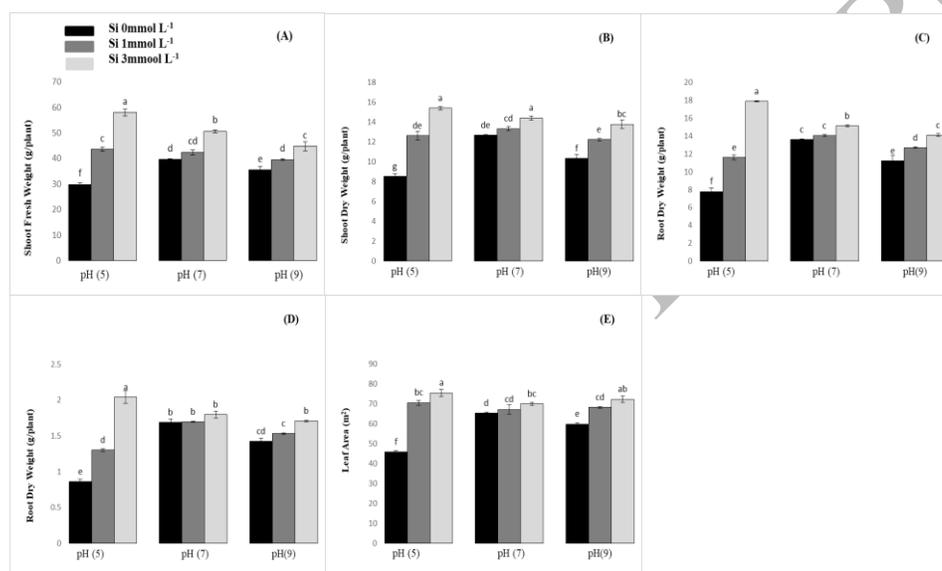
Fig. 1: Effect of silicon application on the shoot and root fresh and dry weights, and leaf area of wheat sown under varying pH levels
Means ±SD (n=9) with different letters in the column indicate significant ($P \leq 0.05$) differences between treatments

Reactive oxygen species (ROS) production and lipid peroxidation

The activities of ROS (hydrogen peroxide; H_2O_2, malondialdehyde; MDA, electrolytic leakage; EL) in roots and leaves were significantly increased by both acidic pH (high H^+ activity) and alkaline pH (high OH^- activity) as compared to control (pH 7; $P < 0.05$) (Table 2). The concentration of H_2O_2 was 106 and 62% higher, MDA was 273 and 252% higher, and EL was 30 and 11% higher in leaves at acidic (pH 5) and alkaline (pH 9) solutions respectively, as compared with control (pH 7; $P < 0.05$). Besides, all of these parameters were significantly decreased by Si 1 and 3 mmol L^{-1} in acidic and alkaline pH, but there was no significant effect of Si at neutral pH. The non-significant effect of Si application on ROS at neutral pH showed that no oxidative stress was produced in wheat plants at neutral pH. Si concentration with 1 and 3 mmol L^{-1}, in acidic solution, reduced H_2O_2 in leaves by 30 and 39%,

MDA in leaves by 37 and 69%, and EL in leaves by 28 and 57%, respectively as compared with alone acidic pH. Similarly, Si concentration with 1 and 3 mmol L^{-1}, in alkaline solution, reduced H_2O_2 in leaves by 13 and 23%, MDA in leaves by 16 and 26%, and EL in leaves by 23 and 31%, respectively for pH 9 as compared with alone alkaline pH. The same trend of ROS concentrations was recorded in roots of wheat plants with the application of Si against acidic as well as alkaline stresses (Table 2).

Nutrients concentration

The concentration of macro and microelements like nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) in roots and leaves were significantly decreased by both acidic pH (high H^+ activity) and alkaline pH (high OH^- activity) as compared to control (pH 7; $P < 0.05$) (Table 3 and 4). In acidic pH, the concentration of N decreased by 25 and 47%, P decreased by 21 and 33%,

Table 2: Effect of silicon application on of the activities of MDA, H$_2$O$_2$, EL and Proline contents in wheat sown under varying pH levels

Treatments	Leaves				Roots		
	MDA (μmol mg^{-1} FW)	H$_2$O$_2$ (μmol mg^{-1} FW)	Proline (μg g^{-1})	EL (%)	MDA (μmol mg^{-1} FW)	H$_2$O$_2$ (μmol mg^{-1} FW)	Proline (μg g^{-1})
pH 5 + Si0	64.88 ± 2.34a	94.49 ± 2.37a	0.061 ± 0.001c	99.05 ±1.12a	24.76 ± 1.21a	58.58 ± 1.79a	0.039±0.003ef
pH 5 + Si1	40.62 ± 3.24c	65.94 ± 2.61c	0.081 ± 0.005b	71.39±1.32cd	17.10 ± 0.32c	38.07 ± 0.33d	0.068±0.001bc
pH 5 + Si3	19.91 ± 0.58de	57.43 ± 1.49d	0.128 ± 0.008a	42.68 ±0.92h	10.97 ± 0.55d	30.21 ± 0.93e	0.083 ±0.004a
pH 7 + Si0	17.38 ± 0.87e	45.78 ± 1.97e	0.030 ± 0.001d	75.65 ±2.37c	8.85 ± 0.04e	25.75 ± 1.33f	0.037 ±0.002f
pH 7 + Si1	23.14 ± 0.23d	39.58 ± 0.42f	0.031 ± 0.001d	63.31±2.80ef	7.56 ± 0.27e	23.09 ± 1.55fg	0.040±0.004ef
pH 7 + Si3	21.80 ± 1.99de	34.84 ± 0.07g	0.025 ± 0.001d	54.73 ±2.19g	7.10 ± 0.10e	21.97 ± 0.98g	0.046 ±0.007e
pH 9 + Si0	61.28 ± 0.91a	74.32 ± 2.01b	0.051 ± 0.004c	84.35 ±1.69b	21.70 ± 0.99b	48.23 ± 0.33b	0.057 ±0.001d
pH 9 + Si1	51.41 ± 1.01b	64.84 ± 2.36c	0.061 ± 0.001c	64.84±1.66de	16.96 ± 0.55c	43.49 ± 1.74c	0.061±0.003cd
pH 9 + Si3	45.61 ± 1.18c	57.06 ± 1.10d	0.075 ± 0.001b	57.84±0.98fg	15.89 ± 0.45c	38.57 ± 0.27d	0.071 ±0.004b

Means ±SD (n=9) with different letters in the column indicate significant (p≤ 0.005) differences between treatments
MDA, H$_2$O$_2$, and EL stand for malondialdehyde, hydrogen peroxide, and electrolytic leakage, respectively
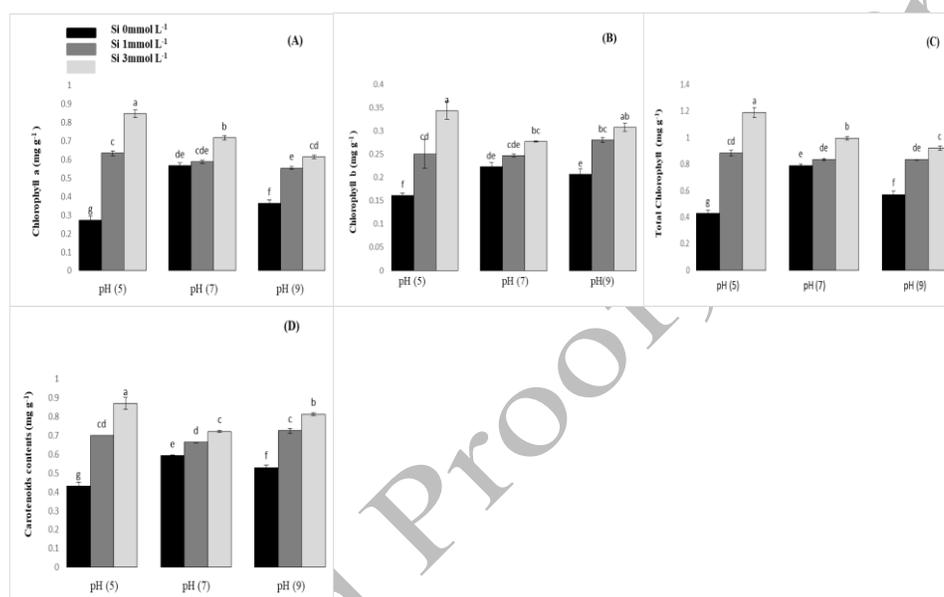
Fig. 2: Effect of silicon application on chlorophyll a, chlorophyll b, total chlorophyll and carotenoids contents of wheat sown under varying pH levels
Means ±SD (n=9) with different letters in the column indicate significant ($P \leq 0.005$) differences between treatments

K decreased by 40 and 34%, Ca decreased by 12 and 17%, and Mg decreased by 3 and 16% in shoots and roots of wheat plants as compared with control (pH 7). Similarly, in alkaline pH, the concentration of N decreased by 12 and 11%, P decreased by 45 and 19%, K decreased by 35 and 28%, Ca decreased by 8% and 0.2%, and Mg increased by 3 and 4% in shoot and roots of wheat plants as compared with control (pH 7). Contradictory results were recorded in the concentration of zinc (Zn) in both shoots and roots, and its concentration was increased with the decrease of pH from 9 to 5 (Table 4). The addition of Si with the concentration of 1 and 3 mmol L^{-1} at acidic and alkaline pH growth medium significantly elevated the concentration of macro and microelements as compared to neutral pH (7). The recorded data showed that Si with 1 and 3 mmol L^{-1} at acidic pH as compared with neutral pH elevated N by 2 and 20%, K by 25 and 49%, P by 19 and 68%, Ca by 33 and 51%, Mg by 10 and 20% and Zn by 20 and 53%, respectively in shoots

of wheat plants. Similarly, Si with 1 and 3 mmol L^{-1} concentration in alkaline solution increased N by 15 and 32%, K by 80 and 85%, P by 21 and 48%, Ca by 7 and 11%, Mg by 3 and 5% and Zn by 14 and 32%, respectively in shoots of wheat plants as compared with alone alkaline pH (Tables 3 and 4).

Tissue-specific silicon concentration

The absorption and accumulation of Si in roots and shoots at varying levels of pH (5, 7, and 9) and Si (1 and 3 mmol L^{-1}) are shown in Table 4. The recorded data showed that Si concentration in roots and shoots of wheat varying with different levels of pH, while the optimum pH for Si uptake and accumulation was recorded at acidic pH 5 (Fig. 3). Si concentration was increased with a decreased pH from pH 9 to 5, as shown in the correlation between Si concentration in plants and pH levels (Fig. 3). Si concentration at pH 5 along

Table 3: Effect of silicon application on the concentration of nitrogen, phosphorus, potassium in shoots and roots of wheat plants sown under varying pH levels

Treatments	Leaves			Roots		
	Nitrogen (mg g⁻¹)	Phosphorus (mg g⁻¹)	Potassium (mg g⁻¹)	Nitrogen (mg g⁻¹)	Phosphorus (mg g⁻¹)	Potassium (mg g⁻¹)
pH 5 + Si0	21.31 ± 0.35g	3.01 ± 0.003f	13.24 ± 0.62e	18.11 ± 0.26f	4.38 ± 0.10f	11.67 ± 0.33f
pH 5 + Si1	32.47 ± 0.57c	4.45 ± 0.03b	26.54 ± 1.19bc	39.54 ± 0.69c	8.83 ± 0.04b	20.42 ± 0.24b
pH 5 + Si3	43.71 ± 0.25a	7.54 ± 0.32a	39.63 ± 0.29a	47.63 ± 1.26a	9.74 ± 0.08a	27.25 ± 0.41a
pH 7 + Si0	28.57 ± 0.30e	3.81 ± 0.07c	22.21 ± 0.62d	34.65 ± 0.68d	6.56 ± 0.13d	17.87 ± 0.49d
pH 7 + Si1	30.49 ± 0.53d	3.75 ± 0.03c	24.56 ± 0.67c	41.31 ± 0.65bc	7.56 ± 0.13c	18.54 ± 0.49cd
pH 7 + Si3	37.18 ± 0.38b	4.03 ± 0.04bc	26.27 ± 0.36bc	43.65 ± 0.85b	8.43 ± 0.17b	19.08 ± 0.62c
pH 9 + Si0	24.98 ± 0.55f	2.09 ± 0.04f	14.42 ± 0.54e	30.73 ± 0.37e	5.26 ± 0.18f	12.83 ± 0.13f
pH 9 + Si1	28.75 ± 0.52e	2.55 ± 0.11e	25.97 ± 0.35bc	35.67 ± 1.16d	6.1 ± 0.05d	15.52 ± 0.11e
pH 9 + Si3	33.09 ± 0.26c	3.12 ± 0.06d	26.78 ± 0.23b	40.31 ± 0.52c	7.47 ± 0.24c	16.09 ± 0.44e

Means ±SD (n=9) with different letters in the column indicate significant ($P \leq 0.005$) differences between treatments
Here N, P, and K stand for nitrogen, phosphorus, and potassium, respectively

Table 4: Effect of silicon application on the concentration of Si, calcium, magnesium and zinc contents in wheat plants sown under varying pH levels

Treatments	Shoots				Roots			
	Si (mg Kg⁻¹)	Zn (mg g⁻¹)	Ca (g kg⁻¹)	Mg (g kg⁻¹)	Si (mg Kg⁻¹)	Zn (mg g⁻¹)	Ca (g kg⁻¹)	Mg (g kg⁻¹)
pH 5 + Si0	0.001 ± 0.00f	383.56 ± 6.08c	14.66 ± 0.06g	2.28 ± 0.01g	0.01 ± 0.005g	431.56 ± 11.21c	12.84 ± 0.33f	1.04 ± 0.01f
pH 5 + Si1	215.26 ± 8.27b	463.69 ± 4.46b	19.56 ± 0.12cd	2.62 ± 0.04b	152.11 ± 6.30d	522.09 ± 11.74b	16.64 ± 0.05bc	1.45 ± 0.02b
pH 5 + Si3	295.42 ± 3.89a	588.30 ± 3.32a	22.16 ± 0.11a	2.99 ± 0.02a	286.08 ± 4.42a	685.16 ± 6.52a	19.46 ± 0.38a	1.55 ± 0.00a
pH 7 + Si0	0.002 ± 0.00f	264.87 ± 17.64ef	16.64 ± 0.33f	2.36 ± 0.00f	0.004 ± 0.003g	354.06 ± 18.89de	15.57 ± 0.01e	1.24 ± 0.03e
pH 7 + Si1	154.97 ± 3.34d	322.36 ± 11.05d	19.09 ± 0.06d	2.45 ± 0.02e	125.12 ± 3.41e	432.53 ± 15.44c	15.93 ± 0.19de	1.33 ± 0.02d
pH 7 + Si3	215.19 ± 8.70b	366.93 ± 2.94c	19.91 ± 0.04bc	2.52 ± 0.02d	218.17 ± 2.34b	493.53 ± 4.06b	16.93 ± 0.02b	1.39 ± 0.01c
pH 9 + Si0	0.001 ± 0.00f	221.36 ± 9.42g	18.02 ± 0.18e	2.45 ± 0.03e	0.002 ± 0.001g	309.15 ± 3.07f	15.54 ± 0.28e	1.30 ± 0.00d
pH 9 + Si1	131.00 ± 8.93e	252.28 ± 9.95f	19.45 ± 0.24d	2.54 ± 0.02cd	105.42 ± 3.96f	345.00 ± 13.65ef	16.23 ± 0.24cde	1.32 ± 0.01c
pH 9 + Si3	177.33 ± 2.79c	294.22 ± 4.62de	20.12 ± 0.21b	2.59 ± 0.01bc	172.44 ± 3.12c	385.73 ± 6.03d	16.58 ± 0.03bcd	1.38 ± 0.01c

Means ±SD (n=9) with different letters in the column indicate significant ($P \leq 0.005$) differences between treatments
Here Si, Zn, Ca, and Mg stand for silicon, zinc, calcium, and magnesium, respectively
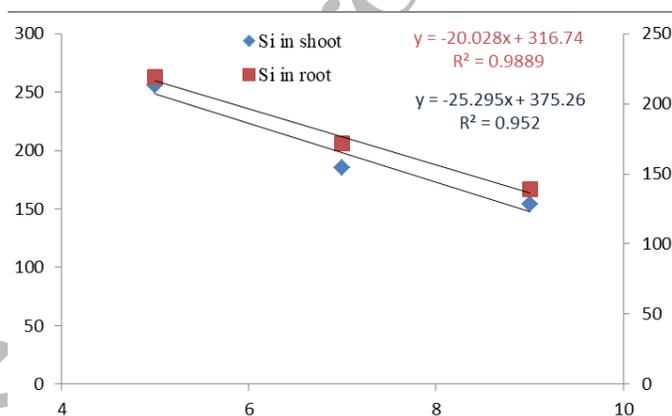
Fig. 3: Correlation between the concentration of available Si and different pH levels of nutrient solution

with Si (1 and 3 m*M*) was 35 and 44% higher in shoots and was 7 and 219% higher in roots as compared to neutral pH along with the same levels of Si (1 and 3 mmol L⁻¹). Similarly, Si concentration at pH 7 along with Si (1 and 3 mmol L⁻¹) was 67 and 63% higher in shoots and was 24 and 63% higher in roots as compared to highly alkaline pH 9 with the same levels of Si (Table 4).

Discussion

Results of this hydroponic study disclosed that all recorded growth and biochemical parameters were not significantly affected at neutral pH (7) with or without Si supplementation in wheat seedlings. However, a significant decline in recorded physiological parameters was observed in acidic as well as alkaline pH. For instance, the leaf area (Fig. 1), plant dry biomass (Fig. 1), chlorophyll contents (Fig. 2), macro and microelements (Tables 3 and 4) were significantly low while, antioxidant enzymes activities (Table 1), membrane injury contents measured as MDA (Table 2), oxidative stress contents measured as H_2O_2 (Table 2), cellular membrane damage contents measured as cell electrolytes (Table 2) were significantly high at both acidic and alkaline nutrient solutions. It showed that alkaline

and acidic stresses are one of the significant abiotic strains that can affect plant growth and development (Yang *et al.* 2008; Odiyi and Bamidele 2014; Wyrwicka and Skłodowska 2014).

Addition of Si at the concentration of 1 and 3 mmol L^{-1} in nutrient solution significantly alleviated harmful effects of acidic and alkaline stresses by further elevating enzymatic (CAT, POD and SOD) and non-enzymatic (proline) antioxidants and restricting the production of various reactive oxygen species like MDA, H$_2$O$_2$, and EL in both roots and shoots of wheat seedlings. These findings are parallel with previous studies (Ju *et al.* 2017; Aldesuquy *et al.* 2018). The interesting thing in present research that two levels of Si 1 and 3 mmol L^{-1} were used against three levels of pH (5, 7, and 9) while, the most significant results were recorded at Si 3 mmol L^{-1} at acidic pH (Fig. 1–2; Tables 1–4).

Acidic and alkaline pH (5 and 9) significantly lowered the leaf area, whole plant dry weight and photosynthetic rate (Fig. 1 and 2), as compared to neutral pH (7). The reduced growth of wheat crop plants in acidic and highly alkaline pH may be due to H$^+$ and OH$^-$ ions toxicities, as indicated by the deficiencies of macro and microelements (Tables 3 and 4). In this study, uptake of N, P, K, Ca, Mg, and Zn were significantly affected by the pH (Table 3 and 4). Uptake of N, P, K, Ca, and Mg significantly increased with the increase of pH, but uptake of Zn showed a significant decrease with the increase of pH. These findings were in line with previous studies that the availability of all essential nutrients is highly dependent on the pH of nutrient solution (Abbasi *et al.* 2017; Gentili *et al.* 2018). For instance, plants absorb N in the form of ammonium (NH$_4^+$) and nitrate (NO$_3^-$) in the sub-acidic environment (5.87), which plays a central role in plant biomass synthesis (Gentili *et al.* 2018). The plant absorbs Mg in neutral to slightly alkaline pH (7.28), which plays a central role in carbon fixation and chlorophyll biosynthesis (Hermans and Verbruggen 2005). Plants absorb Ca in neutral to slightly alkaline pH (7.28), which regulates the biochemical and physiological responses and maintains cell wall structure and membrane functions in plants under various stresses (Reddy *et al.* 2011). In our finding Si application with a preference of 3 mmol L^{-1} increased plant biosynthesis by enhancing N concentration, increased photosynthetic rate by enhancing Mg concentration, and increased cell wall structure and membrane functions by enhancing Ca concentration in roots and shoots of wheat plants under hostile conditions of acidic and alkaline stresses (Table 3 and 4). It might be due to Si buffering capacity in low and high pH environments, which leads the pH of the nutrient solution to an appropriate range and made the nutrients available to wheat plants. Our results were in the line of previous findings, where silicates minerals were found as pH-buffering agents to maintain pH to the neutral range (Lacroix *et al.* 2014). Moreover, in our findings, Si enhances the concentration of phosphorus (P) in both acidic and alkaline pH stresses, which might be due to the decline of P-sorption in the nutrient solution. Koski-

Vähälä *et al.* (2001) reported that in an acidic environment, Si increases plant-available P portion by reducing soil sorption of P. In contrast, Zn concentration was highest at acidic pH as compared to neutral and alkaline pH (Table 4). It might be due to the higher adsorption of Zn in slightly alkaline to highly alkaline environments. These results are supported by Long *et al.* (2017) that acidic pH significantly increased the concentrations of Zn up to the toxic level in *C. grndis* and *C. sinensis*. Although, Si fertigation in nutrient solution with a preference of 3 mmol L^{-1} increased Zn concentrations in roots and shoots of wheat crops in alkaline and neutral pH environments (Table 4). It showed that Si reduces the adsorption of Zn and makes it available to plants in slightly alkaline to highly alkaline environments. In conclusion, Si application significantly alleviated both acidic and alkaline stresses by enhancing the availability of all macro and micronutrients in wheat crop plants to the balanced amount (Table 3 and 4). Similar results have been documented previously (Ju *et al.* 2017; Aldesuquy *et al.* 2018).

Under various environmental stresses, reactive oxygen species (ROS), containing malondialdehyde (MDA), hydrogen peroxide (H$_2$O$_2$), and electrolytic leakage (EL) are accumulated in plants can cause oxidative damage to vital biomolecules (Foyer and Shigeoka 2011). ROS causes lipid peroxidation, DNA mutation, and protein denaturing by oxidation of lipids, nucleic acid, and proteins, respectively (Ahmed *et al.* 2008). In previous findings, MDA contents are considered as an indicator of lipid peroxidation (Shi *et al.* 2006), which causes membrane permeability and damage (Ahmad *et al.* 2010). Besides, excess accumulation of MDA causes the linkage of nucleic acid, lipid, protein, and sugar. As a result, the function and structure of the plasma membrane were damaged (Shi *et al.* 2006). Moreover, higher production of H$_2$O$_2$ in leaves lowered the assimilation of CO$_2$ in leaves, resulting in inhibition of photosynthesis (Long *et al.* 2017). Additionally, EL has been established as the indicator of the damage of membrane-associated fatty acids, which ultimately destabilize membranes in abiotic stresses (Kim *et al.* 2016). In this study, acidic and alkaline stresses boosted up the concentrations of ROS (MDA, H$_2$O$_2$, and EL), resulting in lipid peroxidation, membrane permeability and damage, and oxidative burst (Table 2). While, application of Si with a preference of 3 mmol L^{-1} significantly encountered the adverse effects of acidic as well as alkaline toxicities by hindering the production of MDA, H$_2$O$_2$, and EL in both roots and shoots of wheat plants (Table 2).

Moreover, plants have various enzymatic and non-enzymatic antioxidant defense systems to encounter the negativity of ROS (Wyrwicka and Skłodowska 2006; Zhang *et al.* 2015). Enzymatic antioxidant defense system comprises of catalase (CAT), superoxide dismutase (SOD), and peroxidases (PODs) (Şen 2012). The plant survival in oxidative stress is associated with the upregulation of SODs. The SOD, as first cell defense line (Michiels *et al.* 1994),

converts superoxide anions (O_2^-) into oxygen and hydrogen peroxide molecules, hydrogen peroxide further decomposed into water and molecular oxygen by catalyzing CAT and POD to maintain the level of hydrogen peroxide inside the cells (Kusvuran *et al.* 2016). Alike SODs, peroxidases (PODs) also play important roles in scavenging and consuming H_2O_2 through a series of metabolic processes to modify the levels of ROS (Howladar *et al.* 2018). Rather than SODs and CAT, PODs are more affinitive to H_2O_2; however, PODs may also produce H_2O_2 through the oxidation of NAD(P)H like molecules (Ranieri *et al.* 2005). Numerous studies have been established the dependence of the enzymatic antioxidants on pH (Jin *et al.* 2006; Ismaiel *et al.* 2016). They reported that at extreme pH levels (4 and 11), antioxidant machinery began to collapse or fail to function normally. In contrast of their findings, our study showed minimum activity of SOD and CAT at neutral pH (SOD; 19.6743 and CAT; 3.12 μmol g^{-1} SFW) followed by alkaline pH (SOD; 26.0443 and CAT; 4.62 μmol g^{-1} SFW), while maximum activity at acidic pH (SOD; 33.21 and CAT; 4.31 μmol g^{-1} SFW). The minimum activities of SOD, POD, and CAT at neutral pH indicate the non-limiting behavior of neutral pH for wheat plants. Moreover, our results emphasized Si as an effective beneficial element to encounter the toxicities of acidic as well as alkaline pH by further elevating the activities of antioxidants (Table 1). These results are in line with Ju *et al.* (2017) reported that Si could enhance rice tolerance to acidic induced oxidative stress by increasing the efficiency of antioxidant enzymes. Aldesuquy *et al.* (2018) showed in their findings that Si triggers antioxidant phenols in sorghum (*Sorghum bicolor* L.) plants to encounter alkaline stress.

Another defense system is non-enzymatic antioxidants included various osmoprotectants (proline) and compatible solutes (Rios *et al.* 2017). Proline, as an osmoprotectant, created a balance between cytosol and vacuole osmotic strengths and external environmental osmotic strength to protect plant cells under abiotic stresses (Gadallah 1999; Howladar *et al.* 2018). Furthermore, proline produces a response to osmotic pressure by contributing to osmotic adjustments in plant cells (Zhang *et al.* 2017). In this study, proline contents were highest at acidic and alkaline pH as a response of oxidative stress caused by overproduction of ROS as compared to neutral pH (Table 2), while proline contents were then further increased with Si incorporation in nutrition solution to alleviate oxidative stress in acidic and alkaline toxicities.

Conclusion

Being an effective beneficial element, Si application improved leaf area, plant biomass, membrane characteristics, photosynthetic rate, and increased nutrient availability under acidic and alkaline stress. Furthermore, Si-supplemented wheat plants exhibited more tolerance to acidic and alkaline stresses through hindering ROS production accompanied by MDA and EL activity and by improving CAT, SOD, POD, and osmolyte (proline) contents that are the primary line of defense to scavenge oxidative stress.

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