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Research article

Silicon ameliorates cadmium (Cd) toxicity in pearl millet by inducing antioxidant defense system

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ABSTRACT

Cadmium (Cd) stress is a significant environmental pollutant that can negatively impact crop yield and growth, and is a serious global issue. However, silicon (Si) has been shown to have a potential function in alleviating the effects of several abiotic stress conditions on crops, including Cd stress. This study investigated the effectiveness of applying silicon to soil as a method for reducing cadmium toxicity in pearl millet (IP14599) seedlings. Seeds of IP14599 were treated with Si + Cd element which cumulated to a combination of 9 treatments. Different Cd concentration of (0, 200, and 300 mg/kg-^{1}) was taken and manually mixed into a sieved soil prior to planting and Si (0, 100 and 200 mg/kg-^{1}) was selectively introduced till after attaining 12 days of seedling emergence. The physiochemical parameters of Cd stressed plants investigated includes chlorophyll, gas exchange attributes, proline, relative water contents, malondialdehyde (MDA) content and antioxidant enzymes (superoxide dismutase (SOD),catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD). Our result revealed that the metal (Cd) caused serious oxidative impairment thereby reducing photosynthetic performance, increased activity of MDA and Cd content in the roots and leaves of IP14599.In addition, Si increased the growth pattern and antioxidant defense action thereby mitigating the Cd toxicity. The results revealed that at Si 200, Si significantly increased the chlorophyll, carotenoids and plant height at 122 %, 69 % and 128 % under the Cd 200 and Cd 300 mg/kg-^{1} treatment, respectively. The single treatment at Si100 and Si 200 decreased ROS by 29 %, and 37 % respectively and MDA decreased by 33 % and 43 % in contrast to Cd 200 and 300 treatments, respectively. However, Si200 showed significant increase in the activities of APX 97 %, SOD by 89 %, CAT 35 % and POD 86 % as compared to single Si, Cd or combine Cd + Si treatment. Also, a gradual decline in Cd level in both the leaf and root was present when exposed to high concentrations of Si at Si200 and 300 mg/kg-^{1}. Our findings revealed that Si might significantly increase the capacity to tolerate Cd stress in crop plants. Therefore, the study revealed that Si has the potential to alleviate Cd-induced toxicity by reducing Cd assimilation and enhancing the growth attributes of IP14599 plants.

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1. Introduction

Many abiotic stress factors are becoming more prevalent worldwide, and they can have a detrimental effect on crops. The abiotic stress factors like cold, drought, salt and metals affects crop development. Climate and soil are regarded as two factors that make them distinct from other abiotic stress and become more severe to be monitored [1]. Soils which are contaminated by heavy metals and toxic elements is a huge problem for food sustainability in the near future. Pollution in soil is a major challenge to food production worldwide, as it negatively impacts plant biomass and growth [2]. The element such as cadmium (Cd) is a highly letal metal that is found in arid soils as a result of anthropogenic and natural origin. Cadmium (Cd) is a toxic element that is commonly found in our environment, often as a result of the use of phosphatic fertilizers and industrial effluents [3–5]. Cadmium inhibits translocation and assimilation of water and nutrients. Many plants have the ability to reduce the negative effects of cadmium (Cd) stress by activating their antioxidant defense machinery. Antioxidants can modulate the gene expression pattern of stromal proteins and thylakoids in Cd-stressed plants, leading to an improvement in photosynthetic performance [6]. Farooq et al. [7] found that Cd stress can be lethal to certain plants, leading to a decrease in antioxidant enzyme levels and an increase in malondialdehyde content. Moreover, certain plants face lethal Cd stress showing decreased level of antioxidant enzymes in relation to higher strength of malondialdehyde content. The chemical form of Cd is cadmium oxide (CdO). Man-made causes, like smelting and metal mining, phosphate fertilizer and wastewater irrigation treatment are the main gateway of soil contamination with Cd [8,9]), which give 8 fold change increase in Cd compared to those from natural sources [10,11]. When plants are sown in soils contaminated with Cd metal, Cd have the potential to accumulate in plants resulting to yield decrease in photosynthesis efficiency and nutrient imbalances [12]. Liu et al. [9] reported that the increase in mobility and solubility of Cd makes it very potent in the soil-plant–water relationship. Therefore, immediate strategies are required to mitigate Cd absorption and accumulation in crop plants. The grass, Pearl millet [*Pennisetum glaucum* (L.) R.Br.] is regarded to be a drought resistant crop which is important for silage, hay and grazing and it is sown in sub Saharan Africa and Southern Asia [13–15]. The crop is majorly grown for grains and forage usage to feed humans and animals due to that it contains zinc and iron in comparison to most grasses and it is also useful for biogas production [16,17]. Pearl millet possess the capacity to survive under heat and water stress [18,19]. However, the silicon-mitigating Cd stress resistance process remains unclear in IP14599. Silicon (Si) is one of the valuable elements naturally present in plants. Silicon (Si) is the second most abundant element on Earth's surface after oxygen. However, most silicates and oxides of this essential metal are not readily available to plants [20]. It is a complex growth supporter and a significant phytonutrient required for normal metabolic and physiological processes of crop plants exposed to abiotic stresses. The presence of Si in the epidermal, vascular, and tissue structures enhances strength and rigidity, thereby regulating physiological activities of plants and protecting them from notable stress-inducing factors [20,21]. Although Si is a beneficial element, it is not considered to be an essential element for plant health. The chemical form of Si is silicon dioxide (SiO2) or as silicates.The element goes into the roots of plants in available forms like mono silicic acid [H_4SiO_4] [22]. There are two main Si transporters, which are Si Transporter 1 and Si Transporter 2 [23]. They are valuable for transporting Si in plants generally. Si revealed a positive impact on crop development, chlorophyll pigments content and decreased the fluoride induced membrane damage in *Oryiza sativa* [24]. Generally, Si stabilizes ROS combating systems which include antioxidants like SOD (superoxide dismutase),APX (ascorbate peroxidase), POD (peroxidase), CAT (catalase), also other antioxidants such as glutathione, flavonoid, ascorbic acid in relation to oxidative cell damage [25,26]. Formerly, Zargar et al. [22] found that Si maintains the uptake and absorption, movement and rapid mobility of elements, like metals, permitting most plants to live in lethal polluted environments. Our findings on Cd stress ameliorating ability of Si, showed that single or combined application of Si element might reduce Cd uptake and cause beneficial effect on the growth and development of *P. glaucum* plants. Our research aimed to evaluate the effect of single and combined Si on the growth pattern, photosynthetic performance, and antioxidant defense action of Cd-exposed pearl millet seedlings. The findings of this research provide valuable information on Si-assisted Cd tolerance in P14599 seedlings, which could be advantageous for most grass crops exposed to Cd toxicity, thereby contributing to food security and sustainability.

2. Materials and methods

2.1. Plant sample and treatment

The whole experiment was conducted in a greenhouse facility at Federal University, Oye Ekiti State, Nigeria. The seeds of pearl millet (IP14599) were sourced from International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, India. Pearl millet is used widely grown on a huge scale due to its adaptability to high humidity and tolerance to drought stress. The variety (IP14599) is known for high yield and our previous studies have identified that this variety has the ability to tolerate drought stress [18,19]. The method of Khan et al. [15] was employed to surface sterilize the seeds of IP14599 with 0.5 % NaCl solution for 12

Table 1
Treatment combinations of different levels of cadmium and silicon (9 treatments).

Silicon (Si) (mg kg^{-1})	Cadmium (Cd) (mg kg^{-1})		
	0 Cd (mg kg^{-1})	200 Cd (mg kg^{-1})	300 Cd (mg kg^{-1})
0 Si (mg/kg $^{-1}$)	0 Si + 0 Cd (mg kg^{-1})	0 Si +200Cd (mg kg^{-1})	0 Si +300Cd (mg kg^{-1})
100 Si (mg/kg $^{-1}$)	100Si + 0 Cd (mg kg^{-1})	100Si+200Cd ($mgkg^{1}$)	100Si+ 300Cd ($mgkg^{-}$)
200 Si (mg/kg $^{-1}$)	200Si + 0 Cd (mg kg^{-1})	200Si+ 200Cd ($mgkg^{-}$)	200Si+ 300Cd ($mgkg^{1}$)

min. The seeds were then air dried and sowed in clay pots. Four-kilograms of soil was measured into each of the clay pots and then three seeds were sown into each pot. The soil composition comprised of sand 31 %, clay 34 %, silt 52 % and organic matter content 2.14 %. A completely randomized design layout was used for the experiment. Afterwards, IP14599 were thinned after twelve days and three seedlings were transferred in every pot. Each pot contained a mixture of soilrite (1:1) and 2 kg soil, then treated with varying Cd concentrations (0, 200 and 300 mg/kg) using $CdCl_2$. The clay pots was kept in the screen house at a temperature of 26±3 °C, 16 h photoperiod and relative humidity of 60–65. Some pearl millet plants were used as control and only irrigated with double distilled water. The Cd treatment was administered to the plants in the form of $CdCl_2$ which was directly added to the soil at varying concentrations of 0, 200, and 300 (mg/kg-^{1}) to the IP14599 plants. Si (soil treatment) was applied to plants at varying concentrations of Si (0, 100 and 200 mg/kg-^{1}) at 22–30 days after sowing. The experiment had 9 combinations of treatments as found in Table 1. Biochemical assays and physiological analysis of IP14599 was carried out plants 30 days after sowing.

2.2. Growth indices

Plant weight of IP14599 was measured to determine the biomass according to the formular of Guo et al. [27]. The height of the seedlings was measured using a tape ruler, which was placed at the base of the pot and extended to the height of the tallest leaf. The biomass was measured using a weighing balance. The plant was cut at the base of the soil surface, ensuring that all leaves and stems were on the balance. Plant biomass was read from the weighing balance and recorded in grams.

2.3. Gas exchange parameters and photosynthetic pigments

After the stress treatments, the extraction of photosynthetic pigments was done with 80 % acetone. A clear supernatant portion was collected for measuring total chlorophyll content and carotenoid after centrifugation for 3 min at 480×*g* by using the extinction coefficients according to the procedure of [28]. Gas exchange parameters like transpiration rate, stomatal conductance (gs) and photosynthetic rate (Pn) were determined by using LI-COR (Lincoln, NE, USA) at 30 days after germination between 9:00 to 10:00 a.m. Five individual plants were selected from the treatments after being measured. To estimate photosynthetic efficiency, the leaf was removed and clipped to the surface area using an image analysis system called Delta-T Scan (Cambridge, CB50EJ). The values recorded from the gas exchange attributes were collected based on the efficient photosynthetic area.

2.4. Malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and proline content

The MDA content was determined with thiobarbituric acid (TBA), following the procedure of [29].The leaves were homogenized in 1 % (w/v) trichloroacetic acid (TCA). After centrifugation, 4 mL of 20 % TCA containing 0.5 % (w/v) TBA was added to 1 mL of supernatant. The mixture was incubated at 95 °C for 30 min. The absorbance was measured at 532 nm and 600 nm and the concentration of MDA was calculated by using the extinction coefficient of 155 mM per cm.

The protocol of [30] was used to determine H_2O_2 concentration using a spectrophotometer (UV752 N, Shanghai Precision & Scientific Instrument Co.). A blank use of 1 mL sodium carbonate buffer solution pH 10.2 in a 1.5 mL reaction tube, then preparation of ten reaction tubes per sample with 998 μL was used for a working solution to which 2 μL of the catalase-treated sample were added, homogenizing and immediately quantifying the emitted chemiluminescence (CL). The emitted photons of the samples are measured with a luminometer in suitable tubes at 425 nm for 5–8 s. The difference between catalase-treated and untreated samples (ΔCL) is considered H_2O_2-specific CL. The H_2O_2 content of the samples was calculated from the standard curve.

The protocol of Bates et al. [31] was used to estimate proline content using a spectrophotometer140 (UV752 N, Shanghai Precision & Scientific Instrument Co.). Approximately 0.5g of plant material was homogenized in 10 mL of 3 % aqueous sulfosalicylic acid and the homogenate was filtered through Whatman 2 filter paper. Two mL of filtrate was mixed with 2 mL of acldninhdrin and 3 mL of glacial acetic acid in a test tube for 1 h at 100 °C, and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 mL toluene and mixed vigorously with a test tube stirrer for 15–20 s. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene for a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows: Proline (mg. g−1 FW) = [(μg proline/mL × 4 mL toluene)/(0.5 g sample/2.5)]/1000.

2.5. Relative water content (RWC)

The measurement of RWC was conducted following the formulae of [32], RWC═(FW–DW)/(TW–DW) x 100 %. Leaves were weighed to calculate the fresh weight (FW) of stressed leaves; the same biological leaves were immediately placed in 100 mL of double distilled water for 12 h to estimate the turgid weight (TW) of the leaves. The leaves were further kept in envelopes and oven dried at 65 °C for 48 h to estimate the dry weight (DW).

2.6. Activities of peroxidase (POD), superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX)

Following Lu et al. [33], the liquid fraction resulting from the procedure was used to determine the ascorbate peroxidase (APX), peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) activities. Antioxidant enzyme activities were estimated using a spectrophotometer (UV752 N, Shanghai Precision & Scientific Instrument Co.). The methods of [34] were used to measure POD. The enzyme extract (0.5 mL) was mixed with 1 mL phosphate buffer (0.1 M), 1 mL pyrogallol (0.01 M) and 1 mL H_2O_2 (0.05 M) and then incubated (5 min 25 °C). To stop the reaction, H_2SO_4 (2.5 N) was added and the absorbance of the samples was immediately calculated at 420 nm.

Catalase (CAT) activity was measured by the method of [35]. The total mixture (3.0 mL) comprised of enzyme extract (100 μL), H_2O_2 (100 μL; 300 mM) and 50 mM phosphate buffer (2.8 mL) with 2 mM EDTA (pH 7.0) used for the determination of the CAT activity by taking the absorbance readings at 240 nm using the reduction of H_2O_2.Ascorbate peroxidase (APX) activity was evaluated using Nakano and Asada [36] technique. The reaction mixture contained enzyme extract (100 μL), 100 μL ascorbate (7.5 mM), 100 μL H_2O_2 and 2.7 mL 25 mM potassium phosphate buffer and 2 mM EDTA (pH 7.0). The absorbance at 290 nm was measured based on the oxidation of ascorbate.

Superoxide dismutase (SOD) activity was determined by Ref. [37]. One unit of SOD activity was defined as the amount of enzyme required to cause 50 % inhibition of the rate of nitro blue tetrazolium (NBT) reduction at 560 nm.

2.7. Analyses of Cd content

The protocol of [38] was employed to analyze the Cd content in root and leaf parts of IP14599. The samples were oven dried and homogenized to fine powder with a mortar and pestle. The dried powder of (5 g) was further stored in cellophane bags for other use. The samples were then subjected to acid digestion following a mixture of nitric acid and sulphuric acid (2:1 ratio), the treated samples were now filtered and diluted with double distilled water. The use of an atomic absorption spectrophotometer was then used to analyze the Cd content.

2.8. Phytoremediation potential

To verify the phytoremediation activity of IP14599 plants at varying concentration of Si when exposed to Cd stress, the translocation factors were investigated according to the formular of [39]. $BCF = C_{plant}/C_{Cd} + C_{Si}$ Where C_{plant} control plant and $C_{Cd} + C_{Si}$ are the concentrations of elements in plants treated with single and combined Cd and Si.

2.9. Statistical analysis

The results used in this work were all analyzed with the use of one-way analysis of variance (ANOVA). The data had means of four replicates for each treatment with standard error (SE) at $P < 0.05$. The differences among the treatments were achieved through post hoc Turkey's test. Non similar alphabets letters signify the significance differences that exist among the 9 different treatments. The bar chart was drawn by using sigma plot version 12. Principal component analysis (PCA) was used to measure variables of IP14599 by with "past" software.

3. Results

3.1. Growth attributes

In Table 2, our results showed how Cd stress affects the growth pattern of IP14599, while Si in soil treatment ameliorated the negative effects of Cd. The Cd-200 (mg/kg) decreased leaf area, dry biomass of shoot and root, plant height by 62 %, 74 %, and 71 %, 72 % more than in the control (0). The Si at Si-100 enhanced the leaf area 34 %, shoot weight by 62 %, root dry weight 84 % and plant height by 90 % in comparison to Cd300 (mg/kg). Similarly, Si at Si200 revealed a remarkable significant increase in the dry weight of shoot and root, leaf area and plant height by 149 %, and 151 %, 57 %, 137 % in contrast to Cd-200 (mg/kg). However, Si gradually increased IP14599 growth when exposed to Cd stress. The two varying concentrations (Si100 and Si200) of Si showed an increase in the growth of IP14599 in contrast to combined treatment of Si under Cd stress or to corresponding Cd-single or combine treatments. (Table 2).

Table 2
Effect of silicon application on growth indices attributes of IP14599 exposed to Cd stress.

Treatment	Plant height (cm)	Leaf area (cm^{-2})	Shoot dry weight (g)	Root dry weight (g)
0	82.4 ± 1.24a	54.7 ± 2.11a	39.4 ± 0.89a	12.3 ± 0.16a
Si100	57.6 ± 1.35b	48.5 ± 2.75c	37.8 ± 1.69b	10.5 ± 0.15c
Si200	53.8 ± 1.34d	52.4 ± 1.69b	39.1 ± 2.51a	11.7 ± 0.17b
Cd200	41.8 ± 1.45c	44.7 ± 1.69e	29.5 ± 0.79e	7.6 ± 0.11d
Cd300	36.7 ± 2.11e	39.2 ± 1.84f	25.1 ± 0.52f	6.7 ± 0.21e
Cd200+Si100	59.4 ± 2.06b	45.4 ± 2.14c	32.7 ± 0.87d	7.9 ± 0.32d
Cd200+Si200	63.2 ± 1.89c	48.6 ± 1.86b	33.5 ± 0.92dc	8.2 ± 0.49b
Cd300+Si100	54.3 ± 1.53b	43.2 ± 1.95d	31.7 ± 1.05c	7.1 ± 0.49d
Cd300+Si200	58.3 ± 1.99d	45.6 ± 2.10b	33.7 ± 1.06b	7.5 ± 0.37e

Values presented are mean ± SE (n = 4); Different alphabet letters denote significant mean difference using ANOVA analysis ($P \leq 0.05$) by Tukey's test, while similar alphabet letters show no significance difference.

3.2. Gas exchange parameters and photosynthetic pigments

It was observed that the Cd stress caused a negative impact on photosynthetic performance of IP14599 (Fig. 1). Our result revealed a significant (Cd200 (mg/kg) reduction in total chlorophyll, transpiration rate, carotenoids, net photosynthetic rate and stomata conductance by 73 %,73 %, 54 %,77 % and 81 % in comparison to the control (0) exposed to Cd stress. Si applied at different levels enhanced the photosynthetic activities of IP14599 (Table 3; Fig. 1). Moreover, Si at Si-100 increased the chl by 96 % (Table 3), carotenoids 42 % (Table 3), stomatal conductance 98 % (Fig. 1B), transpiration rate 97 % (Fig. 1C) and net photosynthetic rate 95 % (Fig. 1A), in comparison to Cd-200 (mg/kg) and to the corresponding Cd treatments alone. Also, at Si200 was shown to significantly enhance chl, net photosynthetic rate, carotenoids, transpiration rate and stomatal conductance and by 95 %, 99 %,73 %,129 % and 147 % respectively in contrast to Cd-300 (mg/kg) (Fig. 1A,B,C). We observed that the highest concentrations of Si (Si-100 and Si-200) considerably increased the photosynthetic performance of IP14599 when exposed to Cd stress. Generally, Si has proven to increase the gas exchange characters and photosynthetic activities of IP14599 when exposed to Cd stress in this study.

3.3. Relative water content (RWC) and proline

Cd stress showed a negative impact on RWC and proline activity in IP14599; Si enhanced RWC and proline at the varying

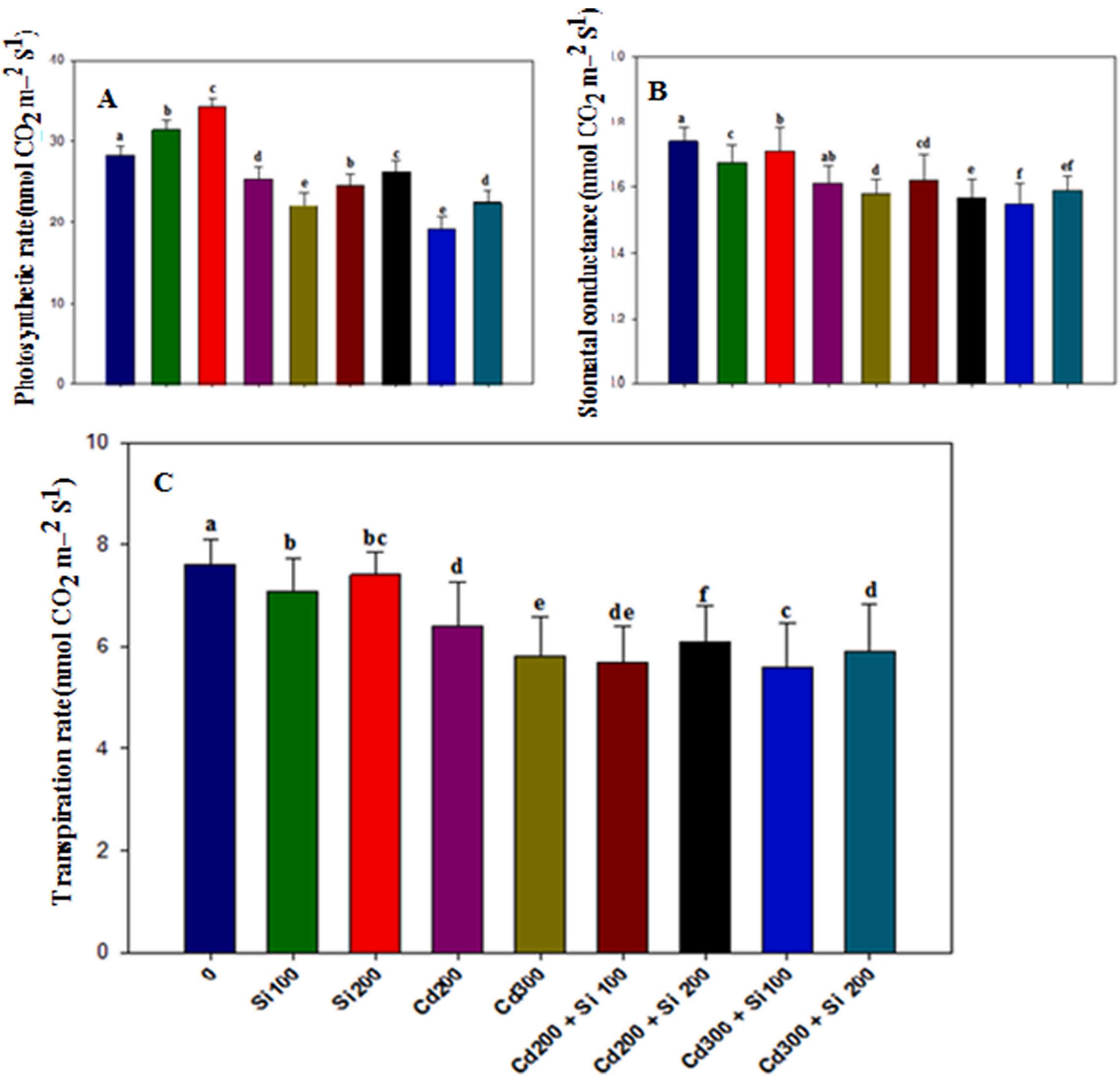

Fig. 1. Effect of silicon application on gas exchange parameters of IP14599 exposed to Cd stress. Photosynthetic rate (A) Stomatal conductance (B) and Transpiration rate (C). Values presented are mean ± SE (n = 4). Non similar alphabet letters denote significant mean difference using ANOVA analysis ($P \leq 0.05$) by Tukey's test, while similar alphabet letters show no significance difference. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Effect of silicon application on chlorophyll pigments of IP14599 exposed to Cd stress.

Treatment	Total Chl (mg g^{-1}FW)	Carotenoid (mgg^{-1}FW)
0	4.86 ± 0.72a	5.32 ± 0.74a
Si100	3.81 ± 0.76b	4.11 ± 0.83c
Si200	3.94 ± 0.81c	4.35 ± 0.85b
Cd200	2.68 ± 0.52d	3.11 ± 0.27d
Cd300	2.19 ± 0.34e	2.82 ± 0.18cd
Cd200+Si100	2.11 ± 0.17f	2.64 ± 0.16e
Cd200+Si200	2.69 ± 0.24c	2.71 ± 0.19BCE
Cd300+Si100	2.37 ± 0.21b	2.69 ± 0.23c
Cd300+Si200	2.42 ± 0.31cd	2.82 ± 0.25d

Values presented are mean ± SE (n = 4); Different alphabet letters denote significant mean difference using ANOVA analysis ($P \leq 0.05$) by Tukey's test, while similar alphabet letters show no significance difference.

concentration of Cd stress (Fig. 2). The treatment with Cd200 (mg/kg) clearly elevated the level of proline activity by 27 % (Fig. 2A) and cause a decrease in RWC by 46 % (Fig. 2B) against the control. However, all concentrations of Si enhanced the level of proline and the leaf RWC in IP14599 at Cd treatment. Moreover, we observed a remarkable improvement at Si-100. Similarly, treatments Si-200 significantly enhanced the proline level by 59 % (Fig. 2A) and RWC by 27 % (Fig. 2B) in IP14599 as compared with those plants under Cd-300 (mg/kg). Overall, the treatment of Si at Cd200+Si100 and Cd200+Si200 was shown to be more preferred in comparison to other Cd + Si concentrations to enhance RWC and proline of IP14599 under Cd single or as compared to combine Cd and Si treatments (Fig. 2).

3.4. Antioxidants enzyme activities and oxidative damage

The Cd stress significantly increase cell membrane damage in the activities of MDA content and H_2O_2 and revealed a negative influence in the activities of antioxidative enzyme as shown in (Fig. 2C and D). Contrastingly, Si showed a decrease in oxidative stress from Cd thereby increasing the enzymes activities when exposed to Cd stress condition. Overall, Cd enhanced MDA by 174 % (Fig. 2C) and H_2O_2 by 96 % (Fig. 2 D) as compared with their control. The gradual increase in the different Si concentrations revealed a significant upregulation in enzymes activities during Cd stress treatment. The Si alone treatments revealed a decrease in MDA content and

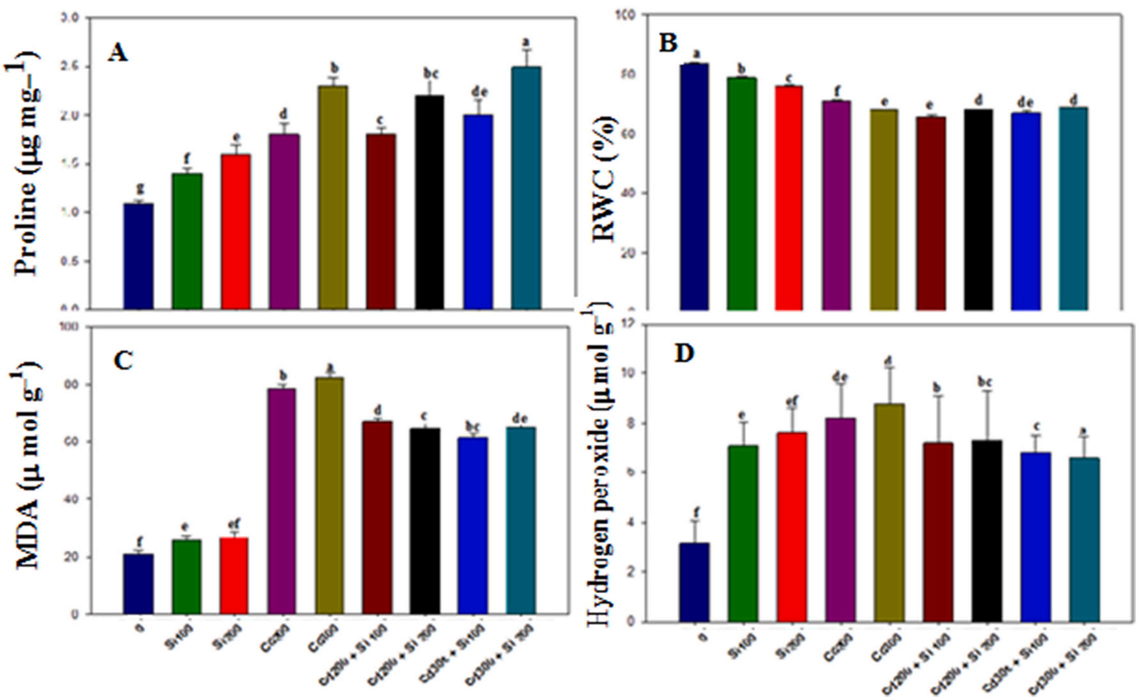

Fig. 2. Silicon treatment mitigate membrane damage in IP14599 exposed to Cd stress. Proline (A) RWC (%) (B) MDA (C) and Hydrogen peroxide (D). Values presented are mean ± SE (n = 4). Non similar alphabet letters denote significant mean difference using ANOVA analysis ($P \leq 0.05$) by Tukey's test, while similar alphabet letters show no significance difference. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

H_2O_2 in IP14599 leaves moreover, Si100 and Si200 showed a decline in H_2O_2 by 45 % and 58 %, and MDA by 57 % and 63 % in contrast to Cd-200 (mg/kg) (Fig. 2C and D). Also, the elevation in the activities of enzymes was revealed at Cd200+Si100 and at Cd300+Si200 when in comparison to other Si concentrations during Cd treatments. However, Cd-200 (mg/kg) increased CAT, SOD, APX and POD activities by 62 %, 31 %,30 % and 24 % in contrast to their control and to other corresponding Cd-single and combine Cd + Si treatments (Fig. 3). We observed that CAT, POD, APX and SOD activities showed a gradual increase by 91 %, 82 %, 95 % and 64 %, at Si-200 against the Cd-300 (mg/kg) (Fig. 3A,B, C and D).Furthermore, other Si + Cd treatments were also positive to enhance the antioxidant defense system as compared to other Cd treatments alone or combine (Fig. 3A,B, C and D).Therefore, the Si application has proven to be beneficial to enhance the antioxidative defense system of IP14599 when under Cd stress.

3.5. *Estimation of Cd in leaf and root*

The amount of Cd was estimated in the leaf and root parts of IP14599 sown under varying concentration of Cd (Fig. 4). The treatment of Si showed a different impact on the level of Cd in leaves and root of IP14599 plants. Also, Si treatments at all levels showed a linear decrease in Cd concentration in IP14599 plants. The Si-100 and Si200 treatment considerably reduced the Cd content in leaf and root (Fig. 4).The Cd values was observed as 14.6 % in leaf (Figs. 4A) and 17.4 % in root (Fig. 4B) of IP14599 sown during Cd stress alone. However, Si at Si100, Si-200, Cd200+Si100 and Cd300+Si200 significantly decreased the Cd in leaf by 11 %, 31 %, 37 % and 39 % (Fig. 4A) and Cd in root by 22 %, 34 %, 39 % and 53 % (Fig. 4B) against Cd 300 mg/kg. In the same vein, the Cd treatments at Cd200, Cd300 and Cd + Si combination reduced the Cd concentration in leaf by 42 %, 48 %, and 52 % and root by 12 %, 25 %, and 31 % as compared with that of Cd-300 mg/kg (Fig. 4A and B). The Si treatments remarkably reduce the Cd content in leaves and root and therefore; Si at Si100 and Si200 gradually decrease the Cd content in root and leaf of IP14599 in comparison to Cd 200 alone or in combination with Si.

3.6. *Translocation factors*

Our results revealed that varying concentration of Si affected the translocation factor of Cd in leaf and root of IP14599 (Fig. 4). The application of Si at Si-200 gradually decreased the Cd translocation in IP14599. Moreover, the Si treatments revealed a significant reduction in Cd translocation factor available in the leaf as compared to the Si application in root (Fig. 4C and D). In addition, the same pattern in translocation factors was noticed in leaf and root, that showed the low Cd content in the leaf (Fig. 4C) in contrast to roots

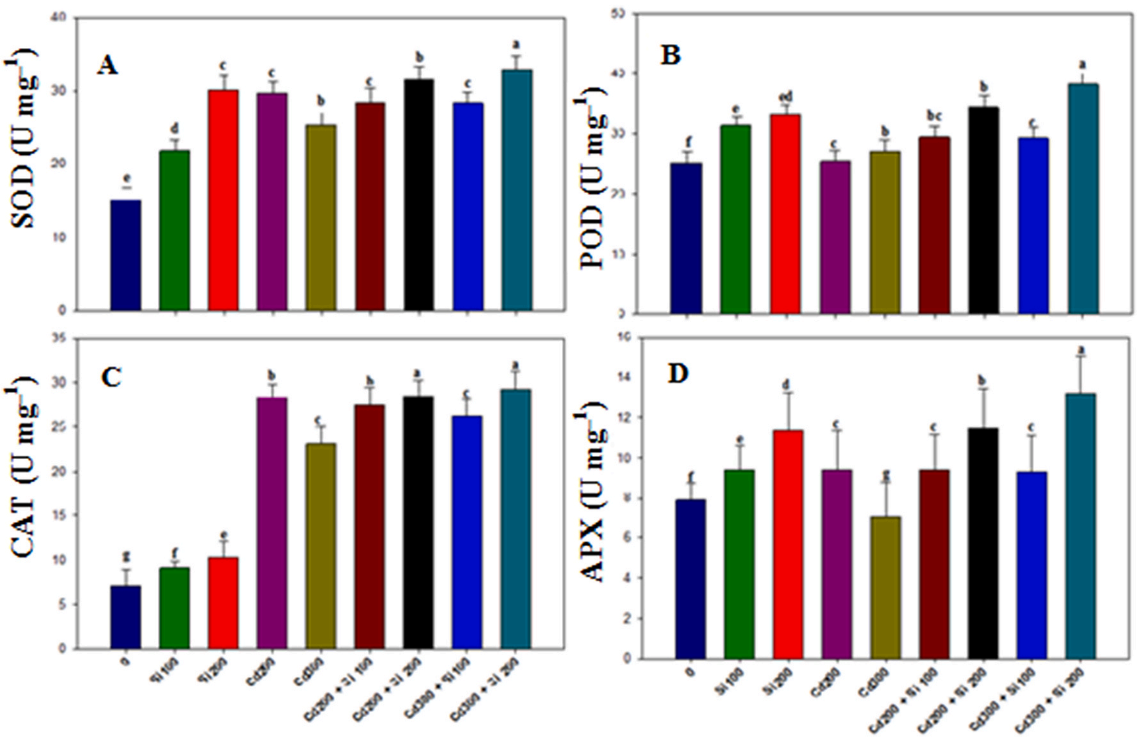

Fig. 3. Effect of silicon in antioxidant enzymes of IP14599 exposed to Cd stress. Superoxide dismutase (SOD) (A) Peroxidase (POD) (B) Catalase (CAT) (C) and Ascorbate peroxidase (APX) (D). Values presented are mean ± SE (n = 4). Non similar alphabet letters denote significant mean difference using ANOVA analysis ($P \leq 0.05$) by Tukey's test, while similar alphabet letters show no significance difference. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

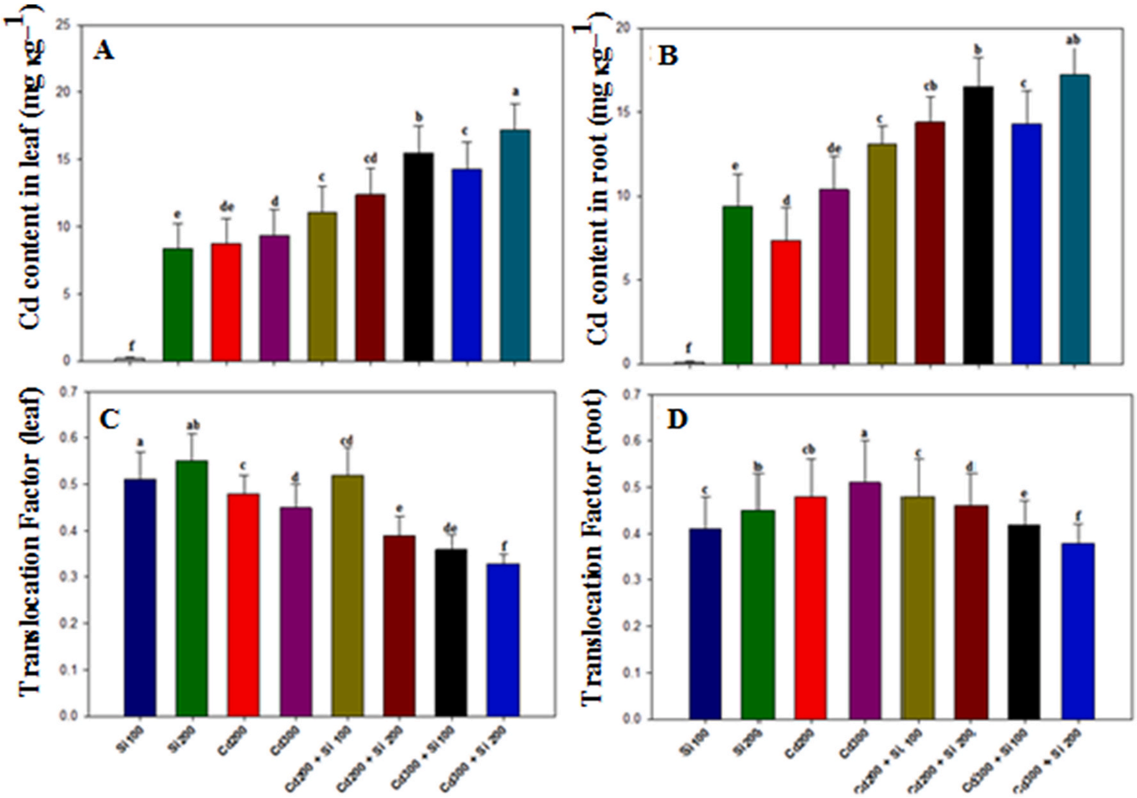

Fig. 4. Impact of silicon on the accumulation and translocation factor of IP14599 exposed to Cd stress. Cd content in leaf (A) Cd content in root (B) Translocation factor in leaf (C) and Translocation factor in root (D). Values presented are mean ± SE (n = 4). Non similar alphabet letters denote significant mean difference using ANOVA analysis ($P \leq 0.05$) by Tukey's test, while similar alphabet letters show no significance difference. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

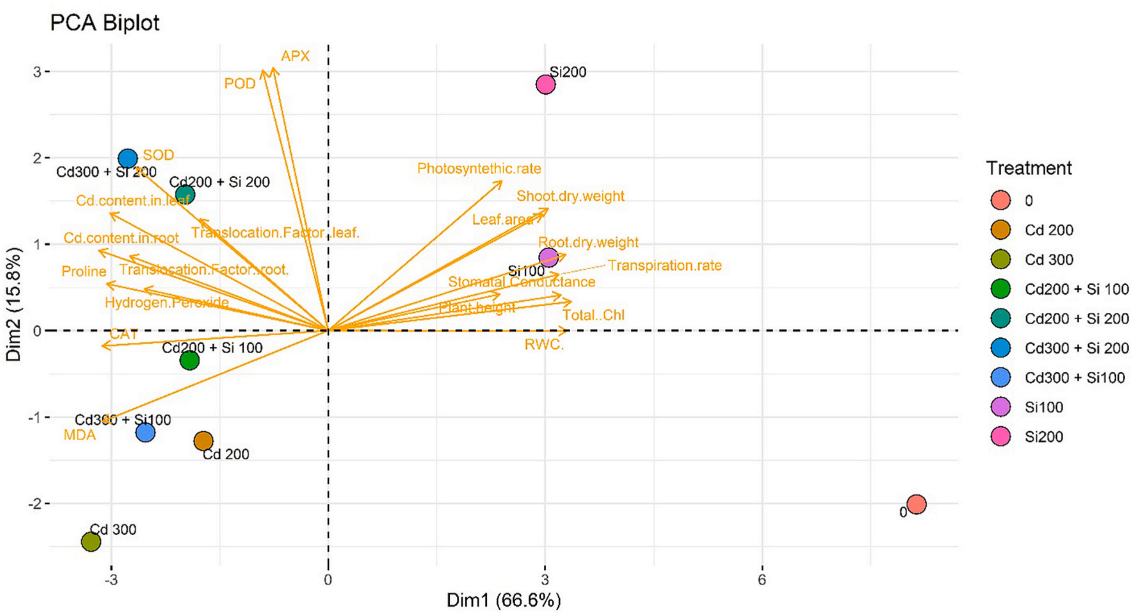

Fig. 5. The principal component analysis (PCA) of measured parameters of IP14599 at varying concentrations of Si under Cd stress.

(Fig. 4D) of IP14599. Generally, Si treatment showed a pronounced decrease in translocation of Cd in leaf and root parts; the introduction of Si showed dose dependent. These due to that, high dosage of Si showed a gradual decrease in Cd in contrast to low doses.

3.7. Principal component analysis (PCA)

PCA was employed to analyze the association of the tested parameters (Fig. 5). PCA reveals that the two groups sum up to 82.4 % of the entire variation in (Fig. 5). The first variable is 66.6 % and 15.8 % (Fig. 5). The Si and Cd stress synergy in this work shows that Si alleviates the deleterious effect on the growth of pearl millet. Growth attributes, gas exchange attributes, CAT, APX and POD activities were joined collectively and revealed a useful relationship among the investigated parameters. However, MDA, protein and oxidative stress activities were categorized and showed a positive relationship between each other.

4. Discussion

The element, Cadmium (Cd) is regarded as a lethal pollutant that impacts negative challenge on living systems.

4.1. Si increased the growth parameters of IP14599 exposed to Cd stress

Currently, Si-mitigated Cd-stress resistance by decreasing Cd accumulation in leaf and root parts of crops has proven to be an effective approach. Herein, Cd treatment poses an adverse impact on the growth attributes of IP14599. However, Nazar et al. [40] found that Cd revealed a significant decline in the growth pattern of plant that might be because of irregular morphological changes and alteration in element uptake and absorption in crop plants. Previous research works have described decrease growth in plants exposed to Cd toxicity [20,41]. Haider et al. [42] reported on how Cd-induced stress led to an inhibition in the development of wheat (*Triticum aestivum* L.) seedlings under exogenous Si application. Our study revealed that application of Si at Si-100/200 gradually increased IP14599 growth when exposed to Cd stress in combination with Si treatments. Former studies reported on gradual enhancement in plant development at varying levels of Si during salinity, drought and metal stress [43–47].

Si-caused development in IP14599 biomass might be because of limited accumulation of Cd from it root part and translocation from the root to the leaf. Furthermore, exogenous application of Si hasten the translocation of metal ions through the plant vacuole that might be associated to the enhancement in plant growth and tolerance capacity [48]. Other research observed that the growth increasing role of Si treatment at a low concentration could indicate that Si application is applied on a dose-dependent nature [43,49]. The enhancement in growth indices seen in IP14599 might be allocated to decrease in oxidative cell damage and modulation in stress-linked enzymes arising from Si treatment. Similar with former literature, our study revealed a progressive and pronounced increase in growth indices when exposed to exogenous Si under Cd stress treatment.

4.2. Si regulates the photosynthetic pigments and gas exchange parameters of IP14599 exposed to Cd stress

Chlorophyll is said to be a vital constituent of chloroplast that takes up light signal necessary for photosynthetic activities [15,50]. Moreover; Rizwan et al. [51] found that the disruption in chlorophyll synthesis adversely impacted the photosynthetic processes in crop plants that resulted to low yield of crops. Ahammed et al. [52] found that Cd adversely affect the chlorophyll molecule biosynthesis, this is the main signal of Cd toxicity level. Ahmad et al. [53] described carotenoid as osmoprotectants which permits enormous energy and preserve the cell membrane from oxidative damage. Also, Cd metal have been found to negatively impact the enzymes present in Calvin cycle, thereby leading to degradation of chloroplast, blockage of the electron transport chain, decrease in stomata conductivity that resulted to a drastic decrease in photosynthesis function of crop [54–56]. Likewise like Haider et al. [56], reported that the effect of Cd stress gradually reduced the carotenoids and chlorophylls and adversely affected the photosynthetic parameters in IP14599 leaves. Moreover; Si decrease the effect of Cd metal and enhanced the gas exchange parameters and carotenoids, chlorophyll when exposed to Cd stress. Our findings are in agreement with former results showing detrimental impacts of Cd toxicity during all stages of plant growth and development [57]. The disruption in normal morphological and physiological processes in plants resulted to increased biosynthesis of ROS thereby causing oxidative cell damage in Cd-treated plants [58,59]. Si improved the functions of photosynthetic attribute by protecting chlorophyll molecule, enhancing stomata opening that increase the carbon dioxide and water mobility and consequently enhanced plant biomass during stress conditions [55,60]. In this present study, decreased chlorophyll and carotenoid contents recorded are observed because of Cd toxicity, thereby inhibiting the activities of enzymes participating in the performance of photosynthetic pigments [61]. Plants treated with Si revealed higher stomatal conductivity and relative water contents in plants exposed to Cd stress in this current study. The gas exchange parameters arising from Si augmentation increased in plants' strength or hardiness, extending in Cd spiked media [62].Thereby, Si might have a direct function in photosynthesis processes during Cd stress.

4.3. Si ameliorate the Cd induced membrane damage in IP14599 under Cd stress

Furthermore, proline which is an osmoprotectants possess an alleviating functions in most plants and improve their stress tolerance capacity to environmental stressors [63]. The current study revealed an increased level of proline because of Si that might be attributed with Cd alleviating by maintaining the enzyme activities, protecting membrane integrity, adapting osmotic equilibrium and stabilizing protein enzymes as reported in former research works like [54,64–66]. Dawood and El-Awadi [67] describe proline as having the ability to produce stress-proteins and thereby stabilizing cell turgidity in plants. Siddique et al. [68] reported that the high accumulation of proline combats oxygen radicals permitting osmotic control in plants under stress. Our work demonstrates higher activities of antioxidant enzymes together with increased accumulation of proline in IP14599 treated seedlings are similar to the results of Shah et al. [4]. Our study suggests that Si might be environmental friendly and efficient approach to enhance Cd-stress resistance capacity in *Pennisetum glaucum* seedlings. Rizwan et al. [51] states that numerous ROS production could induce lethal damage to cell tissues in respect of maliondialdehyde content, this is the normal situation from environmental stress conditions. Current study revealed a gradual increase in the measure of MDA and H_2O_2 because of Cd stress. Moreover, most plants use different approaches to decrease the production of ROS when exposed to stressful condition such as the generation of metabolites, uptake of phenols and enzymes activities [69,70].

4.4. Si modulates the activities of antioxidative enzymes in IP14599 subjected to Cd stress

The investigative enzymes like CAT, POD, APX and SOD perform an essential function in the metabolic responses of environmental stress function in crop [53,64,71]. Liu et al. [72] reported that the increased in the activities of SOD and CAT in Si treated plants present enhanced modification of O to H_2O_2 as similar to Si treatment in Refs. [73,74]. The increased and improved activity level of antioxidant enzymes in Si treated plants is an alternative approach of plants to ameliorate Cd toxicity. Sogarwal et al. [75] reported that Si is a signaling molecule that induces physiological, biochemical or molecular tolerance under stress condition. Similarly to other previous results, Si decrease the excess MDA and ROS in *P. glaucum* leaves by enhancing the antioxidative defense system during Cd stress treatment. Khan et al. [76] reported that SOD is an indicator of defense action that stabilize hydrogen radicals and thereby transform these oxygen radicals to H_2O_2 in crops. Cui et al. [77] reported that APX and CAT convert H_2O_2 into oxygen and water. Nazir et al. [78] found that the activities of enzymes is always depending on the increased form of radical response genes and hindrance of metal genes. This current work observed that exogenous Si enhanced the activities of POD, APX, SOD and CAT in *P. glaucum* seedlings subjected to Cd alone or in combination with Si might be associated to induction in antioxidants enzymes activities. Our results revealed that Si treated plant enhanced antioxidant defense system in Cd treated IP14599 plants. The remarkable increase in the activities of antioxidant enzymes decrease MDA level and mitigate stress in most plants [79,80]. Therefore, exogenous Si plays a very important function in crop plants to enhance their Cd tolerance ability [81,82]. In this research, Si decrease Cd toxicity level in IP14599 plants caused by increased activities of antioxidative enzymes and through reduction in MDA content level.

4.5. Impact of Si on the uptake of Cd in leaf and root of IP14599 seedlings

Our study found that Si hindered the uptake of Cd in leaf and root of *P. glaucum*. The low accumulation of Cd in crop might be because of the production of phytochelatins that mobilize Cd metal into the plant cells and decrease their movement from the root to shoot when exposed to Si [83–85]. Furthermore, Mitani-Ueno et al. [86], reported Si mediated enhancement in element uptake could decrease the absorption of metallic ions, leading in low uptake of Cd in leaf and root in plants. Similarly with other findings, our result revealed that Si particularly decreased the Cd content in leaf and root of *P. glaucum*. Yan and Chen [87] also suggested that Si limits the metals mobility from the root to shoot parts in *Oryiza sativa*. Overall, Si significantly decreased the adverse effects of metal by enhancing the pearl millet resistance that might possess an effective approach and protocol for easy alleviation of Cd pollutant in plants.

4.6. Effect of Si on translocation factor in leaf and root of IP14599 seedlings

Si mediated enhancement in metal uptake might decrease the uptake of metallic ions, leading to low accumulation of Cd in leaf and root in maize crop [48,81,83]. Similar with former reports, our results revealed that Si e significantly decrease the Cd in leaf and root parts of IP14599. Ngugi et al. [82] reported that Si hindered metal translocation from root to shoot in leafy vegetables under lead stress. Si mitigates heavy metal accumulation in rice under metal contaminated acid soil [84,85]. However, the uptake and absorption of metals is based on distinct factors like what type of plant species, growth period, soil characteristics and joint ability of metal ions [86,88]. Herein, our result showed the function of Si in decreasing Cd content level and enhancing Cd s tolerance in IP14599. However, Si decreased the negative effects of Cd metal by enhancing pearl millet tolerance, this can be an efficient approach for safer alleviating of metal toxicity in plants.

4.7. Analyzing the responses of IP14599 to Si under Cd stress

The report by EI-Hendawy et al. [89] found that the PCA estimation gives a clear explanation of the interrelationships between all tested parameters, and we noticed what trait can be determined as a single, combined approach for assessing the impact of Cd and Si on distinct parameters of IP14599. The component 1 distributed 66.6 % and component 2 possess 15.8 % of the total data tested. We observed that our analysis showed that all the parameters measured revealed positive and negative correlation at varying concentrations of Si exposed to Cd stress. The measured parameters include MDA, RWC, were found negatively correlated while Cd content level in root and leaf, APX, CAT, SOD, TFR and TFL showed a positive correlation together with other parameters investigated in the entire dataset. In our study, both single and combine concentration of Cd and Si were employed. Therefore, it will be beneficial to examine the combination of several concentrations of stress mediators in the future to alleviate Cd stress in other or underutilized crop plants.

5. Conclusion

The result of the current work showed that Si positively reduced the Cd uptake and accumulation in IP14599 by mitigating any oxidative stress condition. This process is possible due to the up regulation in the activities of SOD, APX, POD and CAT that decreases ROS effect and improved tolerance ability to Cd stress. Moreover, Si-assisted crop development, physiology of plant, antioxidant action, and tolerance capacity might differ with the treatment dose of applying, nature of species and nature of stress treatment. Our work focused on the vital process of Cd tolerance ability in *P. glaucum* via external Si treatment. This result might be a useful approach to ameliorate Cd effect in grasses to ensure food availability. Sequel to our achieved result, we believe that our documented findings will be the first research revealing synergistic treatment of Si on growth enhancement of Cd treated IP14599 plants. The current procedure might be a new management strategy for sustainable crop production. Nevertheless, further studies are needed to clarify the broad stress alleviating occurrence by Si. The findings showed that Si could be used for farming, such instance soil restoration. Moreover, recommended field trials should be performed to assess the economic usefulness of such a method. The immobilization implications and expected environmental issues of Cd discharge must center on future research to guarantee their prolonged use for heavy-metal-polluted soil recovery and restoration.

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Data availability

All data used in this work is not available but only on request.

CRediT authorship contribution statement

Emmanuel Iwuala: Methodology, Investigation, Conceptualization. **Olubunmi Olajide:** Methodology, Data curation. **Isaika Abiodun:** Formal analysis, Data curation. **Victor Odjegba:** Formal analysis. **Obaiya Utoblo:** Writing – review & editing, Writing – original draft, Formal analysis. **Tolulope Ajewole:** Investigation, Formal analysis. **Ayoola Oluwajobi:** Writing – original draft, Methodology. **Sylvia Uzochukwu:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Emmanuel Iwuala reports travel was provided by The World Academy of Sciences. Emmanuel Iwuala reports a relationship with The World Academy of Sciences that includes: non-financial support. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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