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RESEARCH

Modulating Physiological and Antioxidant Responses in Wheat Cultivars via Foliar Application of Silicon Nanoparticles (SiNPs) Under Arsenic Stress Conditions

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Abstract

Globally, heavy metals especially arsenic (As) toxicity in staple crops like wheat has posed serious threats to human health, necessitating conducting fresh studies to find out biologically viable As toxicity mitigation strategies. Therefore, this study aimed to investigate the impact of foliar-applied silicon nanoparticles (SiNPs) at the tillering stage on the activation of physiological and antioxidant regulation in wheat to induce tolerance against varying As toxicity levels. The trial comprised two promising wheat cultivars (Anaaj and Ghazi) and five SiNPs regimes including 0, 30, 60, 90, and 120 ppm doses against As toxicity levels of 0 and 25 ppm. The recorded findings depicted that SiNPs regimes significantly improved morphological characteristics such as root length, fresh and dry weight, as well as shoot length, and fresh and dry weight of wheat cultivars. Additionally, the levels of chlorophyll pigments, including chlorophyll a, chlorophyll b, and total chlorophyll contents, were significantly increased in SiNPs-treated plants, indicating improved photosynthetic activity. The enhanced antioxidant enzyme activities, such as ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), played a vital role in combating oxidative stress induced by As toxicity. Moreover, SiNPs application resulted in a significant reduction in As concentration in both leaves and roots, highlighting the ability of SiNPs to regulate the uptake and accumulation of arsenic and mitigate its toxic effects. In conclusion, the foliar application of SiNPs during the tillering stage of wheat effectively activated physiological and antioxidant regulation, leading to enhanced tolerance against As toxicity.

Keywords Heavy metals toxicity · Silicon nanoparticles · Peroxidase and catalase · Photosynthetic pigments · Antioxidants · Staple crops

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1 Introduction

Wheat (*Triticum astivum*) is a vital food crop that feeds over a third of the world's population and provides a major part of daily calories to the rapidly increasing human population, particularly in South Asian countries [1–3]. It contains fats, proteins, minerals, and vitamins, making it a crucial part of the food chain [4, 5]. Wheat production worldwide amounts to about 650 million tons annually [6], whereas in Pakistan, it serves as a staple food crop and contributes 2% and 9.9% to the national GDP and agricultural value added, respectively. The wheat-growing area in Pakistan covers 9,204 thousand hectares, with a 0.6% increase from the previous year [4, 7, 8]. The genetic composition of maternal genotypes greatly influences wheat crop improvement [9, 10].

Heavy metals (HMs) particularly arsenic (As) contamination in agricultural systems pose a significant concern,

worldwide. The As is a toxic metalloid that can accumulate in crops such as wheat, leading to potential health risks for consumers [11, 12]. Its exposure can lead to reduced seed germination, decreased root length, and stunted shoot growth in wheat seedlings [12, 13]. Additionally, As accumulation in wheat plants can disrupt vital physiological processes, such as photosynthesis and nutrient uptake, ultimately adversely affecting overall plant productivity [14]. Furthermore, As can translocate from the roots to different plant tissues, leading to higher concentrations of As in the leaves and grains of wheat [12, 15]. Long-term consumption of As-contaminated wheat grains can have adverse effects on human health, including an increased risk of cancer and other chronic diseases, which necessitate conducting studies to find out biologically viable strategies to mitigate the accumulation and toxic effects of As in wheat crops.

Recently, foliar application of silicon nanoparticles (SiNPs) has emerged as one of the potent strategies to mitigate the toxic effects of HMs. By their unique physicochemical properties, SiNPs hold the potential to enhance plant growth, improve stress tolerance, and reduce HMs uptake in various crop species, including wheat [16, 17]. In addition, these have been found to enhance the antioxidant defense system, regulate nutrient uptake, and improve photosynthetic efficiency, thereby reducing the adverse effects of As stress [18–20]. Moreover, SiNPs application pronouncedly enhanced the growth parameters, biomass accumulation, and yield of tomato plants under As stress conditions [21]. Furthermore, SiNPs could play a crucial role in reducing the uptake and translocation of As in wheat plants [11, 22, 23]. Thus, foliage-applied SiNPs hold bright perspectives for reducing the health risks associated with the consumption of As-contaminated wheat grains. In As contaminated soils, SiNPs could be applied as a foliar spray that offers several advantages, including easy application, rapid absorption, and targeted delivery to the plant tissues. Moreover, SiNPs can stimulate the expression of genes involved in detoxification mechanisms, enhance the activity of antioxidant enzymes, and modulate the physiological processes in wheat plants exposed to arsenic stress [24, 25]. However, research gaps need to be bridged about dose optimization of SiNPs for wheat cultivars as previous research findings are limited in scope and offer contradictory recommendations which have necessitated conducting site-specific studies.

This study hypothesizes that the foliar application of SiNPs can mitigate the negative effects of As contamination on wheat plants by enhancing the growth and development of wheat plants by modulating physiological processes and antioxidant mechanisms along with reducing As uptake and imparting tolerance against As stress. Thus, the prime objectives of this study were to investigate the potential of foliar application of silicon nanoparticles in alleviating arsenic toxicity in wheat plants by triggering physiological and biochemical responses and examining antioxidant defense, optimizing application parameters, and assessing long-term effects on wheat cultivars in arsenic-contaminated soils.

2 Materials and Methods

2.1 Site of Experiment

A research study was conducted Department of Botany, University of Central Punjab, Constituent Punjab College, Bahawalpur Campus, Punjab, Pakistan (29.3544° N, 71.6911° E).

2.2 Details of Experiment

The experimental research aimed to investigate the effects of silicon nanoparticles (SiNPs) on enhancing tolerance to arsenic toxicity in two varieties of wheat including Anaaj, and Ghazi. The wheat seeds were obtained from the Regional Agricultural Research Institute Bahawalpur (RARI), located in Punjab, Pakistan. The plastic pots were purchased from the local market in Bahawalpur for executing the trial. At the tillering stage of wheat growth, foliar applications of different SiNP treatments were carried out. Five treatments were implemented: a control treatment (T_0) with no SiNPs applied, Si at 30 ppm (T_1), Si at 60 ppm (T_2), Si at 90 ppm (T_3), and Si at 120 ppm (T_4). The SiNPs were applied using a foliar spray technique, ensuring thorough coverage of the wheat plants. The arsenic consecration at the rate of 0 ppm and 25 ppm was applied after the completion of germination of both wheat varieties. The experimental design was a Completely Randomized Design (CRD) with a factorial arrangement and three replications were maintained.

Before sowing the seeds, soil samples were collected and the pots were filled with soil up to a predetermined mark. The field capacity of the soil samples was determined before seed sowing, ensuring optimal conditions for plant growth. Each plastic pot was planted with five wheat seeds, and after the seedlings germinated, a thinning practice was performed to retain three seedlings per pot. This ensured uniformity and reduced competition among the plants. Both the Anaaj and Ghazi wheat varieties were planted in plastic pots, with three replications labeled R1, R2, and R3. This replication ensured reliable and statistically significant data.

To support the overall growth and development of the wheat plants, a basal dose of major nutrients, including nitrogen (N), phosphorus (P), and potassium (K), was applied. These nutrients are essential for plant growth and enhance crop productivity. Following the foliar application of SiNPs, the wheat plants were allowed to grow until the tillering stage. After seven days of SiNP treatment, various parameters were measured and recorded using standard procedures. These parameters included root length,

shoot length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, chlorophyll a content, chlorophyll b content, total chlorophyll content, carotenoid content, and antioxidant levels. Measuring root length and shoot length provided insights into the growth patterns and potential morphological changes induced by SiNPs. Determining the fresh and dry weights of the roots and shoots allowed for a quantitative assessment of biomass accumulation and potential alterations caused by the SiNPs. To assess the impact of SiNPs on the plant's photosynthetic pigment content, chlorophyll a, chlorophyll b, and total chlorophyll levels were measured. These parameters are indicative of photosynthetic efficiency and can reflect any changes induced by SiNPs. Additionally, the carotenoid content was measured as these compounds play a crucial role in plant defense mechanisms and antioxidant activity. Finally, the levels of antioxidants were analyzed, as they are essential in mitigating the harmful effects of arsenic toxicity and oxidative stress. All measurements and analyses were conducted following established standard procedures to ensure the accuracy and reliability of the data obtained.

2.3 Morphological Traits Recordings

To study the morphological traits of plants, five randomly selected plants were removed from the soil with great care to minimize any damage to their root systems. Subsequently, the plants were washed with water to remove any soil particles adhering to the roots. The length of these plants' roots was then measured in centimeters, starting from the point of seed attachment and extending to the tip of the root. Plant shoot measurement encompassed the distance from the seed attachment point to the tip of the highest leaf, often referred to as the blade leaf.

To determine the fresh weight of the roots and shoots, an electronic balance was utilized. After harvesting, plant samples, including both root and shoot, were thoroughly washed to remove any soil particles and then placed in a drying oven set to 80°C for 24 hours to ensure complete dehydration. The dry weight of the samples was then measured using an electronic balance with a precision of 0.01 grams.

2.4 Leaf Chlorophyll Pigments

After careful selection of fresh leaves, the extraction of chlorophyll pigments was the next step in the procedure. A clean mortar and pestle was used for this purpose. A small amount of liquid nitrogen or pre-chilled acetone was added to the mortar to maintain a low temperature during the extraction process, preventing the degradation of chlorophyll pigments. The paste was then transferred to a clean centrifuge tube.

In the centrifuge tube, a suitable volume of acetone (usually 80% acetone) was added to completely submerge the leaf paste. Following the extraction period, the leaf extract was gently swirled to ensure thorough mixing. A spectrophotometer was used to measure the chlorophyll content in the extract. The spectrophotometer was set to the appropriate wavelengths for chlorophyll measurement, typically around 645 nm and 663 nm. Absorbance readings at the specified wavelengths were recorded. Using the obtained absorbance values, the chlorophyll content was calculated using appropriate equations or formulas. Common equations include the Arnon equation [26].

$$\text{Chlorophyll } a\text{(mg)in original tissue sample} = \text{Chlorophyll } a\text{(mg/ml)} \times \text{finalvolume(ml)}.$$

$$\text{Chl } a = [12.7(\text{OD663}) - 2.69(\text{OD645})] \times \text{V}/1000 \times \text{W}$$

$$\text{Chlorophyll } b\text{(mg)in original tissue sample} = \text{Chlorophyll } a\text{(mg/ml)} \times \text{finalvolume(ml)}.$$

$$\text{Chl } b = [22.9(\text{OD645}) - 4.68(\text{OD663})] \times \text{V}/1000 \times \text{W}$$

$$\text{V} = \text{Sample extract volume;}\quad \text{W} = \text{Weight of sample}$$

The total amount of carotenoids in the sample was calculated using the following formula:

$$\text{Carotenoids(g.M1} - 1) = \text{Acar/Em} \times 100$$

where A car represents the absorbance of carotenoids and Em is the molar absorptivity.

2.5 Measurement of Antioxidants Assay

The measurement of antioxidants such as catalase, peroxidase, superoxide dismutase (SOD), and ascorbate peroxidase in wheat plant leaves involves a detailed procedure to evaluate the activity and levels of these enzymes.

2.5.1 Catalase (CAT), Peroxidase (POD), Superoxide Dismutase (SOD) and Ascorbate Peroxidase (APX) assays:

The catalase activity in wheat plant leaves was measured using the method described [27]. Firstly, a leaf extract was prepared by homogenizing the samples in a phosphate buffer. The reaction mixture was then prepared by combining the leaf extract with hydrogen peroxide (H_2O_2). The decomposition of H_2O_2 was monitored by measuring the decrease in absorbance at 240 nm over a fixed time interval using a spectrophotometer. The catalase activity was calculated using the molar extinction coefficient of H_2O_2 and expressed as units per gram of fresh weight.

Likewise, the peroxidase activity in wheat plant leaves was measured following the protocol described [28]. The increase in absorbance at a specific wavelength, corresponding to the oxidation of the substrate, was monitored using a spectrophotometer. The peroxidase activity was calculated based on the change in absorbance per unit time and was expressed as units per gram of fresh weight.

Moreover, the SOD activity in wheat plant leaves was determined by using the method of [29]. Leaf samples were homogenized in a suitable buffer, and the inhibition of chromogen reduction was measured at a specific wavelength using a spectrophotometer. The SOD activity was calculated based on the degree of inhibition and was expressed as units per gram of fresh weight.

Finally, the ascorbate peroxidase activity in wheat leaves was also measured by following the protocol as described by [30]. Leaf samples were extracted in a phosphate buffer containing ascorbate and hydrogen peroxide. The reaction mixture was prepared by combining the leaf extract, ascorbate, and H_2O_2. The decrease in ascorbate concentration was monitored by measuring the absorbance at a specific wavelength using a spectrophotometer. The ascorbate peroxidase activity was calculated based on the rate of ascorbate oxidation and expressed as micromoles per gram of fresh weight per minute.

2.6 Arsenic Concentration in Leaf and Root

The measurement of As concentration in the leaf and root tissues of wheat plants was performed by following the procedure given by [31]. The dried and grounded samples were mixed with an appropriate extraction solution containing concentrated acids. The mixture was heated to facilitate As extraction, and after cooling, it was centrifuged to separate the liquid phase. The extracted samples were then analyzed using atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), or inductively coupled plasma optical emission spectrometry (ICP-OES). Calibration standards of known As concentrations were prepared, and As concentration was determined by comparing their signal intensities to the calibration curve. Finally, As concentration in the leaf and root tissues was calculated based on the sample weight, dilution factor, and calibration curve.

2.7 Statistical Analysis

The recorded data were arranged for statistical analysis by employing the Analysis of Variance (ANOVA) method with the assistance of Statistical 8.1 programming software. To evaluate the significance levels, two different α values were utilized including$\alpha = 0.01$ was applied to identify highly significant effects, while a significance level of $\alpha = 0.05$ was used to identify significant effects. These significance levels are based on established guidelines for statistical analysis [32], ensuring that the results are robust and reliable.

3 Results

3.1 Foliar Silicon Role in Growth Characters of Wheat

The results of the application of foliar SiNPs on two wheat varieties, (Akbar and Ghazi) demonstrated that different treatments of SiNPs and As significantly affected the measured attributes of growth characters of wheat at their tillering stage (Table 1). The results showed that the As reduced the growth attributes of wheat while the application of foliar SiNPs was helpful for the improvement of growth characteristics of wheat under As stress conditions in both varieties.

Table 1 Analysis of variance table for wheat growth, chlorophyll pigments, antioxidant attributes, and arsenic concentration in wheat in response to varying nano silicon levels and arsenic-induced stress

Parameters	Treatments	Stress	Variety	S×T	S×V	T×V	S×V×T
Root length	**	***	**	*	*	*	**NS**
Root fresh weight	***	**	*	**	**	**NS**	**NS**
Root dry weight	**	***	**	**	*	**NS**	**NS**
Shoot length	**	***	*	*	*	**	**NS**
Shoot fresh weight	***	***	*	***	**	*	*
Shoot dry weight	**	***	**	**	*	**NS**	*
Chlorophyll *a*	**	***	*	**	***	**	*
Chlorophyll *b*	**	**	*	**	**	**	*
Total Chlorophyll	**	***	*	*	**	**	**
Carotenoids	**	***	*	***	***	***	**
Ascorbate peroxidase (APX)	*	**	**	*	**	**	NS
Catalase (CAT)	**	**	**	**	**	NS	NS
Peroxidase (POD)	**	*	**	*	**	NS	NS
Superoxide dismutase (SOD)	*	**	**	**	*	**	NS
Arsenic conc. leaf	**	***	**	*	*	NS	NS
Arsenic conc. root	**	***	**	**	*	NS	NS

N.S = non-significant; *'**'***significant at $p \leq$ at 0.05, $p \leq$ at 0.01, $p \leq$ at 0.001, respectively

The highest improvement in the growth characteristics of wheat plants was noted when The application of SiNPs at 120 ppm (T4) was applied compared to all other treatments. This higher concentration of SiNPs led to improved length, fresh and dry weights of both roots and shoots in Akbar and Ghazi varieties. However, it was also observed that Akbar demonstrated superior performance compared to Ghazi in all treatment groups in terms of morphological traits under investigation (Fig. 1).

3.2 Leaf Chlorophyll Pigments

The application of foliar silicon nanoparticles (SiNPs) on two wheat varieties such as Akbar and Ghazi, showed significant results. Various treatments of SiNPs and arsenic (As) had a notable impact on leaf chlorophyll pigments, including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents, during the tillering stage of wheat (Table 1). The findings indicated that As reduced the levels of leaf chlorophyll pigments in wheat. However, the application of foliar SiNPs proved beneficial in enhancing these pigments in wheat under As stress conditions, observed in both Akbar and Ghazi varieties.

The maximum improvement was observed in recoding pigments where foliar SiNPs at the rate of 120 ppm were applied under normal as well as As stress conditions while the lowest values of measured pigments were noted under As stress conditions where As was applied at the rate of 25

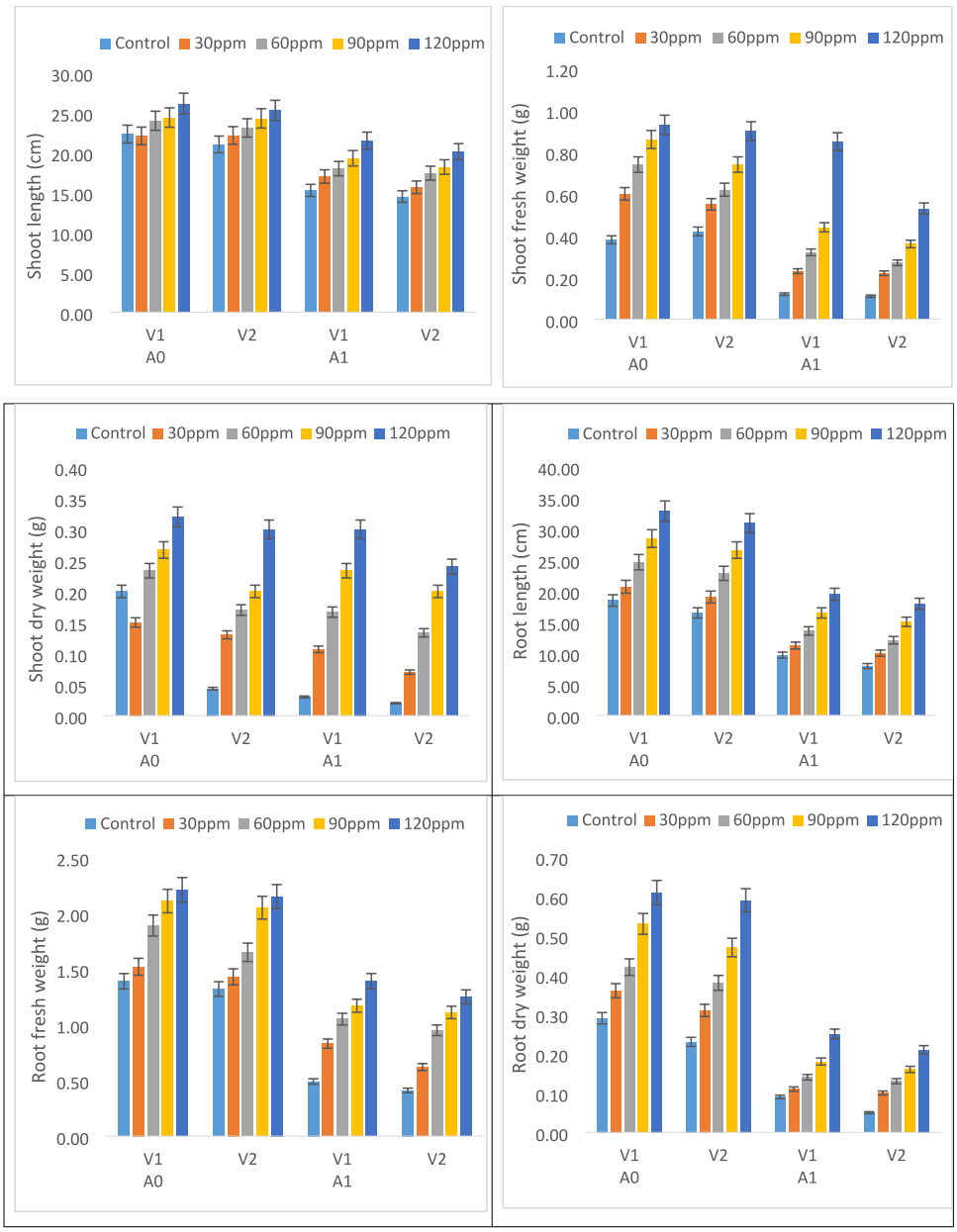

Fig. 1 Impact of Foliar Application of Nano Silicon on Morphological Traits of Wheat under Arsenic-Induced Stress. (V1= Variety 1 Akbar, V2 = Variety 2 Ghazi, A0 = without arsenic control, A1 = Arsenic applied @ 25 ppm)

ppm. A better performance was noted in the Akbar wheat variety in the improvement of leaf pigments as compared to the Ghazi wheat variety (Fig. 2). This finding suggests that foliar application of silicon nanoparticles at a higher concentration can potentially counteract the negative impact of arsenic on plant health.

3.3 Antioxidants Concentration

The role of foliar nano-silicon in the assay of antioxidants such as CAT (catalase), SOD (superoxide dismutase), POD (peroxidase), and APX (ascorbate peroxidase) has been studied. Different foliar silicon rates were tested, including a control group without silicon (T0), SiNPs (silicon nanoparticles) at 30 ppm (T1), SiNPs at 60 ppm (T2), SiNPs at 90 ppm (T3), and SiNPs at 120 ppm (T4). The application of foliar silicon nanoparticles (SiNPs) on two wheat varieties such as Akbar and Ghazi, showed significant results. Various treatments of SiNPs and arsenic (As) had a notable impact on antioxidant activity, including APX, CAT, POD, and SOD, during the tillering stage of wheat (Table 1). The findings indicated that As reduced the levels of antioxidant activity in wheat. However, the application of foliar SiNPs proved beneficial in enhancing these antioxidant activity in wheat under As stress conditions, observed in both Akbar and Ghazi varieties.

The maximum improvement was observed in recoding antioxidant activity where foliar SiNPs at the rate of 120 ppm were applied under normal as well as As stress conditions while the lowest values of measured antioxidant activity were noted under As stress conditions where As was applied at the rate of 25 ppm. A better performance was noted in the Akbar wheat variety in the improvement of antioxidant activity as compared to the Ghazi wheat variety (Fig. 2). The study focused on two wheat varieties, (Akbar and Ghazi) to evaluate their performance. Among the two wheat varieties, Akbar demonstrated better results in the assay of antioxidants compared to Ghazi. The addition of foliar nano-silicon at different concentrations also played a significant role. However, it was observed that the highest

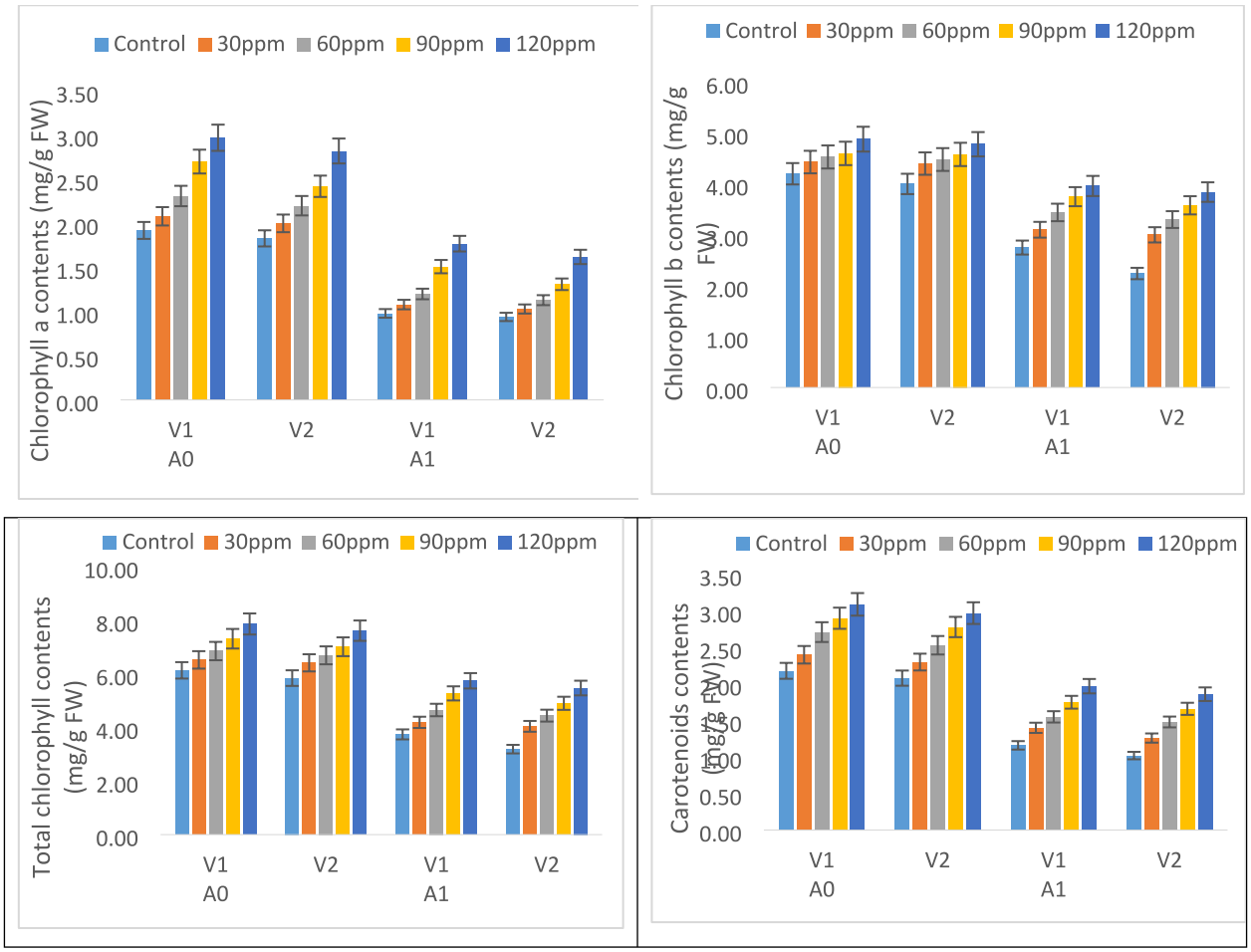

Fig. 2 Impact of Foliar Application of Nano Silicon on leaf photosynthetic pigments of Wheat under Arsenic-Induced Stress. (V1= Variety 1 Akbar, V2 = Variety 2 Ghazi, A0 = without arsenic control, A1 = Arsenic applied @ 25 ppm)

concentration of SiNPs at 120 ppm (T4) performed better than all other concentrations in terms of enhancing the activity of antioxidants (Fig. 3). Furthermore, it is worth noting that the study involved the use of arsenic heavy metal. While the specific details of the study regarding arsenic heavy metal are not mentioned, it can be inferred that the presence of arsenic might have influenced the overall antioxidant activity and the performance of the wheat varieties and foliar nano-silicon treatments.

3.4 Arsenic Concentration in Wheat Leaf and Root

The role of foliar nano-silicon in controlling arsenic concentration in wheat leaf and root has been studied using different foliar silicon rates. The rates used were: foliar nano-silicon control (T0), SiNPs at 30 ppm (T1), SiNPs at 60 ppm (T2), SiNPs at 90 ppm (T3), and SiNPs at 120 ppm (T4) presented in Table 1. Two wheat varieties, namely Akbar and Ghazi, were compared to assess their performance in this context. The results showed that Akbar variety performed better in controlling arsenic levels compared to Ghazi. Among the foliar silicon rates, SiNPs at 120 ppm (T4) demonstrated the most effective control of arsenic concentration in both wheat leaves and roots (Fig. 4). This suggests that higher concentrations of foliar nano-silicon can effectively reduce arsenic accumulation in wheat plants, with the Akbar variety exhibiting superior tolerance to this heavy metal.

3.5 Principle Components Analysis (PCA)

The PCA was performed based on control and stress treatment in wheat crops to determine the relationship using different silicon as nanoparticles against arsenic toxicity studied (Table 2). The results suggested 0.615 eigenvalues and 99.40% variance of the total at PC4 respectively. However, PC1 contributes 67.27% of total diversity, followed by 29.36 in PC2.

A biplot analysis was conducted on the mean data from control and stress treatments (Fig. 5), with PC1 and PC2 used as the axes. The results revealed that several significant characters, including SDW, SFW, RFW, RDW, Chla, Chlb,

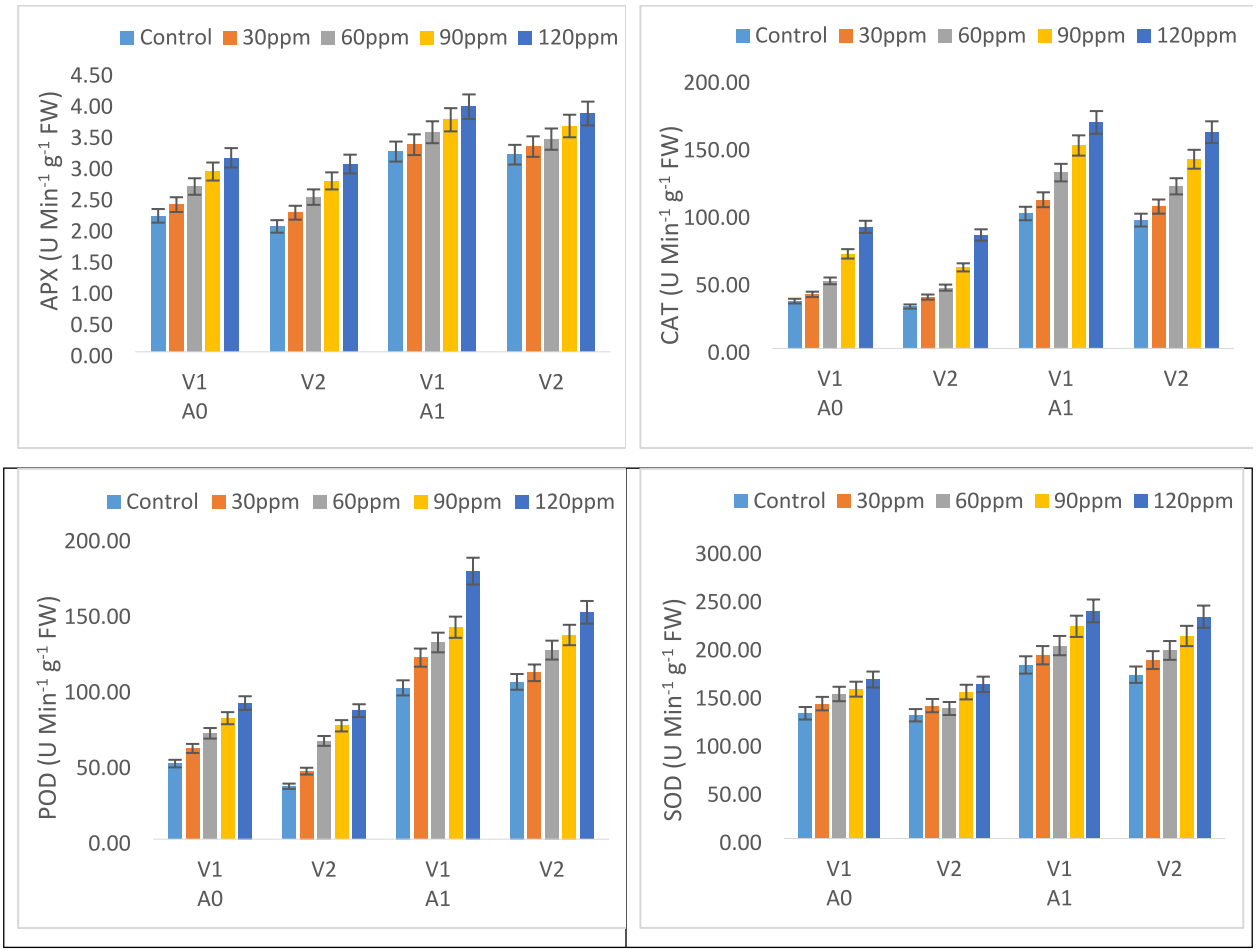

Fig. 3 Impact of Foliar Application of Nano Silicon on the antioxidant assay of Wheat under Arsenic-Induced Stress. (V1= Variety 1 Akbar, V2 = Variety 2 Ghazi, A0 = without arsenic control, A1 = Arsenic applied @ 25 ppm)

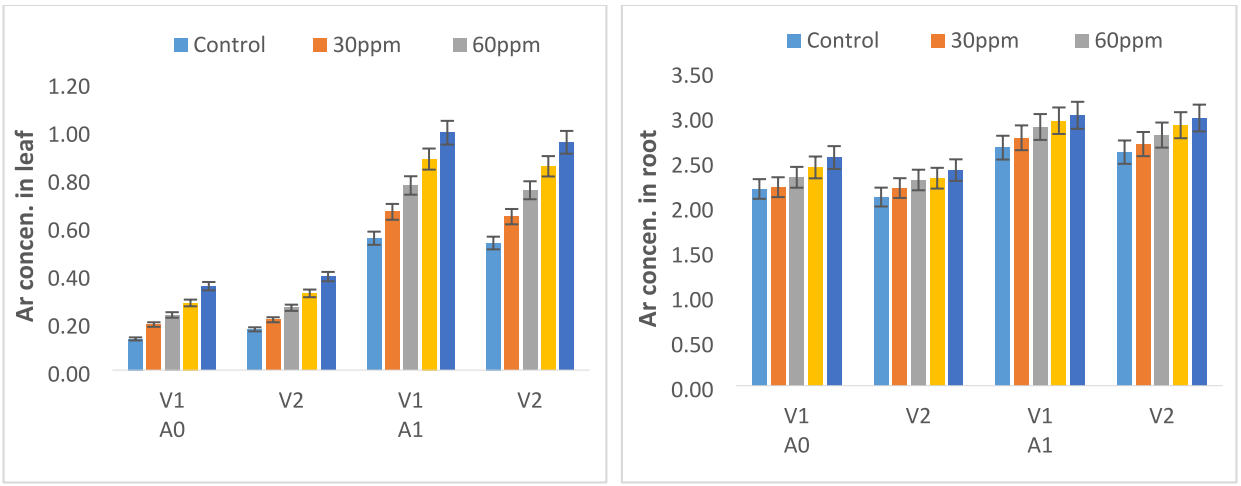

Fig. 4 Impact of Foliar Application of Nano Silicon on Arsenic Concentration of Wheat under Arsenic-Induced Stress, (V1= Variety 1 Akbar, V2 = Variety 2 Ghazi, A0 = without arsenic control, A1 = Arsenic applied @ 25 ppm)

Table 2 Principle components analysis (PCA)

PC	Eigen value	% Variance	Cum. Eigen	% Variance
1	0.416538	67.272	0.416538	67.272
2	0.181848	29.369	0.598386	96.641
3	0.013088	2.1137	0.6114735	98.7547
4	0.004029	0.6507	0.61550255	99.4054

and RL, were associated with PC1. Among the genotypes, V4C, 2V4C, V3C, 2V3C, V2C, 2V2C, V1C, 2V1C, 2V1C, and VOC performed well under PC1. In contrast, the traits Arl, CAT, POD, SOD, and AxR were found to contribute more to PC2 (Fig. 6). Furthermore, the genotypes vs treatments (2V4s, V2S, V3S, 2V3S, V2S, 2V2S, and V1) showed greater contributions to PC2 across different treatments. The most significant characters that contributed more in PC1 are SL, SFW, SDW, RL, RFW, Chla, Chlb, and Car, however,

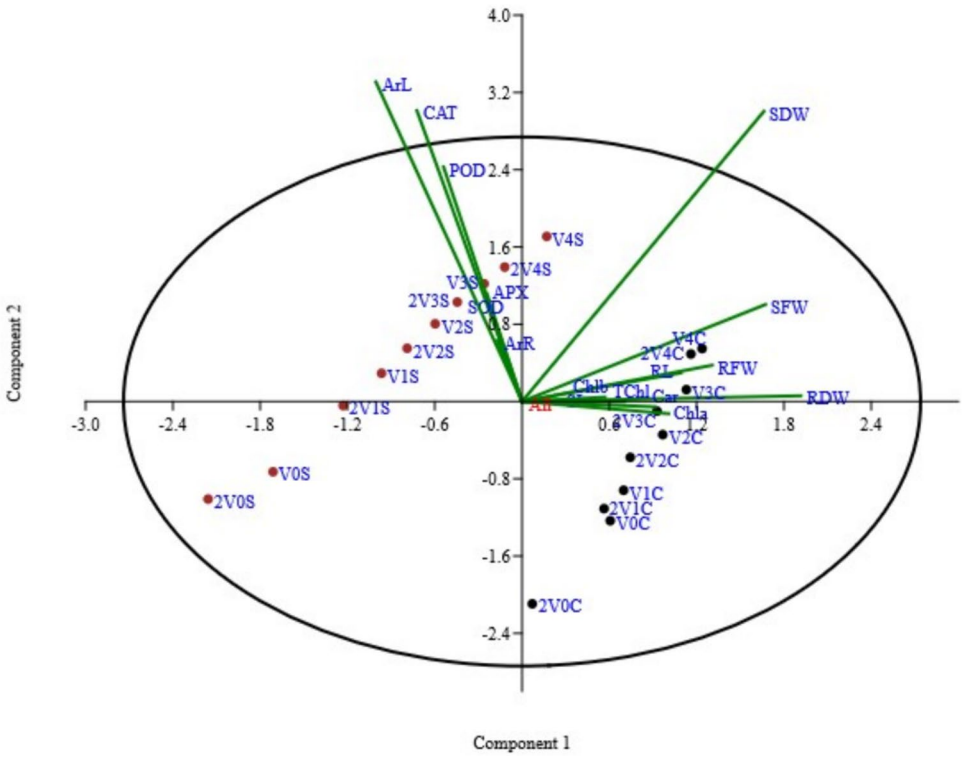

Fig. 5 Biplot analysis of control (black dot) and stress (brown dot)

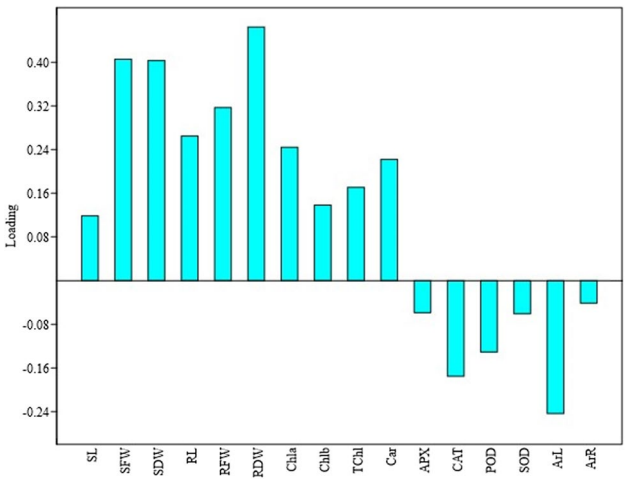

Fig. 6 Biplot traits loading under control and stress treatments

Table 3 Distribution of genotypes and treatments loading under PCA analysis

	PC 1	PC 2	PC 3	PC 4
$\mathbf{V_1T_0C}$	0.39137	-0.5264	-0.3239	0.070726
$\mathbf{V_1T_1C}$	0.45059	-0.3918	-0.0497	-0.0191
$\mathbf{V_1T_2C}$	0.62405	-0.1462	-0.0461	-0.00185
$\mathbf{V_1T_3C}$	0.72891	0.05256	0.0508	0.059638
$\mathbf{V_1T_4C}$	0.7987	0.2335	0.1130	0.1047
$\mathbf{V_2T_0C}$	0.0454	-0.8933	0.1753	-0.12527
$\mathbf{V_2T_1C}$	0.3639	-0.4732	-0.0473	-0.08723
$\mathbf{V_2T_2C}$	0.4801	-0.246	-0.0036	-0.01803
$\mathbf{V_2T_3C}$	0.5987	-0.0425	0.09065	0.01107
$\mathbf{V_2T_4C}$	0.749	0.2095	0.11413	0.065018
$\mathbf{V_1T_0Si}$	-1.104	-0.3103	0.1094	0.12456
$\mathbf{V_1T_1Si}$	-0.6231	0.1246	-0.11274	-0.00547
$\mathbf{V_1T_2Si}$	-0.3852	0.3430	-0.09814	-0.02439
$\mathbf{V_1T_3Si}$	-0.1679	0.5199	-0.03227	-0.01928
$\mathbf{V_1T_4Si}$	0.1095	0.7288	0.086562	-0.0922
$\mathbf{V_2T_0Si}$	-1.392	-0.4309	0.11407	0.042201
$\mathbf{V_2T_1Si}$	-0.7935	-0.0180	-0.02506	-0.02579
$\mathbf{V_2T_2Si}$	-0.5093	0.2349	-0.08012	-0.01828
$\mathbf{V_2T_3Si}$	-0.2871	0.4389	-0.07215	-0.01538
$\mathbf{V_2T_4Si}$	-0.0768	0.5934	0.037131	-0.02563

V_1= Annaj, V_2= Ghazi; C=control; (T_0), Si = Silicon @ 30 ppm (T_1), Si @ 60 ppm (T_2), Si @ 90 ppm (T_3), Si @ 120 ppm (T_4)

APX, CAT, POD, SOD, ArL, and ArR contributed more in PC2 (Fig. 6).

Table 3 describes the distribution of genotypes along with different silicon levels were presented. Both the genotypes under control condition showed a positive and significant association in PC1, however, under silicon application, only the Anaaj wheat genotype was present in PC1 at 120ppm however, the rest of the treatment combination was present in PC2. Table 4 presents the correlation coefficients between different variables, such as APX, ArL, ArR, CAT, Car, Chla, Chlb, POD, RDW, RFW, RL, SDW, SFW, SL, SOD, and Tchl. The coefficients represent the strength and direction of the linear relationship between the variables. The values range from -1 to 1, with 1 indicating a perfect positive correlation, -1 indicating a perfect negative correlation, and 0 indicating no correlation.

4 Discussion

The recorded findings of this study revealed that foliar application of SiNPs at the tillering stage of wheat demonstrated significant improvements in various morphological parameters, including root length and their fresh and dry weight, as well as shoot length and their fresh and dry weight. Several previous research findings by [17, 18, 33] also demonstrated that foliar application of SiNPs triggered root length and enhanced the fresh and dry weight of the roots and shoots of wheat seedlings. It was also inferred that these improvements were crucial that enabled wheat plants to absorb more nutrients from the soil solution which led to increased biomass growth and grain yield. absorption and overall plant growth. Furthermore, a study conducted [16] revealed that SiNPs treatment significantly increased shoot length and improved the fresh and dry weight of the shoots in wheat plants by increased photosynthesis rate and partitioning of assimilates that resulted in taller plants having significantly higher weight.

It has been established that chlorophyll pigments are crucial components of the photosynthetic machinery and play a vital role in capturing light energy for photosynthesis, especially in a stressful environment. Several studies have demonstrated that foliage-applied SiNPs remained instrumental in promoting the chlorophyll pigments in wheat leaves which enabled crop plants to overcome HMs toxicity [20, 34]. Similarly, in our study, the total chlorophyll content was also enhanced, indicating improved photosynthetic efficiency in SiNP-treated plants suggesting that SiNPs positively influenced the photosynthetic capacity of the plants under As stress. By improving chlorophyll pigment content, SiNPs application during the tillering stage can play a crucial role in maintaining optimal photosynthetic activity, even under arsenic stress conditions [24]. Previously, it was inferred that increased chlorophyll content caused by SiNPs might increase carbon assimilation, improve energy utilization, and ultimately, trigger plant growth and significantly multiply grain yield and biological productivity [35, 36]. The findings of these studies highlight the potential of SiNPs for activating different vital physiological and antioxidant regulations

Table 4 Pearson correlation matrix under control and silicon application

	APX	ArL	ArR	CAT	Car	Chla	Chlb	POD	RDW	RFW	RL	SDW	SFW	SL	SOD
ArL	0.95**														
ArR	0.98**	0.98													
CAT	0.98**	0.98	0.98**												
Car	-0.45*	-0.58	-0.55**	-0.49*											
Chla	-0.44	-0.57**	-0.54*	-0.47*	0.99**										
Chlb	-0.44	-0.51**	-0.51**	-0.44*	0.96**	0.94**									
POD	0.98**	0.97	0.98**	0.98	-0.48*	-0.47*	-0.44*								
RDW	-0.38	-0.55*	-0.51*	-0.44*	0.98**	0.98**	0.91**	-0.44							
RFW	-0.38ns	-0.47*	-0.45*	-0.38	0.98**	0.97**	0.96**	-0.37	0.98**						
RL	-0.28	-0.45*	-0.41	-0.33	0.97**	0.98**	0.93**	-0.33	0.70**	0.98**					
SDW	0.24	0.10	0.155	0.22	0.69**	0.70**	0.71**	0.23	0.91**	0.76**	0.78**				
SFW	-0.14	-0.28	-0.26	-0.17	0.91**	0.92**	0.89**	-0.15	0.95**	0.93**	0.94**	0.83**			
SL	-0.43	-0.54	-0.52**	-0.45*	0.98**	0.97**	0.98**	-0.44	-0.46*	0.98**	0.96**	0.73**	0.92**		
SOD	0.96**	0.98**	0.98**	0.99**	-0.50*	-0.48*	-0.43	0.98**	0.96**	-0.39	-0.35	0.21	-0.17	-0.45*	
Tchl	-0.44	-0.55**	-0.53**	-0.46*	0.99**	0.98**	0.98**	-0.46*		0.98**	0.97**	0.72**	0.92**	0.99**	-0.46

ArL= Arsenic concentration in leaf; ArR= Arsenic concentration in root; CAT = Catalase; Car = Carotenoids; Chla =Chlorophyll *a* content; Chlb = Chlorophyll *b* contents; POD = Peroxidase; RDW= Root dry weight; RFW= Root fresh weight; RL = Root length; SDW =Shoot dry weight; SFW = Shoot fresh weight; SL= shoot length; SOD = Superoxide dismutase; Tchl = Total chlorophyll contents

in wheat, thereby enhancing the tolerance of crop plants to As toxicity.

Antioxidant enzymes play a crucial role in scavenging reactive oxygen species (ROS) and minimizing oxidative damage in plants. The SiNPs provision through foliar sprays enhanced the activity of peroxidase, catalase, superoxide dismutase, and ascorbate peroxidase in wheat plants which assisted wheat seedlings to detoxify ROS [37, 38]. Similarly, the activities of catalase and superoxide dismutase were also elevated in our study and results revealed that these assisted crop plants to survive under As-induced oxidative stress. Additionally, the activity of ascorbate peroxidase, an important enzyme involved in the ascorbate-glutathione cycle, was found to be significantly higher in SiNPs-treated wheat plants in this study. This enzyme plays a crucial role in the detoxification of hydrogen peroxide and the maintenance of cellular redox homeostasis. The enhanced activity of ascorbate peroxidase suggests a more efficient antioxidant system in SiNPs-treated plants, contributing to improved tolerance to arsenic toxicity. The findings of these studies emphasize the potential of SiNPs in activating physiological and antioxidant regulation in wheat. By enhancing the activity of key antioxidant enzymes, SiNPs application during the tillering stage can effectively mitigate the harmful effects of arsenic-induced oxidative stress [23, 34].

Previously, it has been demonstrated that exogenous application of SiNPs holds the potential to mitigate the As toxicity in wheat. For instance, a study by [39] investigated the impact of SiNPs application on As accumulation in wheat plants and reported that it remained effective in reducing the As concentration in both the leaves and roots of wheat plants. The ability of SiNPs to reduce As accumulation needs further in-depth studies to reveal the underlying mechanisms. It has become even more important because As in wheat seedlings tend to disrupt cellular processes and inhibit the biosynthesis of many vital enzymes [40, 41]. Therefore, reducing As accumulation in staple crops has become crucial for maintaining optimal plant functioning and reducing its adverse effects on human health. However, SiNPs critical role in regulating the uptake, translocation, and accumulation of As within the plant system has been elaborated in concurrence with our findings [11].

During the initial stages of plant growth, HMs (particularly lead and As) contamination in agricultural lands poses a serious threat to plant productivity because these hinder essential metabolic and biochemical processes, ultimately impeding the growth and development of young seedlings [42–44]. Therefore, owing to the carcinogenic properties of As, it is the need of time to reduce its accumulation in wheat [45]. This study utilized exogenous silicon to examine its potential interaction with wheat plant in mitigating the toxic effects of arsenic, as previous research has demonstrated its stress-alleviating properties in various studies [46]. Our study also revealed that As has a severe detrimental impact on the growth of wheat shoots and roots. Arsenic significantly affected cellular proliferation in both the shoots and roots, leading to inhibited phototrophic and geotropic expansion, as well as the induction of necrosis. However, when exogenous silicon was applied, it promoted growth in both normal and stressed conditions by enhancing

mechanical strength. The introduction of SiNPs resulted in improved morphological and growth parameters, including elongation of shoots, longer roots, and enhanced root architecture. Notably, the stress-mitigating effects of silicon were particularly pronounced in wheat treated with silicon in combination with arsenic (Si + As), compared to those exposed to As alone and the control group. Under As stress, leaves exhibited dryness and sunken appearance, whereas Si remained effective in maintaining plant turgidity and preserved leaf growth under both normal and stressful conditions. The underlying reason for these beneficial effects of silicon could be attributed to its potential to provide mechanical strength during stressful conditions, thereby promoting optimal growth [47]. The current study was designed to group the wheat genotypes based on silicon application against toxification on morphology and antioxidant characteristics. For that purpose, principal components analysis (PCA) and biplot analysis were performed on the group, and the wheat genotypes were in different groups. The current study also described the appropriate silicon under control (without arsenic) and with arsenic toxicity. Similar principal component analysis (PCA) and biplot loading for PC1 and PC2 were performed in wild tomatoes under salinity conditions to group the genotypes based on salinity tolerance [48]. In our study, PCA and biplot analysis effectively differentiated the genotypes of wheat under arsenic stress, identifying those with higher tolerance. Our results align with another study that suggested multivariate analytical techniques, such as PCA and biplot analysis, are significant in identifying genotypes with notable stress tolerance under various conditions [49].

5 Conclusions

The results of this study were by the research hypothesis as morphological parameters, such as root length, shoot length, and their fresh and dry weights of both wheat cultivars (especially Akbar) were significantly enhanced by SiNPs foliar application in different doses (120 ppm surpassed rest of doses). This improvement in root and shoot development indicates better nutrient uptake and overall plant growth, which assisted crop plants in coping with arsenic toxicity. Moreover, SiNPs application resulted in increased leaf chlorophyll pigments, including chlorophyll a, chlorophyll b, and total chlorophyll contents suggesting an improved photosynthetic efficiency and energy utilization in SiNPs-treated plants. Another crucial aspect of SiNPs treatment is its impact on the antioxidant system of wheat. SiNPs application increased the activity of key antioxidant enzymes, such as ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). This augmentation of antioxidant activity assisted crop plants to mitigate oxidative stress induced by arsenic toxicity, thereby protecting the plant from damage. Furthermore, SiNPs treatment significantly reduced the arsenic concentration in both wheat leaves and indicated a vital role in regulating the uptake, translocation, and accumulation of arsenic within the plant system. Overall, the findings suggest that the activation of physiological and antioxidant regulation in wheat through SiNPs application during the tillering stage might be explored further to impart tolerance against arsenic toxicity. These findings provide valuable insights for developing sustainable agricultural strategies to enhance wheat crop resilience against arsenic contamination to ensure food and nutritional security of the rapidly increasing human population in the wake of arsenic toxicity in agricultural soils.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval Not applicable, manuscript does not report on or involve the use of any animal or human data or tissue.

Consent to Participate All authors participate in the preparation of the manuscript.

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