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Unravelling the role silica nanoparticles to ameliorate chromium heavy metal stress in Mexican marigold (*Tagetes erecta* L.)

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Abstract

The influence of environmental contamination on different ecosystems has become a major problem worldwide. Pollution of heavy metals in soil has become a serious global concern. The purpose of the present experimental work was to assess the effect of silica nanoparticles (SiNPs) on the growth, biochemical parameters, and physiological responses of *Tagetes erecta* L. plants under chromium (Cr) stress. In the current investigation, we implemented the CRD experiment, and three sets of replicates were employed, each comprising nine unique treatments; control, Cr-I (50 mg kg⁻¹), Cr-II (100 mg kg⁻¹), SiNPs-I (100 mg L⁻¹), SiNPs-II (200 mg L⁻¹), Cr-I + SiNPs-I, Cr-I + SiNPs-II, Cr-II + SiNPs-I, Cr-II + SiNPs-II). When plants were exposed to Cr, the oxidative damage was noticeable. Cr contamination markedly decreased the growth characters of the plants including shoot and root length, fresh and dry weights as well as photosynthetic pigment but increased the level of proline, hydrogen-peroxide (H₂O₂), malondialdehyde (MDA) and relative membrane permeability in plants. Significantly, the impacts of Cr on plants were avoided by SiNPs application. SiNPs applied externally lessened the concentrations of H₂O₂, MDA, and relative membrane permeability. Conversely, SiNPs enhanced the content of

chlorophyll *a*, *b*, carotenoid and improved the growth of plant. They also elevated the antioxidant enzyme activity. Furthermore, SiNPs promote the defensive mechanisms of plants against the stress of toxic metals by boosting the absorption of particular elements such as Ca^{2+} and K^{+} . Based on our research, treating plants with SiNPs can potentially help them to overcome abiotic stresses. Our findings suggest that SiNPs have the potential to effectively regulate stress indicators and antioxidants, which can significantly lower the adverse impact of abiotic stresses in plants.

Introduction

Plants face numerous environmental stresses that can be categorized as biotic or abiotic. Plants undergo stress in several ways in their natural environments. The productivity of a number of crops is reduced by many factors, including water shortages, extreme temperatures, and toxicity from heavy metals [1]. Since plants cannot move, they have to face several harsh stresses that negatively influence physiological growth and development [2]. Heavy metal toxicity in the soil is one of the biggest challenges faced by the world today [3]. The growth and reproductive development of plants can be severely affected by heavy metal (HM) poisoning [4]. In addition to harming plants, HM pollution interferes with the elements' normal cycle by affecting the activity of microbes [5]. The heavy metal chromium (Cr), which is symbolized as Cr, can be poisonous and dangerous to human beings, plants as well as animals [6]. A major and rapidly growing ecological problem is the pollution of Cr in the soil. Because of its high accessibility to different plant organs, Cr (VI) could be more dangerous in comparison to Cr (III) [6]. The fact that Cr is widely employed in mining operations, substantial quantities of Cr (VI) are becoming part of our surroundings, creating problems all over the globe [7].

The primary form of Cr found in agricultural soils, particularly those susceptible to flooding, is trivalent Cr(III) [8]. The leather industry has been utilizing extremely toxic heavy metal Cr (Cr) in numerous ways, particularly in the process of chrome-based tanning of skin [9]. The existence of toxic metals retards the growth of plants. These metals replace the essential nutrients in the soil which may lead to a decrease in yield rate. This is particularly true in the case of Cr(VI) contamination; it may cause the economic yield to drop significantly. Plants that have elevated Cr levels have altered ultrastructural characteristics [10]. When plants face Cr stress, it produces oxidative stress and destabilize the integrity of cell membranes. Reactive species produced as a result of oxidative damage may block the physiological and structural properties of plants [11].

Extended exposition to Cr may lead to cytotoxicity and other consequences in addition to negatively affecting various processes that occur in plants, such as the process of photosynthesis, and metabolic activities along the germination of seeds [12].

Innovative approaches in a variety of agricultural areas have been developed as a consequence of the effective nanotechnology application in the farming industry with the aid of nanoscience. In the past, agribusiness has been the industry of greatest significance since it supplies the raw materials needed by the industries for food and feed [13]. The latest nanotechnology methods hold great promise for resolving the challenges and inefficiencies associated with traditional agriculture operations. For example, to enhance agricultural output and protect plants from stresses, researchers require the use of nanopesticides, nanosensors as well nanofertilizers [14].

Whenever plants are stressed by toxic substances, the administration of nanoparticles also strengthens their defenses and increases their absorption of nutrient-rich minerals [15]. Numerous nanoparticles, including metal-oxide nanoparticles, demonstrated excellent efficacy in reducing a variety of stresses in plants [16]. Additionally, NPs may be utilized to lessen the toxicity of heavy metals [17]. Silica nanoparticles (SiNPs) have been shown to protect plants from *C. arietinum* from aluminum toxicity [18]. SiNPs encourage photosynthesis, plant growth, and absorption of silicon, which lessens the intensification of toxic metals in plants [19]. By promoting plant defensive systems and reducing the accumulation of harmful metals and oxidative stress in plants, SiNPs improve plants' resilience to heavy metals [18].

Tagetes erecta L. often called Mexican marigold, it is a member of the *Asteraceae* family and is a significant economic flower. About 40–50 species make up the genus *Tagetes*, which comes from the family, *Asteraceae* (Composite) [20]. The petals of marigolds possess a significant amount of carotenoids and phenolic compounds, they are believed to have therapeutic uses against various diseases [21]. The pigment that is lutein imparts marigold flowers with distinct orange and yellow colors. Lutein, which is derived from a substance known as xanthophyll, is crucial for preventing diseases and eye-related problems, especially in protecting the third layer of the eye which is the retina [22]. This research was conducted to investigate the growth of *T. erecta* in response to chromium stress as well as this research focuses on the defensive mechanisms adopted by *T. erecta* when subjected to chromium-contaminated soil.

Materials and methods

Preparation of SiNPs

The precursor sodium silicate (Na_2SiO_3) was combined with distilled water in the presence of HCl for the production of SiO_2 . For this purpose, sodium silicate (Na_2SiO_3) 10 mL was combined with 500 mL of distilled water. In the meantime, a 0.5 M HCl solution was also prepared in one liter of distilled water. Then the solution of sodium silicate (Na_2SiO_3) and water was allowed to stir continuously using a magnetic stirrer for 4 hours, and in the process of continuous stirring, HCl was added dropwise until the pH shifted from 11.8 to 7. Four hours were given to the solution to make sure the reaction was completed. Leave the sample overnight to form silica gel. Now wash the gel with pure water to counteract the acidic effect. Pour the gel into the petri dish and let the gel completely dry at room temperature. The gel was subjected to the process of drying for 12 hours in an oven at 60 °C. The dried substance was then ground into a fine powder see Fig. 1 [23]. For characterization of synthesized SiNPs, SEM, EDX, UV-VIS spectrophotometry, FTIR and XRD techniques were employed.

Experimental design and layout

Two-factor (Cr and SiNPs) CRD experiment with three replicates was conducted at Botanical Garden, University of Education, Lahore, Pakistan. Experimental was conducted in natural conditions during September 2023. Average relative humidity during the experiment was 55% with average day and night temperature of 35/25 °C. *T. erecta* seeds were procured from Punjab Seeds Centre, Lahore. Pots having 15 cm length and 20 cm diameter were filled with garden soil. Seeds were surface sterilized and then sown in filled pots. For Cr stress, CrCl_3 was purchased from Sigma-Aldrich and mixed in soil at 50 and 100 mg kg^{-1} . SiNPs were applied via foliar spray at 100 and 200 mg L^{-1} . Tween 20 (2%) was utilized as surfactant. Firstly, SiNPs were applied at 4 leaf stage of plants, three times in total with one week interval. Following that growth, physiological and biochemical attributes were measured as mentioned below.

Sampling and Growth Parameters

One plant was chosen from each treatment group, washed with tap water and wiped with fabric. Subsequently, the shoots and roots were cut down with the help of a sharp blade. Growth parameters of the plant studied by using weighing balance and ruler. Counted the number of leaves,

then measured root and shoot length, root and shoot fresh weight. After drying the roots and shoots at temperature of 65 °C, dry weight was also noted.

Chlorophyll and Carotenoid Content Determination

The procedures outlined by Arnon [24] were implemented to figure out the amount of carotenoids and chlorophyll. By carefully selecting fully grown and recently harvested leaf samples from every replicate, the samples were accurately weighed to 0.5g and then ground consistently in a mortar and pestle. The ground material was uniformly mixed with acetone of concentration 80% (10 mL) using homogenization. The mixture was passed through 42 Whatman filter papers using a filtration process. The resulting filtrate was then stored in the refrigerator for duration of 24 hours at 4°C. Absorbance of samples were noted at three wavelength 480, 645, and 663 nm,

Given formula was employed to analyze the values of chlorophyll a and b.

$$Chl. a (mg\ g^{-1}\ FW) = [12.7 (OD_{663}) - 2.69 (OD_{645})] \times V/1000 \times W$$

$$Chl. b (mg\ g^{-1}\ FW) = [22.9 (OD_{645}) - 4.68 (OD_{663})] \times V/1000 \times W$$

The amount of carotenoid content in plant was measured by the formula below described by Lichtenthaler [25].

$$Carotenoids = (1000 \times OD_{480}) - (1.9 \times Chl\ a - 63.14 \times Chl\ b)/214$$

SPAD value measurement

A leaf meter was employed to determine the SPAD value of the leaf. Three measurements were obtained from each leaf (above, center, and bottom) after one leaf was chosen from every pot. The average of these values was used to get the SPAD reading of each leaf.

Determination of Chlorophyll Fluorescence

The distinctive features of chlorophyll fluorescence were measured and fast light response curves were obtained using an OS30p+ Chlorophyll Fluorometer (Opti-sciences, Inc. | Hudson, NH 03051, USA). One leaf was taken from each replicate and placed in a chlorophyll fluorometer for examination after the replicates had all been exposed to darkness for fifteen minutes. After a period of 15 minutes in the dark, measurements were taken accurately without exposing the leaves to light. Fv/Fm, the highest quantum efficiency of PSII photochemistry, was computed.

Assesement of Relative Water Content (RWC)

Identical size leaves were carefully chosen, weighed on weighing balance, and immediately placed into water that is purified for floating of leaves. The leaves, then soaked in distilled water at temperatures ranging from 25 to 26 °C for three hours. Following a duration of 3 hours, the leaves were filled with water and attained a state of turgidity, and then, their weight was recorded. The oven was set at 80°C to dry the leaves for 24 hours, and their dry weights were then determined.

The formula described by Jones & Turner [26] was used to measure the RWC of samples.

$$RWC (\%) = [(FW - DW) / (TW - DW)] \times 100$$

Determination of Relative Membrane Permeability

From every replicate, mature, uniformly sized as well as fully grown leaves were taken. Chopped leaves were added into test tubes containing 20 milliliter of distilled water. After five seconds of vortexing the mixture, an EC meter was used to calculate the electrical conductivity (EC_0). EC_1 was observed after the tubes were kept in the refrigerator for a full day at 4 degrees Celsius. To derive EC_2 values, the same samples were autoclaved for a duration of 20 minutes at 120°C. By using the given method, the % RMP was calculated [27].

$$RMP (\%) = [(EC_1 - EC_0)/(EC_2-EC_0)] \times 100$$

Determination of Hydrogen Peroxide

Hydrogen peroxide was calculated by following the earlier specified procedure [28]. Fresh and young leaves were collected from every replicate, weighing 0.5 gram, and combined with 5 milliliter of trichloroacetic acid having concentration 0.1% in a pre cool mortar and pestle. The conical tubes were utilized to hold the extracts. The extracts were then centrifuged at 4°C and 12,000 g for 15 minutes. 0.5 milliliter of potassium phosphate buffer (pH 7), potassium iodide of 1 milliliter and 0.5 milliliter of the produced supernatant was added in the test tubes. After the mixture had been vortexed, the absorbance was read at 390 nm with the help of spectrophotometer.

Detection of Malondialdehyde

With very minor modifications, MDA was calculated using the procedure as stated [29]. 0.5 gram fresh and clean leaves were collected from each pot and blended with 3 mL of trichloroacetic acid having temperature 4 °C and concentration 1.0%. After being moved to tubes, the centrifugation

of resulting paste was done for 15 minutes at 20,000 g. once the centrifugation was done, 0.5 milliliter of the filtrate was transferred into test tubes along with 3 mL of a solution of 0.5% thiobarbituric acid prepared in 20% trichloroacetic acid. After that, each sample was heated for 50 minutes by using water bath at 95 °C with shaking. The process was terminated right away by rapidly chilling in a water bath having ice cold water. Then, for ten minutes, all samples were centrifuged again at 10,000g. Absorbance was detected at 532 and 600 nm. The MDA level (nmol) for every sample was calculated by using the formula below.

$$MDA\ level\ (nmol) = [(A_{532nm} - A_{600nm})/1.56 \times 10^5] \times V/W \times 1000000$$

Biochemical Attributes

Estimation of Total Soluble Protein content

From each replicate, a set of fresh leaf samples was gathered, the weighed of sample leaf was 0.5 gram, and subsequently combined with 10 milliliter of pre-cooled phosphate buffer having concentration 50 milli molar. The buffer was carefully prepared to maintain a pH of 7.8. After collecting the extracts in a conical tube, they were centrifugation was done at 6,000 g for duration of 20 minutes at 4 °C. After being removed from the leftover, the supernatant was kept in a deep freezer. The previously indicated methodology was used to quantify the protein level in samples [30]. To make Bradford solution, 100 milligrams of Coomassie Brilliant Blue were dissolved in 50 milliliter of 95% ethanol, Afterwards, combine the above-mentioned mixture with 85% phosphoric acid (100 ml). To get a one-liter total volume, distilled water was added. Now mix Bradford solution (5 milliliter) along with 0.1 milliliter of ready-made leaf sample that were stored in refrigerator. Wavelength was calculated at 595 nm by using spectrophotometer

Antioxidant Activity

Determination Catalase (CAT) Activity

To figure out the activity of catalase, 0.1 milliliter of leaf extract, 1.9 milliliter of 5.9 milli molar H₂O₂, and 1 milliliter of 50 milli molar phosphate buffer (pH 7.0) was combined to form a solution. Catalase absorbance was measured at a wavelength of 240 nm for every 30 seconds over 120 seconds [31].

Detection Peroxidase (POD) Activity

Similarly, for determination of POD, solution was made by combining leaf extract 0.1 mL, guaiacol 0.6 milliliter of 20 milli molar concentration, phosphate buffer (pH 5.0) 0.7 mL of 50 milli molar concentration and 0.6 milliliter of 40 mM H_2O_2 . By using spectrophotometer, absorbance of POD was measures at a wavelength of 470 nm for every 30 seconds interval for 150 seconds [32].

Assessment Ascorbate Peroxidase Activity (APX)

To assess the ascorbate peroxidase activity, 3 milliliter solution was prepared for each replicate. It contained 2.70 milliliter of 50 milli molar phosphate buffer, ascorbic acid about 0.10 milliliter of 7.50 milli molar concentration, hydrogen peroxide (H_2O_2) about 0.10 milliliter of 300 milli molar concentration, and enzyme extract of 0.10 milliliter was added. APX absorbance was recorded for 60 seconds at a wavelength of 290 nm for every 30 seconds [33].

Determination of Superoxide Dismutase (SOD) Activity

To prepare the sample for determination of SOD, take 0.3 milliliter of 130 milli molar methionine, 0.3 milliliter of 50 micro molar nitro blue tetrazolium, 0.3 milliliter of 100 micro molar EDTA- Na_2 and 0.3 milliliter 20 micro molar riboflavin were combined. After adding 0.05 mL sotion of enzymes to the reaction solution, the mixture was exposed to light of 4000 lux for at least 20 minutes. After preparation of samples, absorbance was checked at the wavelength of 560 nm [34].

Assessment of Inorganic Mineral Ions Content

After obtaining samples of dried shoots and roots, they were thoroughly mixed in a pestle and mortar. Samples of roots as well as shoots were digested by using H_2SO_4 , weighing 0.1 g. Test tubes were filled with samples, and two milliliters of H_2SO_4 were added. Following 24 hours, samples was heated by using hot plate and gradually mixed in H_2O_2 until the liquid took on no color. After that, water that had been distilled was added. A final volume of 50 mL of distilled water was added. Following the filtration of this mixture, a flame-photometer was employed to measure the levels of Na^+ , K^+ , and Ca^{++} [35].

RESULTS

Characterization of silica nanoparticles.

SEM analysis

To observe and analyze the size, shape, and surface properties of SiNPs is scanning electron microscopy or SEM. SEM images provide three-dimensional, high-resolution images important for applications in chemistry, materials science, and nanotechnology. An electron beam is applied on the surface of the nanoparticle; it releases an electron that is collected to make an image. The shape of SiNPs depends on the method of synthesis. Our results revealed that, the size of SiNPs is 20 38.5 nm , and the shape of the particle will be nearly spherical (Fig. 2A).

EDX analysis

The elemental composition of nanoparticles can easily be determined by the utilization of the EDX technique in our synthesis, EDX analysis was used to validate the presence of silica in nanoparticles sample synthesized by the sol-gel method. The percentage of silicon is 29.52% and oxygen is 45.09% respectively. The highest peaks are of silicon and oxygen as compared to other additional components confirming the presence of silica (Fig 2B).

UV-vis spectroscopy

Ultraviolet-visible (UV-Vis) spectroscopy is commonly used to determine the size of nanoparticles because nanoparticle size influences their absorption properties while nanoparticles are smaller and their absorption spectra shift to shorter wavelengths. As in our results, the peak is found at 277 nm which proves that the size of silica nanoparticles lies within the range of 100 nm (Fig. 2C).

FTIR analysis

FTIR analysis is used to identify the functional groups of metabolites involved in the synthesis of nanoparticles. Figure (D) presents the FTIR analysis results for silica nanoparticles within the wavenumber range of 4000-540 cm^{-1} . The spectrum shows various peaks corresponding to different functional groups.

Distinct absorption bands between 3500 and 2000 cm^{-1} indicate characteristic OH and N-H stretching of aldehydes. The peak at 3243 cm^{-1} is attributed to polyphenols, the OH groups of alcohols, or amines. Peaks in the region of 2500 to 2000 cm^{-1} signify $\text{C}\equiv\text{C}$ and $\text{C}\equiv\text{N}$ vibrations. Absorption bands observed between 1400 and 1000 cm^{-1} correspond to methylene and C-N stretching vibrations. The presence of a peak at 1068 cm^{-1} is consistent with the formation of silica

nanoparticles as reported in the literature. These spectral features collectively reveal the presence and involvement of various functional groups on the surface of silica nanoparticles, providing insights into their composition and molecular interactions (Fig. 2D).

XRD analysis

The X-ray diffraction pattern presented in Figure (E) offers valuable insights into the crystalline nature of the silica nanoparticles. The pattern exhibits distinct, well-defined broad peaks, indicating the presence of a nanocrystalline phase. The primary diffraction peaks are observed at 2θ values of 21.93° , 28.41° , 31.55° , 36.11° , 41.36° , 45.39° , 48.41° , 53.67° , and 56.78° , corresponding to the (101), (111), (102), (200), (201), (112), (202), (113), and (220) planes, respectively. These peaks confirm the crystalline structure of the silica nanoparticles and align with the Joint Committee on Powder Diffraction Standards (JCPDS) database, specifically file No. 39-1425.

Using the Scherrer formula, the average crystallite size of the synthesized silica nanoparticles was determined to be 38.5 nm. This X-ray diffraction analysis provides essential information about the crystallinity and structure of the synthesized silica nanoparticles (Fig 2E).

Effects of SiNPs on growth parameters of *T. erecta* under Cr stress

Cr stress had great influence on growth of *T. erecta* plants. Conversely, SiNPs application reversed the impact caused by Cr stress on growth of plants as shown in Fig. 3. Chromium stress significantly decreases the shoot length by 21% and 67% when grown in toxic conditions under 50 mg kg^{-1} and 100 mg kg^{-1} Cr stress, respectively in comparison with control plants. When the treatment of SiNPs was given, they improved the shoot length by 35% and 57% when compared to control. In the case of Cr contaminated plants, application of SiNPs significantly increase the shoot length in comparison with Cr only contaminated plants (Fig. 4A). With 50 mg kg^{-1} and 100 mg kg^{-1} Cr stress, the root length was significantly lower by 46% and 68% when compared to untreated control, respectively. In relation to non-contaminated, sprays of SiNPs improved the root length by 9% and 35% respectively. Application of SiNPs to the plants growing in contaminated soil remarkably enhanced root length than that of Cr only treated plants (Fig. 4B). Cr toxicity also reduced the number of leaves of *T. erecta* plants by 32% and 40% under 50 mg kg^{-1} and 100 mg kg^{-1} Cr stress, respectively concerning control. when SiNPs was given to the plant by the foliar method they increased the number of leaves by 28% and 42% respectively, as equated to control.

Application of SiNPs enhanced the number of leaves by 69% and 82% respectively as compared to stressed plants Cr 1. SiNPs also boosted the number of leaves by 118% and 136% respectively, as compared to Cr (Fig. 4C).

Cr exerted toxic effect on the *T. erecta* plants by declining the shoot fresh weight (SFW) by 18% and 68% grown under Cr contaminated soil (50 mg kg⁻¹ and 100 mg kg⁻¹) respectively, as compared to non-stressed plant. SiNPs effectively raised the SFW by 37% and 63% respectively, in comparison with non-treated plants. Supplementation of SiNPs increases the SFW by 32%, 43%, 232% and 293% growing under Cr contaminated soil, respectively, when compared to the Cr stressed plants (Fig. 5A). Shoot dry weight (SDW) of *T. erecta* was lowered by the Cr toxicity by 34% and 80% under 50 mg kg⁻¹ and 100 mg kg⁻¹ respectively, in relation to untreated control. Supplementation of SiNPs led to the increment in SDW by 24% and 43% respectively when compared to non-stressed plants. Likewise, in Cr contaminated plants, administration of SiNPs outstanding increase the SDW as compared to Cr alone treatment (Fig. 5B).

Root fresh weight (RFW) reduced by 14% and 37% with 50 mg kg⁻¹ and 100 mg kg⁻¹ respectively, in relation to control. Roots FW was elevated by the application of SiNPs by 42% and 60% respectively, in comparison to untreated control. Moreover, SiNPs treatment enhanced the RFW by 30%, 50%, 74% and 79% respectively, in relation to stress plants (Fig. 5C). Chromium decrease the root dry weight (RDW) by 63% and 79% under 50 mg kg⁻¹ and 100 mg kg⁻¹ respectively, when compared to the untreated control. On the contrary SiNPs increased the RDW by 56% and 89% respectively, as compared to non-contaminated control. In similar manner, foliar spray of SiNPs remarkably improved the RDW in comparison with only Cr treated plant (Fig. 5D).

Impact of SiNPs on photosynthetic pigments of *T.erecta* under Cr toxicity

Soil contaminated with Cr decrease the content of chlorophyll *a* by 20% and 30% under 50 mg kg⁻¹ and 100 mg kg⁻¹ respectively, when compared to control. Opposite to that, supplementation of SiNPs augmented the level of chlorophyll *a* by 10% and 25% respectively, in relation to non-contaminated plants. Moreover, supplementation of SiNPs further enhanced the level of chlorophyll under toxic conditions by 25%, 31%, 50% and 71% respectively, in relation to only Cr-contaminated plants (Fig. 6A). During the present study, 39% and 54% reduction of photosynthetic pigment like chlorophyll *b* was observed in *T.erecta* plants under 50 mg kg⁻¹ and 100 mg kg⁻¹ conditions respectively, than that of control. However, the foliar sprays of SiNPs

enhanced the level of chlorophyll *b* by 17% and 22% respectively, in relation to untreated control. The supplementation of SiNPs led to an additional increment in the level of chlorophyll *b* by 25%, 68%, 162%, and 181% respectively, then that of Cr alone treatment (Fig. 6B).

Chromium toxicity decreases the level of carotenoid by 24% and 34% under 50 mg kg⁻¹ and 100 mg kg⁻¹ conditions respectively, when compared to control. Moreover, the addition of SiNPs raised the level of carotenoid by 14% and 16% respectively, as compared to untreated control. The supplementation of SiNPs augmented the level of carotenoid by 18%, 41%, 63% and 75% respectively, as compared to the concerned Cr alone treatment (Fig. 6C).

Effect of SiNPs on Relative Water Content (RWC) of *T. erecta* plants under Cr stress.

Chromium toxicity reduced the relative water content by 30%, 42% under 50 mg kg⁻¹ and 100 mg kg⁻¹ conditions respectively, as equated to control plants. Foliar supplementation of SiNPs enhanced the RWC by 12% and 17% respectively, in comparison with non-polluted conditions. In case of Cr-contaminated soil, the treatment of SiNPs further increase the RWC by 47%, 63%, 94% and 108% respectively, when compared to Cr polluted conditions (Fig. 6D).

Effect of SiNPs on maximum quantum efficiency of PSII (Fv/Fm) value of *T. erecta* plant under Cr stress

Fv/Fm value of *T. erecta* plant was lowered by 27% and 29% under Cr 50 mg kg⁻¹ and 100 mg kg⁻¹ respectively, corresponding to control. Fv/Fm value of *T. erecta* plant augmented by the supplementation of SiNPs by 9% and 14% respectively, in comparison to untreated control. Moreover, SiNPs treatment enhanced the SPAD value by 25%, 35%, 30% and 35% respectively, concerning stress plants only (Fig. 7A).

Effect of SiNPs on SPAD value of *T. erecta* plant under Cr stress

SPAD value was reduced by 47% and 57% under Cr 50 mg kg⁻¹ and 100 mg kg⁻¹ respectively, compared to control. SPAD value of *T. erecta* plant increased by the application of SiNPs by 21% and 31% respectively, in comparison to untreated control. Moreover, SiNPs treatment significantly enhanced the SPAD values in relation to stress plants (Fig. 7B).

Effect of SiNPs on total soluble protien contents of *T. erecta* plants under Cr stress.

During the current investigation, the Cr toxicity markedly decreases the total soluble protein by 6% and 26% under 50 mg kg⁻¹ and 100 mg kg⁻¹ conditions respectively, in relation to untreated to

control. In opposite to that, the foliar spray of SiNPs elevated the level of by 23% and 46% respectively, over corresponding controls. Similarly, when SiNPs sprayed on the plants, they increase the level of total soluble protein by 18%, 30%, 62% and 96% respectively, then that of only Cr-treated plants (Fig. 7C).

Effect of SiNPs on the antioxidant enzymes (CAT, POD, APX & SOD) activities in *T. erecta* plants under Cr stress

Cr stress enhanced the activity of CAT by 31% and 46% under 50 mg kg⁻¹ and 100 mg kg⁻¹ conditions respectively, when compared to control conditions. Exogenous application of SiNPs increases the activity of CAT by 8% and 54% respectively, in relation to the control group. Moreover, providing SiNPs elevated the activity of CAT by 5%, 19%, 5%, and 7% respectively, when compared to Cr-only contaminated conditions (Fig. 8A).

Toxicity of Cr has enhanced the activity of POD by 27% and 48% under 50 mg kg⁻¹ and 100 mg kg⁻¹ conditions respectively, as compared to given normal conditions. SiNPs application augmented the activity of POD by 55% and 69% respectively, when compared to non-contaminated conditions. In *T. erecta* plants of Cr-contaminated pots, supplementation of SiNPs application further enhanced the activity of POD by 11%, 44%, 9% and 20% respectively, when compared to Cr-only contaminated conditions (Fig. 8B).

Cr toxicity markedly elevated the activity of APX by 32% and 38% under 50 mg kg⁻¹ and 100 mg kg⁻¹ conditions respectively, concerning control. Treatment of SiNPs augmented the APX level by 5% and 14% respectively, as compared to non-contaminated soil. Moreover, SiNPs enhanced the activity of APX by 6%, 8%, 8% and 12% respectively, as compared to Cr-only contaminated conditions (Fig. 8C).

T. erecta plants growing under toxic conditions 50 mg kg⁻¹ and 100 mg kg⁻¹ exhibit increment in the level of SOD by 15% and 37% respectively, in relation to control. Treatment of SiNPs improves the activity of SOD by 34% and 54% respectively, as equated to the control. Furthermore, the treatment of SiNPs enhanced the activity of SOD by 26%, 42%, 8% and 13% respectively, when compared to Cr-only plants (Fig. 8D).

Effect of SiNPs on stress indicators (MDA, H₂O₂ & RMP) and proline contents of *T. erecta* plants under Cr stress.

T. erecta plants growing under toxic conditions 50 mg kg⁻¹ and 100 mg kg⁻¹ exhibit enhancement in the level of MDA by 92% and 99% respectively, when compared with untreated control. However, when SiNPs were applied, this trend was reversed and decreased the MDA by 3% and 27% respectively, as equated to the control. Furthermore, the application of SiNPs markedly lowered the level by 14%, 34%, 18% and 24% respectively, as compared to stressed plants only (Fig. 9A).

Under hazardous conditions 50 mg kg⁻¹ and 100 mg kg⁻¹, H₂O₂ levels of *T. erecta* plants elevated by 83% and 114% respectively, with relation to untreated control. conversly, supplementation of SiNPs decreased the H₂O₂ levels of *T. erecta* plants by 6% and 17% respectively, as compared to the concerned control. likewise, the foliar application of SiNPs significantly reduced the level H₂O₂ by 19%, 30%, 21% and 37% respectively, as compared to stressed plants only (Fig. 9B).

Toxicity of Cr increases the Relative membrane permeability by 23% and 48% under 50 mg kg⁻¹ and 100 mg kg⁻¹ respectively, concerning non-contaminated plants. However, the application of SiNPs decreases the value of Relative membrane permeability by 16% and 34% respectively, as equated to control. Furthermore, the addition of SiNPs further reduce the values of Relative membrane permeability by 19%, 35%, 45% and 54% respectively, in comparison with Cr treatment only (Fig. 9C).

Cr stressed plants had elevated proline contents when compared to non-stressed plants. A gradual increment in proline level of *T. erecta* plants was observed with increase in stress level. Similarly, SiNPs foliar spray at both concentrations also increased proline level in both control and Cr stressed *T. erecta* plants (Fig. 9D)

Influence of SiNPs on nutritional content of *T. erecta* plants under Cr stress.

Cr toxicity reduced the absorption of minerals such as Na⁺ by 12 and 90%, K⁺ by 16 and 39% and Ca²⁺ by 15 and 28% under 50 mg kg⁻¹ and 100 mg kg⁻¹ stress level, respectively, equated to control plants. SiNPs enhanced the mineral absorption of Na⁺ by 41 and 51%, K⁺ by 35 and 52% and Ca²⁺ by 16 and 36% at 100 and 200 mg L⁻¹, respectively, in comparison with non-polluted conditions. Similarly, in the case of Cr-contaminated soil, the application of SiNPs also escalated the mineral

absorption of Na^+ , K^+ and Ca^{2+} at both stress levels in comparison to stress only plants (Fig. 10A-C).

Pearson's correlation and principle component analysis (PCA)

Pearson's correlation in the Fig. 11 revealed that growth and biomass related parameters were positively correlated with chlorophyll pigments and essential mineral ions. This showed that SiNPs application improved photosynthesis related attributes and modulated mineral ion uptake. This ultimately upheld growth of *T. erecta* plants. On other side, growth related parameters were negatively correlated with stress indicators of *T. erecta* plants. It is thus depicted that Cr stress imposed oxidative damage to plants in the form of elevated MDA level, lipid peroxidation and permeability loss. This ultimately hampered plant growth and development. From this it can be assured that SiNPs were much effective in alleviation of Cr imposed toxicity in *T. erecta* plants.

PCA (Fig. 12A-C) also validated these findings. It showed that all the studied variances in this work were explained in 8 principle components (PCs) out of which the first two had maximum contribution 92.01% in total (Fig. 12A). Fig. 12B showed that all the treatments applied in this study were also successfully distributed in the first two PCs. Cr-I and Cr-II represented by numeric 4 and 7 in the graph are separated well from the other treatments. This showed that both stress levels had great influence on all studied attributes of *T. erecta* plants. PCA of parameters (Fig. 12C) depicted that studied parameters were divided into two groups. Parameters of first group were aligned with PC1 and parameters of second group were aligned with PC2. Parameters of PC1 including growth, physiological and mineral ions attributes had positive correlation with each other but had negative correlation parameters of PC2 mainly stress indicators of *T. erecta* plants.

DISCUSSION

In plants, the presence of chromium can be utilized to ascertain the level of stress induced by toxic metals. Normal growth indicates a reduced amount of heavy metals in the plants, and these metals often have fewer harmful impacts on the growth of plants. In the current research, exogenously applied SiNPs significantly minimize the effect of chromium on *T. erecta*.

examined how the growth of *T. erecta* was affected by chromium stress against SiNPs. Our results showed that the growth of *T. erecta* was hampered due to the presence of Cr in the soil. The height of root and shoot and the fresh and dry weight of roots and shoots were greatly affected by the

presence of Cr ions in the region of roots. This Cr metal replaces the essential ions and enter the plant via roots to deactivate the plants regular function resulting in lower growth. Wu et al. [36] clearly mention that the growth of peas was inhibited when Cr stress was applied. Fawe et. al [37] explained that when SiNPs were applied to the gramineous plant they made a thin layer on the plant and that layer protected them from environmental changes. In the present work, the damage that occurred due to Cr was mitigated by providing SiNPs, they enter the plant through aerial parts and shield the plant from damage caused by oxidation reactions, hence encouraging the development of *T. erecta* [38]. Emamverdian et al. [39] also revealed that when bamboo (*P. pygmaeus*) was subjected to lead (Pb) stress, SiNPs preserved its cellular structure and enhanced growth characters.

The rate of photosynthesis decreases as the plant is subjected to Cr stress [40]. The presence of photosynthetic pigments is crucial for enabling plants to effectively harness light energy to form required products. Our findings also describe that the amount of photosynthetic pigments was lowered because Cr disturbs the mechanism for the production of above-mentioned pigments. when a plant undergoes chromium-induced stress. Then these pigments diminished their activity resulting in a low photosynthetic rate as well as stunted growth [41]. The results were reversed by the addition of SiNPs, plants with SiNPs had significant values of chl a, chl b and carotenoids pigments. According to Rizwan et al. [19] when plants were exposed to Cd, chlorophyll contents such as chl a, chl b and carotenoids was significantly increase by the supplementation of SiNPs in rice plants.

plants of *P. oleracea* were subjected to Cr stress, they experienced elevated levels of MDA and H_2O_2 . In our work, the levels of MDA and H_2O_2 were also high due to the higher amount of Cr in the plants. Increased levels of MDA and H_2O_2 indicate that the plants are encountering oxidative stress. The higher values of MDA and H_2O_2 mean a higher degree of oxidative damage, an increment in these values proved not to be beneficial rather it damages the cell membrane and eventually causes cellular death. The roots and leaves are the major organs that experience higher levels of MDA and H_2O_2 [42]. Conversely, MDA and H_2O_2 values significantly decrease in *T. erecta* by the application of SiNPs. The outcomes of our research work are similar to the findings of Ahmad et al. [43] that during dry scenarios, the incorporation of SiNPs considerably decreases the MDA and H_2O_2 concentrations.

In the present work, Cr stress markedly decreases the total soluble protein in *T. erecta*. Ali et al. [44] also investigated that an increase in the chromium concentration further dropped protein content which as a result led to the creation of reactive species in spinach plants. on the contrary, total soluble protein content was augmented by the supplementation of SiNPs in *T. erecta*. moreover, Fatemi et al. [45] also revealed that the level of soluble protein was elevated when SiNPs were applied in Pb-stressed plants.

Absorption of essential nutrients by the plant is hampered due the presence of Cr stress. Roots of the plant contain Cr 100 times more as compared to Cr content in shoots. The uptake of cations for normal functioning of cells is diminished by the Cr accumulation in the rhizosphere region. In the present study, the Cr toxicity decreases the translocations of cations from soil to the roots of the plants. Even when present in very low quantities, it can seriously hinder the absorption of nutrients and water, resulting in a shortage of ions from roots to the shoots of plants [46]. Treatments of SiNPs interestingly augmented the uptake of nutrients into the plant. These findings are confirmed by the Fraceto et al. [47] that enhanced absorption of nutrients enabled by SiO₂ treatment.

when plants encounter stress conditions, they generate ROS at a greater rate, plant activates its antioxidant mechanism to alleviate or scavenge the ROS. Although, chromium has detrimental impact on the plant, but plant uses its antioxidant machinery to deal with stress situation [41]. Our results show that the level of enzymes that convert toxic substances into non-toxic molecules to avoid damage such as CAT, SOD, POD, and APX increases as the plant experiences stress. They tend to accumulate in tissues of the plant to protect the cellular machinery from heavy metal stress. Antioxidant enzymes rapidly activate themselves to detoxify the ROS produced from the Cr stress. However, this strategy is not sufficient to tolerate stress conditions. Exogenously applied SiNPs further elevated the activity of antioxidant enzymes to minimize the impact of Cr stress on the plant. Garg et al. [48] proved by the experiment on maize plants that the activity of all antioxidant enzymes was significantly greater in the presence of SiNP treatments of maize plants. our findings are supported by Fatemi et al. [45] that the stress of lead (Pb) on *E. sativa* seedlings was decreased by increasing the activity of SOD, POD, and CAT under the influence of externally applied SiNPs.

A very effective stress marker in plants is proline, proline is a substance related to indicating the extent of stress in plants [49]. The results of the present research indicate that the proline level of

T. erecta under stress was elevated as compared to untreated control. The high rise in the values of proline is due to the stress, under stress the plants produce proline that is efficiently associated with different signaling molecules to reduce the stress of heavy metals [50]. Our results are very similar to Adhikari et al. [51] findings, as a result of stress, plants accumulate more than enough proline as an adaptive mechanism to cope up with stress situation. In the same pattern, we observed the high values of proline *T. erecta*, when SiNPs were applied. SiNPs improve the proline content by activating the enzymes that protect the plant from oxidative damage. Our results are in line with Zhao et al. [49] that SiNPs elevated the proline under Cd stress in rapeseed seedlings.

CONCLUSION

Reactive oxygen species produced as a result of Cr stress, can be very harmful to plants when they are generated in large quantities. They are well known to initiate oxidative processes that may further degrade biomolecules, block enzyme activity, and eventually cause cellular death. Thus, protecting plants against the toxicity of Cr is crucial to combat cellular damage and oxidative stress brought on by plant absorption of Cr. In this research, we analyze the effect of SiNPs on alleviating Cr stress. Additionally, by employing changing transduction processes and signaling pathways through interaction with ROS, SiNPs can reduce stressful circumstances. Utilization of SiNPs reduced Cd buildup in wheat grains of wheat as well as promoted the proliferation of cells. The biomass of plants rose and accumulations of metals by crops decreased when SiNPs were available in the rhizosphere region.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Statements & Declarations

Ethical approval and consent to participate

We declare that the manuscript reporting studies do not involve any human participants, human data or human tissues. So, it is not applicable.

Our experiment follows with the relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not applicable

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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Author Contributions

HZR; Experimentation and Methodology, AAS & ZN; Conceptualization, Supervision and Validation, SU & SJ; Statistical analysis, writing-original draft preparation, MK & MKG; Resource acquisition and Investigation, and SS; Data curation and Formal analysis. All authors read and approved the final manuscript.

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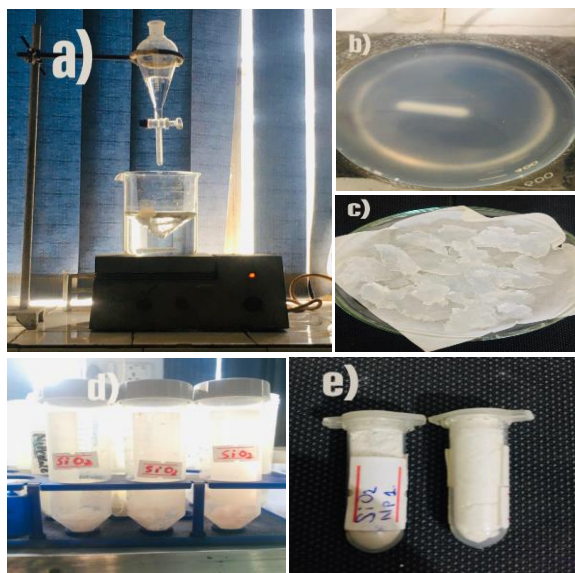


Figure 1. Synthesis process of SiNPs. a) reaction of sodium silicate with HCl, b) cloudy solution was formation, c) gel formation at the end of reaction, d) centrifugation and e) grinding to get powdered SiNPs.

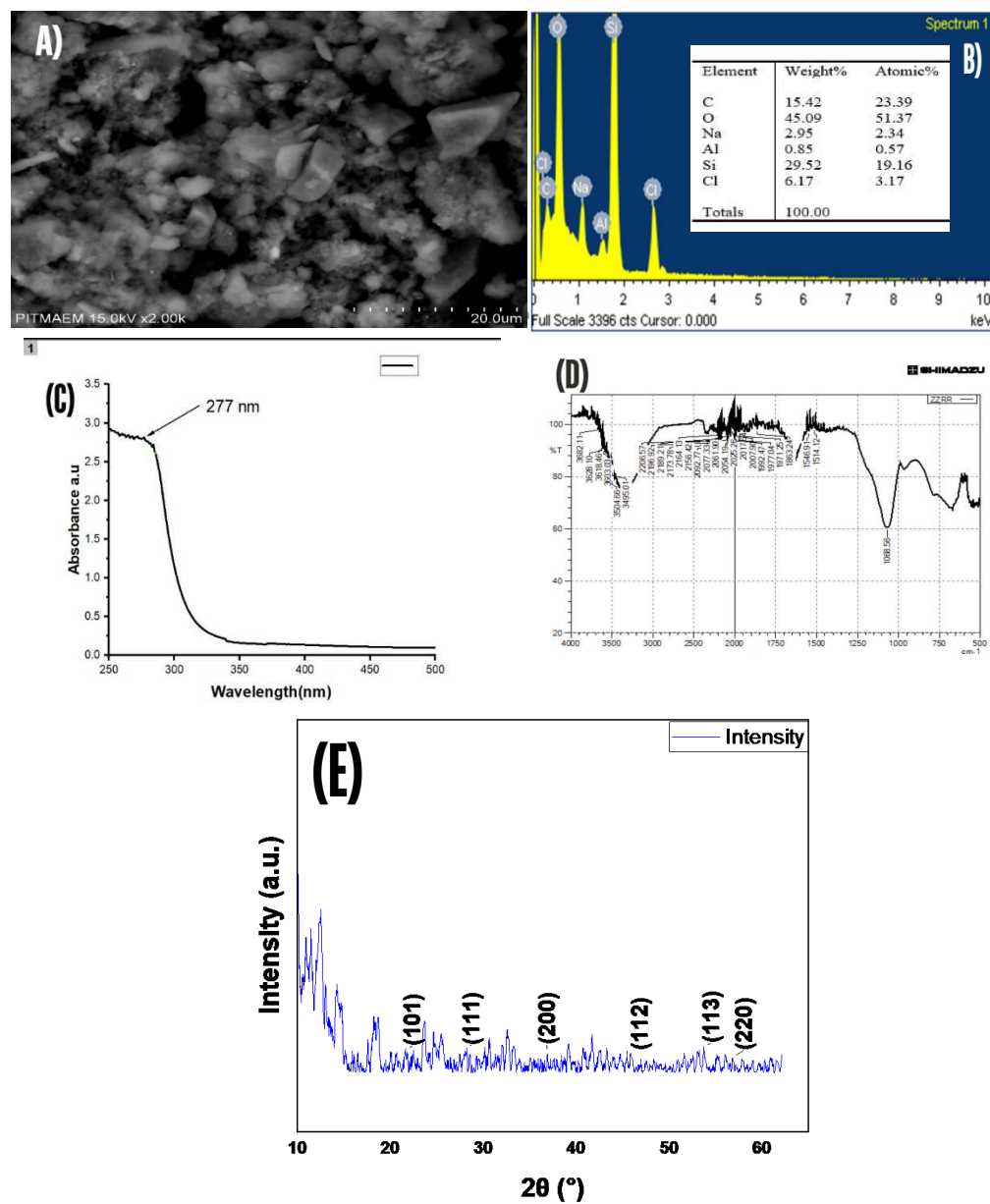


Figure 2. Characterization of synthesized SiNPs. A) SEM micrograph of silica nanoparticle, B) EDX analysis of silica nanoparticle, C) UV-VIS spectrophotometry showing the optical absorption spectra of silica nanoparticles, D) FTIR spectra of silica nanoparticle and E) XRD graph showing the size of silica nanoparticles.



Figure 3. Effect of SiNPs on growth of *T. erecta* plants under Cr stress. Where; Cr 1 = CrCl_3 50 mg kg^{-1} of soil, Cr 2 = CrCl_3 100 mg kg^{-1} of soil, NP 1 = SiNPs 100 mg L^{-1} and Np 2 = SiNPs 200 mg L^{-1} .

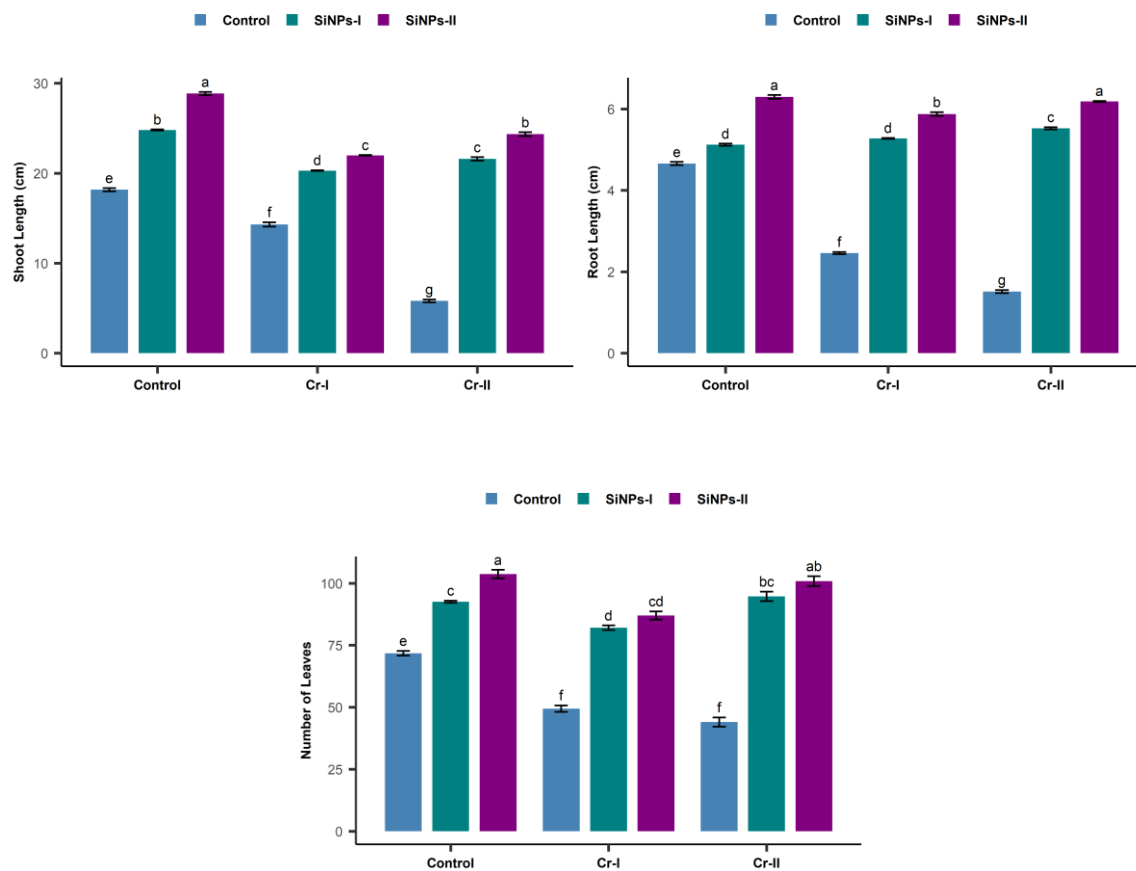


Figure 4. Effect of SiNPs application on growth of *T. erecta* plants under Cr stress. A) shoot length, B) root length and C) number of leaves. Graph bars presented the average of three replicates. Error bars presented standard error of mean. Graph bars sharing same letters are not significantly different at $p \leq 0.05$.

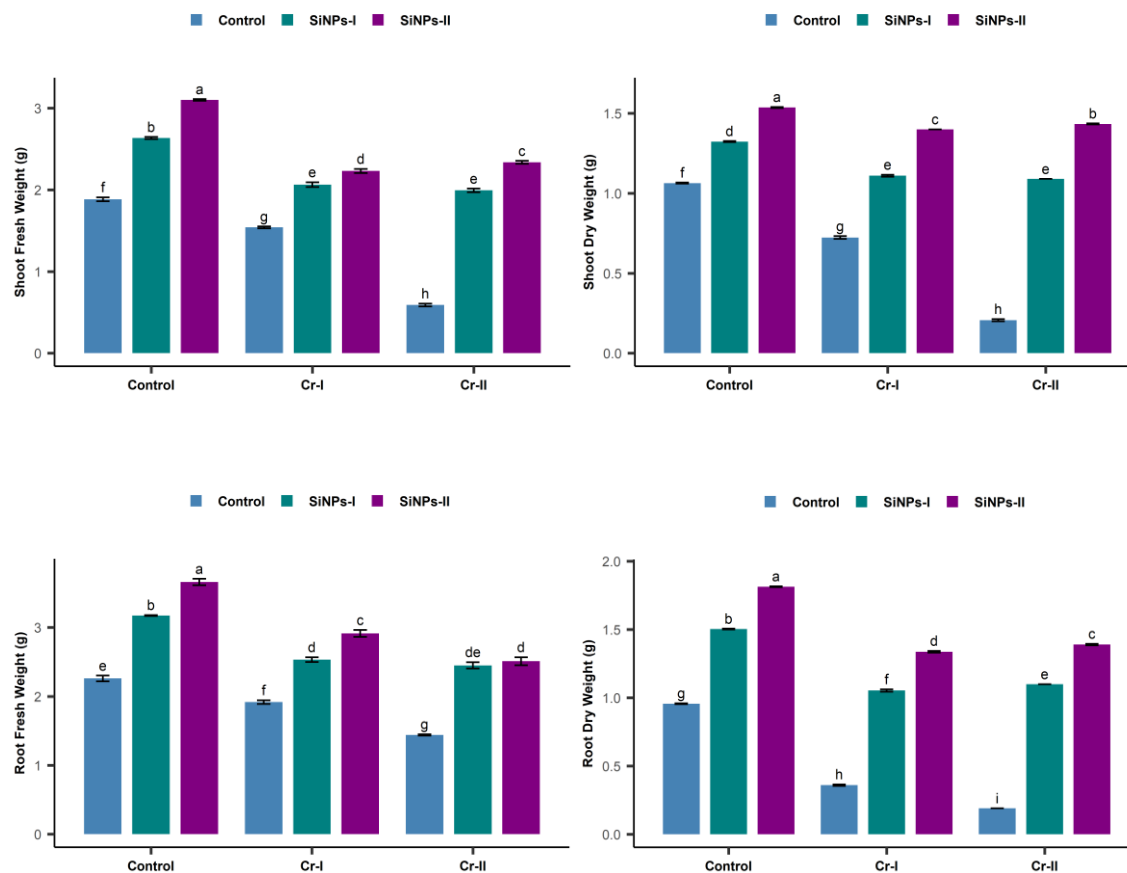


Figure 5. Effect of SiNPs application on biomass of *T. erecta* plants under Cr stress. A) shoot fresh weight, B) shoot dry weight, C) root fresh weight and D) root dry weight. Graph bars presented the average of three replicates. Error bars presented standard error of mean. Graph bars sharing same letters are not significantly different at $p \leq 0.05$.

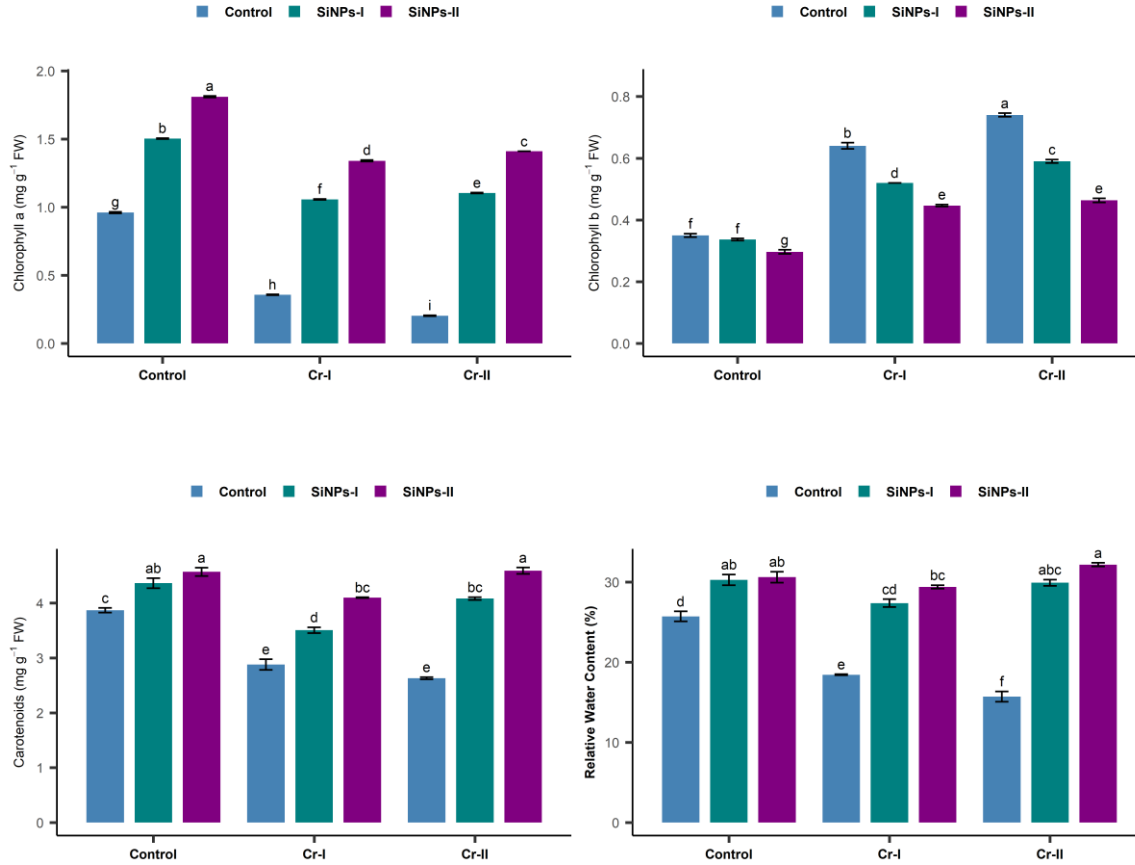


Figure 6. Effect of SiNPs application on physiological attributes of *T. erecta* plants under Cr stress. A) chlorophyll *a*, B) chlorophyll *b*, C) carotenoid and D) relative water contents. Graph bars presented the average of three replicates. Error bars presented standard error of mean. Graph bars sharing same letters are not significantly different at $p \leq 0.05$.

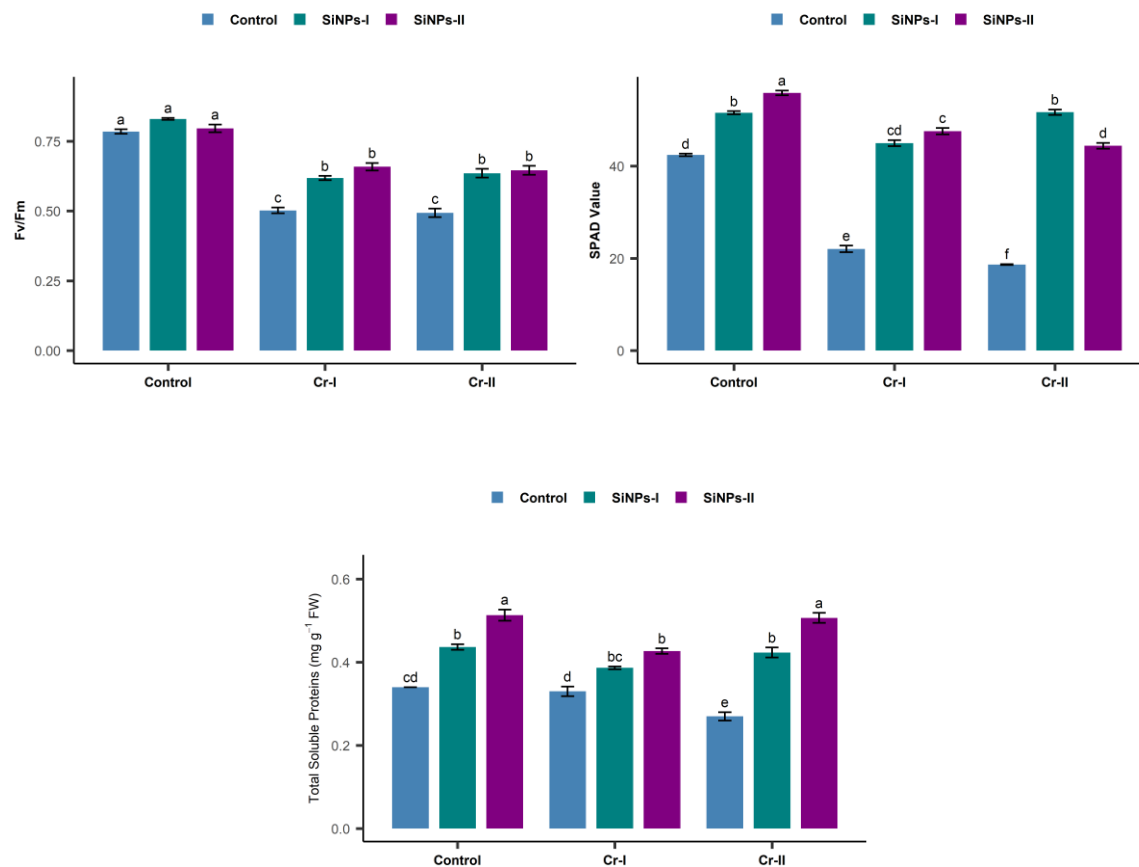


Figure 7. Effect of SiNPs application on A) quantum efficiency of PSII (Φ PSII), B) SPAD value and C) total soluble proteins in *T. erecta* plants under Cr stress. Graph bars presented the average of three replicates. Error bars presented standard error of mean. Graph bars sharing same letters are not significantly different at $p \leq 0.05$.

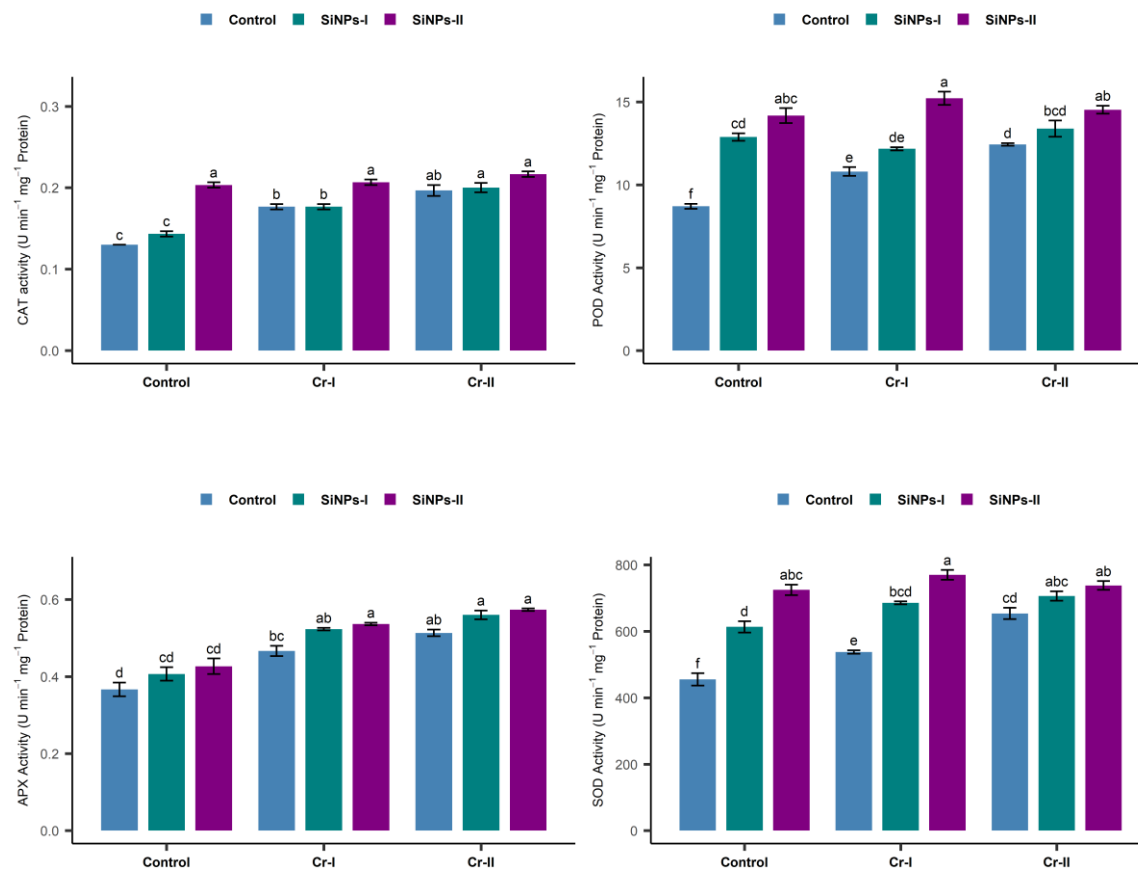


Figure 8. Effect of SiNPs application on antioxidant enzymes activities in *T. erecta* plants under Cr stress. A) Catalase, B) Peroxidase, C) Ascorbate peroxidase and D) Superoxide dismutase. Graph bars presented the average of three replicates. Error bars presented standard error of mean. Graph bars sharing same letters are not significantly different at $p \leq 0.05$.

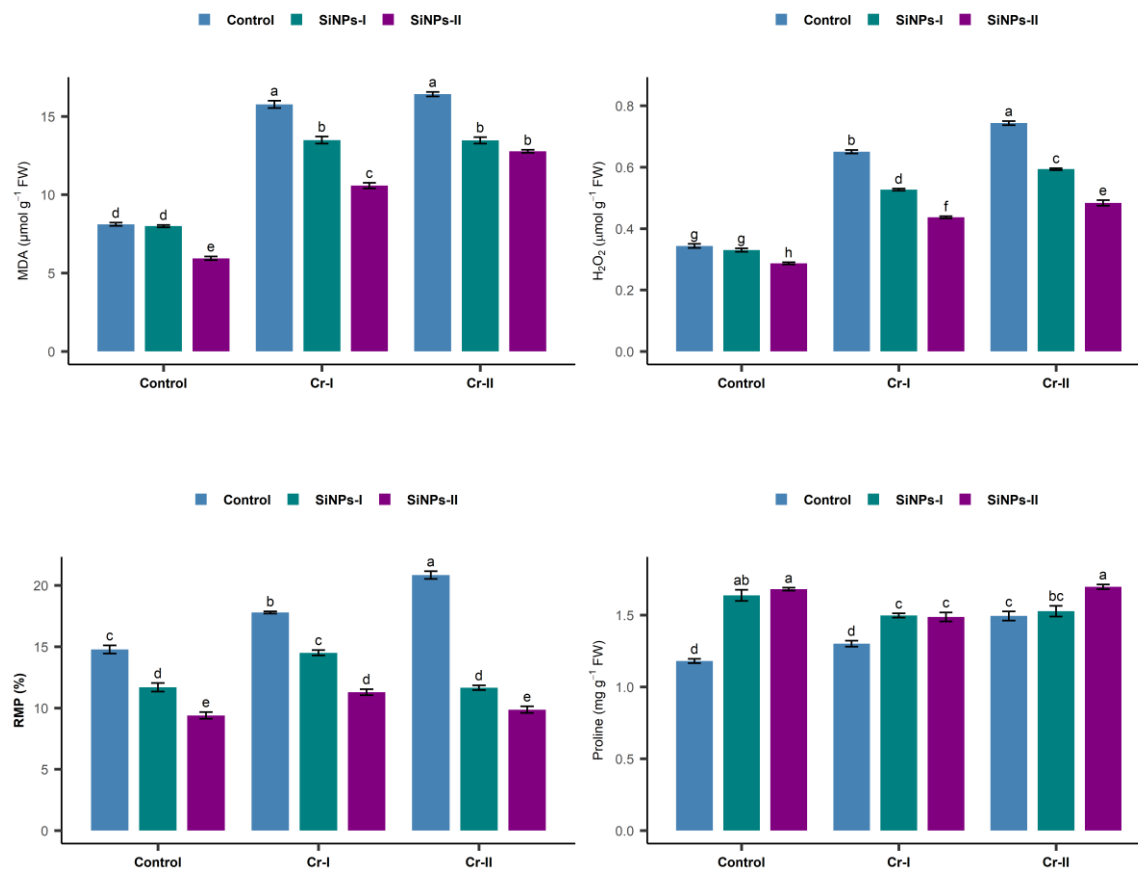


Figure 9. Effect of SiNPs application on A) malondialdehyde, B) hydrogen peroxide, C) relative membrane permeability and D) proline contents of *T. erecta* plants under Cr stress. Graph bars presented the average of three replicates. Error bars presented standard error of mean. Graph bars sharing same letters are not significantly different at $p \leq 0.05$.

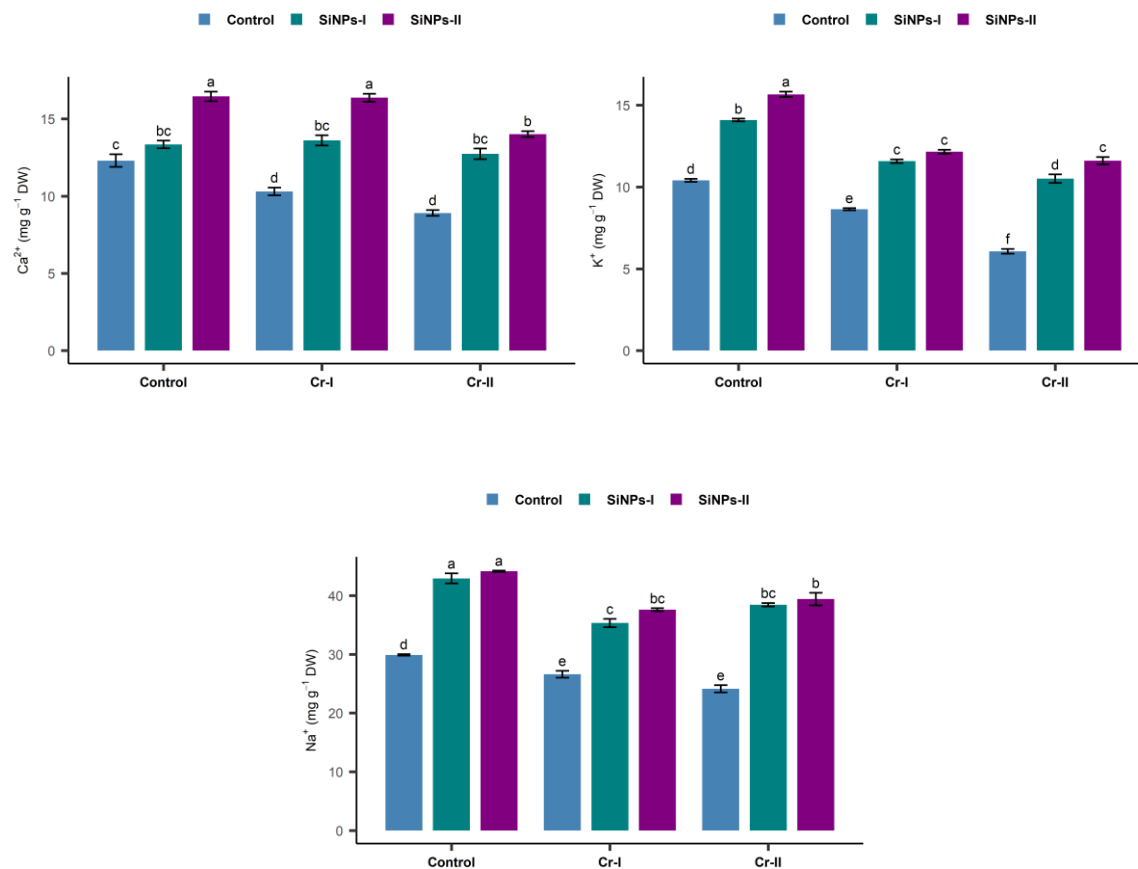


Figure 10. Effect of SiNPs application on mineral ions of *T. erecta* plants under Cr stress. A) calcium, B) potassium and C) sodium ions. Graph bars presented the average of three replicates. Error bars presented standard error of mean. Graph bars sharing same letters are not significantly different at $p \leq 0.05$.

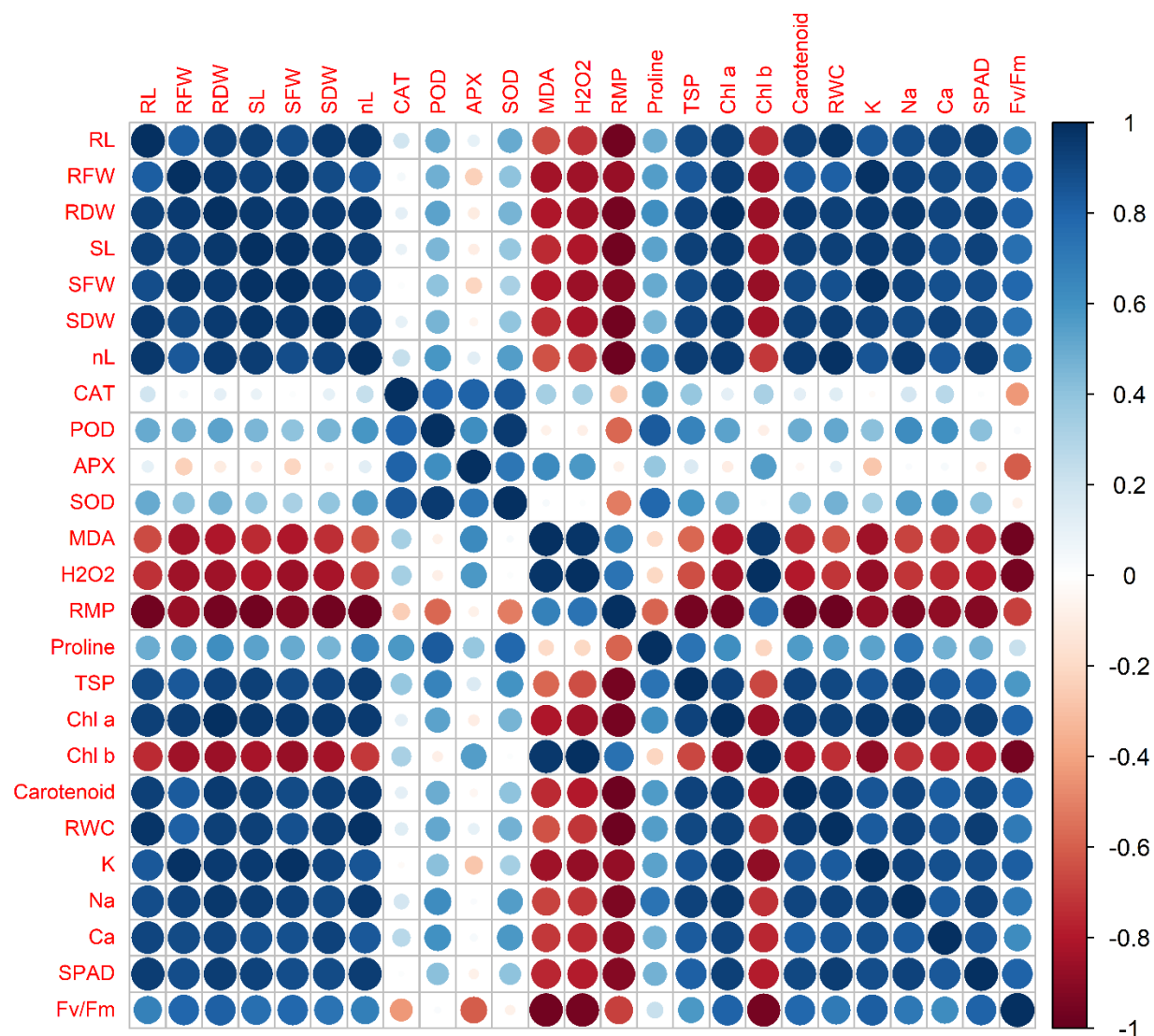


Figure 11. Pearson's correlation for all studied parameters of *T. erecta* plants treated with foliar spray of SiNPs (100 and 200 mg L⁻¹) in control and Cr stress (50 and 100 mg kg⁻¹ of soil). (Various abbreviations used are as follows; RL; root length, RFW; root fresh weight, RDW; root dry weight, SL; shoot length, SFW; shoot fresh weight, SDW; shoot dry weight, nL; number of leaves, CAT; catalase, POD; peroxidase, APX; ascorbate peroxidase, SOD; superoxide dismutase, MDA; malondialdehyde, H₂O₂; hydrogen peroxide, RMP; relative membrane permeability, TSP; total soluble proteins, Chl; chlorophyll, RWC; relative water content, K; potassium, Na; sodium, Ca; calcium and Fv/Fm; maximum quantum efficiency of PSII).

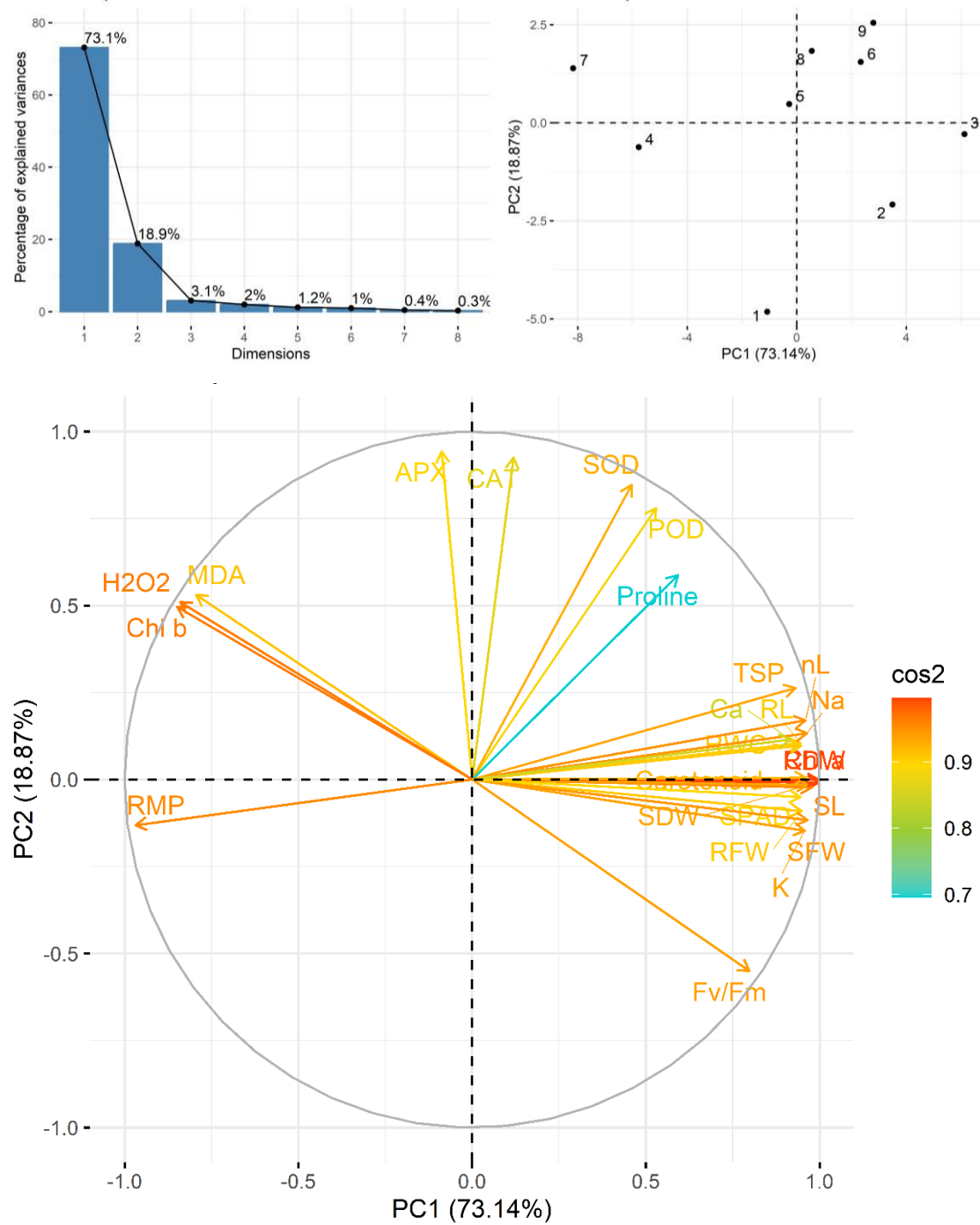


Figure 12. Principle component analysis (PCA). A) percentage of explained variables (parameters) in all principle components, B) PCA for individual treatments where ; 1 = Control, 2 = SiNPs-I, 3 = SiNPs-II, 4 = Cr-I, 5 = Cr-I+SiNPs-I, 6 = Cr-I+SiNPs-II, 7 = Cr-II,

8 = Cr-II+SiNPs-I, 9 = Cr-II+SiNPs-II and C) PCA for all studied parameters (various abbreviations used are same as described in Fig. 11).