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Nanosilicon-based recovery of barley (*Hordeum vulgare*) plants subjected to drought stress†

Mansour Ghorbanpour,*[a] Hamid Mohammadi[b] and Khalil Kariman[c]

The present study explores the potential impact of silicon nanoparticles (Si NPs), in comparison with their bulk counterpart (silicate), on post-stress recovery performance of barley (*Hordeum vulgare*) seedlings under different drought stress intensities during vegetative growth. Barley plants were grown under 100% field capacity (FC), or mild (75% FC), moderate (50% FC) and severe (25% FC) drought stress levels, and were subsequently recovered by different treatments including soil application of 150 mL of Si NPs and silicate (at 125 and 250 mg Si L^{-1}), and water. Si NPs application at 250 mg L^{-1} led to formation of Si NP aggregates in plant tissues, large pores in roots, and also rapid stomata closure in leaves. However, the lower Si NPs dose (125 mg Si L^{-1}) was accompanied by a wider distribution of Si NPs in cells, and formation of a regular porosity pattern in roots *i.e.* more frequent pores of a smaller size. Upon recovery from all the drought stress levels, shoot biomass increased significantly in recovered plants compared to the respective non-recovered controls, and the maximum shoot biomass increase (27.3%) belonged to the moderate-stressed plants treated with Si NPs at 125 mg L^{-1}. Exposure to Si NPs and silicate (at both doses) after all drought stress intensities caused a significant increase in total chlorophyll (up to 17.1%) and carotenoid (up to 24.1%) content of leaves except for the carotenoid content under severe drought stress. Post-drought recovery with Si NPs and silicate was linked to alterations in the plant osmolyte and metabolite profile, cellular injury and membrane stability indices, and the activity of antioxidant enzymes. Soil application of Si NPs (at a low dose of 125 mg Si L^{-1}) showed a promising potential for post-drought recovery of barley plants *via* modifying plant morpho-physiological and antioxidative attributes and synthesis of specific metabolites.

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Environmental significance

Drought is a major environmental stress that causes substantial reductions in crop yield worldwide. "Boosting crop productivity in marginal lands" is an integral component of the second green revolution, and NP-based strategies have already shown the potential to aid plants cope with environmental constraints. Silicon is a beneficial element that can protect plants exposed to drought stress through providing structural stability for cell wall and membrane. However, no research has been performed to investigate the potential of Si nanoparticles (Si NPs) during the post-drought recovery of barley plants, and the affected physiological/antioxidative pathways. Therefore, the present study was conducted to explore the potential effects of Si NPs, and its bulk counterpart (silicate), on anatomy, physiology and antioxidative pathways of barley plants during the recovery from different drought intensities. We employed scan electron microscopy (SEM) to unearth the anatomical changes in roots and leaves, and also performed diverse physiological, metabolic (phenolic acids, carotenoids, flavonoids, amino acids, *etc.*), and antioxidative analyses including the activity of SOD, POD, CAT, and APX enzymes. A UPGMA cluster analysis and biplot analysis were also carried out by which the employed treatments were clustered into three distinct groups based on the investigated traits. Si NPs (at a lower dose) showed a promising potential for recovery of barley plants under drought stress *via* inducing anatomical, physiological and antioxidative alterations.

1. Introduction

Plants are major primary producers of the terrestrial ecological systems, and are often subjected to diverse unfavorable environmental perturbations in the form of abiotic and biotic stresses such as drought, flooding, heavy metals, pesticides, salinity, temperature extremes, pests and pathogens.[1,2] Abiotic stresses are considered as a principal

[a] *Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, 38156-8-8349, Arak, Iran. E-mail: m-ghorbanpour@araku.ac.ir*
[b] *Faculty of Agriculture, Azarbaijan Shahid Madani University, Tabriz, Iran*
[c] *School of Agriculture and Environment, The University of Western Australia, Perth, WA, 6009, Australia*

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constraint of crop failure worldwide, which can potentially dip the average yield of most field crops by more than 50%.[3] Drought stress, in particular, is a significant environmental stress that limits crop performance and yield both quantitatively and qualitatively.[4]

It is well-established that drought can impair a wide range of fundamental physiological and metabolic reactions, which are involved in regulation of plant growth and performance.[5] Drought stress can lead to enhanced production of reactive oxygen species (ROS) including the hydrogen peroxide (H_2O_2), hydroxyl radical (OH), and superoxide anion ($O_2^{\bullet-}$).[6] The generated ROS can result in the oxidation of photosynthetic pigment molecules, membrane lipids, proteins and nucleic acids and thereby disturb cell normal functioning.[7] To mitigate the destructive effects of ROS, plants have developed several adaptations and protection mechanisms that include stimulation of effective enzymatic [superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX)] and non-enzymatic (phenolic acids, carotenoids, flavonoids, ascorbic acid, proline, *etc.*) antioxidant defense pathways.[4,8]

Diverse strategies are employed to improve plant fitness and decrease the risk of environmental stress factors that include nano-based approaches. As a newly emerging frontier of the current century, nanotechnology can be harnessed to tackle the current global challenges in diverse fields including agriculture and environmental research.[9] A variety of nanoparticles (NPs) such as carbon-based NPs and metal-based NPs have been manufactured in recent years. Nanoparticles possess unique physical, chemical and mechanical characteristics due to their high surface and nanoscale size, facilitating their interactions with other particles and living organisms, leading to desirable or undesirable biological functions[10] "Boosting crop productivity in marginal lands" is an integral component of the second green revolution, and NP-based strategies have already shown the potential to aid plants withstand extreme environmental conditions.[11]

Silicon (Si) makes up approximately 25% of the earth's crust, and it exists in the soil solution as monomeric or monosilicic acid [$Si(OH)_4$], which can be absorbed by the root system and transported to the above-ground organs.[12,13] Although Si is not considered as an essential nutrient for plants growth and development, it is classified as a structural element due to its important role in physiological/metabolic pathways, cell structure, and plant survival under different biotic and abiotic stresses.[14] Among the different types of NPs, Si NPs have demonstrated a significant ability to improve plant performance in stressful environments.[15] Many studies have demonstrated the positive effects of Si NPs (*via* foliar spray, soil application, and/or seed priming) on plants exposed to toxic metals,[16,17] UV-B exposure,[18] salinity,[19] drought[20] and oxidative stress.[15] Soil application of Si NPs was shown to increase hawthorn seedlings resistance to drought stress mainly due to enhanced photosynthesis efficiency and stomatal conductance.[20] Furthermore, seed priming with Si NPs not only alleviated the toxicity of heavy metal stress, but also successfully improved the growth and biomass of wheat plants grown in Cd-stressed environment due to activation of seed metabolic pathways and enhanced nutrient supply to plants.[15] However, our knowledge is gravely limited about the potential role of Si NPs on post-drought recovery in plants, and the cellular mechanisms involved.

Barley (*Hordeum vulgare* L.) is one of the eight so-called founder agricultural crops and one of the most cultivated crops worldwide.[21] Irrespective of its agronomical significance, barley is considered as a classical model plant for genetic and physiological studies due to its remarkable genetic diversity and natural tolerance to unfavorable environmental conditions, serving as a gene pool to confer tolerance to crops against abiotic stresses.[22,23] Therefore, in the current study, our objectives were to evaluate: i) the effects of different drought stress levels on plant biomass accumulation, physiology, and biosynthesis of secondary metabolites ii) the comparative impact of Si NPs and silicate (as Na_2SiO_3, hereafter referred to as "silicate") in recovery of drought-treated seedlings, and (iii) anatomical and physiological alterations by which Si NPs or silicate can potentially facilitate the recovery of barley seedlings during the re-watering stage after the drought stress. Assessing the effects of different drought stress intensities on barley plants and post-drought recovery with Si NPs in comparison with their bulk counterpart (silicate) would potentially provide useful information to improve our understanding of the patterns of drought stress response in grain crops as well as induction of drought stress resistance to plants by Si-based materials of nanoscale or bulk type.

2. Materials and methods

2.1. Preparation of silicon nanoparticles (Si NPs) characterization

In the present study, we used silicate in the form of sodium silicate (Na_2SiO_3; Sigma-Aldrich), and Si NPs as silicon dioxide (SiO_2) nanopowder (purity: 99+%, size: 20–30 nm, amorphous, US Research Nanomaterials, Inc, Houston USA). The Si NPs powder was suspended in deionized water and sonicated using an ultrasonicator (Powersonic, UB-405, 10 MHz for 30 min), causing a fairly homogeneous solution. The characterization of Si NPs was carried out by various analytical techniques such as scanning electron microscopy (SEM) (Hitachi S-4160, Japan), transmission electron microscopy (TEM) (LEO 906E, Zeiss, Germany), X-ray diffraction (XRD) (Philips-X'Pert MPD X-ray diffractometer) analysis, and Fourier-transform infrared spectroscopy (FTIR) (Spectrum 400, Perkin Elmer, USA). Zeta potential and hydrodynamic diameter values were determined using dynamic light scattering (DLS) techniques by Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK) apparatus.

2.2. Plant materials, growth conditions and treatments

Seeds of cultivated barley (*Hordeum vulgare* L.) cv. Kavir, a spring six-row cultivar of the USA Ministry of Agriculture origin, were supplied by the Seed and Plant Improvement Institute of Iran. Seeds were visually inspected for any morphological damage, and surface sterilized by incubation in sodium hypochlorite solution (0.5%) for 10 min, followed by three times washing, and overnight soaking in deionized (DI) water. Five seeds were sown in each pot containing a mixture of 6 kg loamy-clay soil (pH: 7.2; EC: 2.16 dS m^{-1}; OM: 0.78%; available nitrogen (N): 46.8 mg kg^{-1}; available phosphorus (P): 14.72 mg kg^{-1}, available potassium (K): 148 mg kg^{-1}; silica: 14 mg kg^{-1}) and 2 kg clean sand (3 : 1 ratio; soil/sand). The field capacity (FC) of the soil–sand mixture was determined to be 18% (w/w). Fifteen days after sowing, the seedlings were thinned to three seedlings per pot (third-leaf stage, or growth stage 14, according to Zadoks staging key). Seedlings were grown in a controlled growth chamber with air temperature of 22/18 °C day/night cycle, 16 h photoperiod (220 μmol m^{-2} s^{-1} photon flux density), and 65% relative humidity.

Five-week-old seedlings were exposed to different drought stress levels by a gravimetric method.[24] Pots were weighed twice a day (11:00 a.m. and 5:00 p.m.) and the designated levels of drought stress were maintained. Well-watered (no drought stress: NDS) plants were maintained under optimal water availability conditions *i.e.*, at 100% FC. For drought conditions, plants were maintained at 75% FC (mild drought stress), 50% FC (moderate drought stress), and 25% FC (severe drought stress) by weighing the pots to the required FC level twice a day (11:00 a.m. and 5:00 p.m.). The soil moisture content was measured using a pressure plate apparatus. The induced drought stress continued for 30 days, followed by a seven-day recovery period.[25] A subset of the well-watered plants (NDS treatment) designated as "NR" (non-recovery) was harvested at the beginning of the recovery period for comparison. Within the NDS treatment group, the difference between "NR" and "RW" (rewatering) treatments is that plants in the "RW" treatment grew one week more than those in the "NR" treatment.

Post-stress recovery treatments were carried out using Si NPs and silicate (at two levels: 125 and 250 mg Si L^{-1}) compared to the non-recovery (NR) control, and a recovery control treatment, which received DI water without Si (referred to as "rewatering": RW). The soil moisture content of the recovered plants was maintained at 100% FC during the recovery period. The Si treatments were applied to the soil as aqueous solutions on the 1st and 3rd day of the recovery (150 mL of the corresponding solutions was used per pot). In order to achieve a homogenized and well mixed dispersion, and to minimize aggregation and agglomeration, the Si NPs solutions were ultra-sonicated for 30 min before the application. All pots were randomly distributed on the benches and periodically rotated to minimize the possible effects of environmental heterogeneity. The entire experiment was conducted with three replicates in a completely randomized design.

2.3. SEM observations and Si determination in plant tissues

The presence of Si NPs, and the surface morphology of root and leaf tissues were analyzed by field emission SEM. The leaf and root samples were prepared for SEM imaging based on the reported literature.[26] The sample preparation process is presented in the ESI† (S1.1.).

To determine the leaf Si content of the barley seedlings, the method of Lukacova *et al.* (2013) was used (with slight modifications).[27] Briefly, dried sample (50 mg) was homogenously digested in a mixed acid solution (10 mL of HNO_3 and 2 mL of $HClO_4$, Merck), and heated at 150 °C for 2 h until a colorless solution was formed. Then, 3 mL of H_2O_2 and 2 mL of HF were added to the solution, heated at 150 °C for 2 h, and finally 30 mL of 4% H_3BO_3 was added. The Si content of leaf samples was measured by atomic absorption spectrometer (Shimadzu, model AA-7000F Series, Japan). Details of the analysis are provided in the ESI† (S1.2.).

2.4. Plant biomass and water status during the drought stress and recovery periods

The plant materials were harvested and dried in an oven at 80 °C, weighed for dry matter determination, finely ground with an electric blender, and stored in designated containers for further analyses. To estimate the leaf relative water content (RWC), fresh weight (FW) of the top-most fully expanded leaves was measured, and then the sample was immediately hydrated with DI water to full turgidity for 24 h at room temperature (23 °C). The turgid weight (TW) of leaves was noted, and samples were then oven-dried at 80 °C for 24 h and weighed (DW). The following equation was used for calculation of RWC:[28]

$$\mathrm{RWC} = (\mathrm{FW} - \mathrm{DW})/(\mathrm{TW} - \mathrm{DW}) \times 100$$

2.5. Photosynthetic pigments

The chlorophyll and carotenoids were extracted from 0.5 g of fresh leaf samples in 5 mL of acetone (80% V/V). Total chlorophyll (TChl) and carotenoid contents (mg g^{-1} FW^{-1}) of leaf tissues were estimated using the following formula:[29,30]

$$\mathrm{TChl} = (18.54 \times A_{649} + 6.87 \times A_{665}) \times V/(1000M)$$

$$\mathrm{Carotenoids} = 4.69 \times A_{470} - 0.268 \times (20.2 \times A_{649} + 8.02 \times A_{665})$$

where A_{649}, A_{665} and A_{470} exhibit the absorbance at 649 (chlorophyll a), 665 (chlorophyll b), and 470 (carotenoids) nm, V = extract volume (mL) and M = sample weight (mg).

2.6. Leaf soluble sugar and protein contents

The soluble sugars content of leaf samples was determined by anthrone method using glucose solutions as standard samples.[31] The total protein content was measured by

Bradford assay-based method using a series of bovine serum albumin standard.

2.7. Determination of H_2O_2, MDA, ELI, and MSI

The H_2O_2 content of shoot samples was estimated following the procedure of Sergiev *et al.* (1997) compared with a previously constructed calibration curve of H_2O_2.[32]

Malondialdehyde (MDA), an end-product of polyunsaturated fatty acids (PUFA) peroxidation of cellular membrane, is commonly known as oxidative stress biomarker. The MDA content of shoots was determined as described by Kuk *et al.* (2003).[33]

To check the cell membrane injury, electrolyte leakage index (ELI) of leaf samples was measured following the method of Lutts *et al.* (1996).[34] Briefly, 50 mg of fresh leaf sample was washed and kept in approximately 15 mL of DI water at 4 °C for 24 h and electrical conductivity (EC1) was recorded. Then, the sample was fully damaged by boiling at 100 °C for 20 min. The final electrical conductivity (EC2) was recorded using conductivity meter, and ELI was calculated as:

$$\mathrm{ELI}(\%) = (\mathrm{EC1}/(\mathrm{EC2}) \times 100$$

The cell membrane stability index (MSI) was calculated using the following equation as previously described by Sairam (2003):[35]

$$\mathrm{MSI} = [1 - (\mathrm{EC1}/\mathrm{EC2})] \times 100$$

The detailed procedures for determination of H_2O_2, MDA, ELI, and MSI are shown in the ESI† (S1.3.).

2.8. Determination of antioxidant enzymes activities

To quantify the activity of antioxidant enzymes, fresh leaf samples (0.3 g) were ground using a chilled mortar and pestle with liquid nitrogen in 1 mL of ice-cold 0.1 M potassium phosphate buffer (pH 7.5), which contained Na_2–EDTA and ascorbic acid (0.5 mM). The homogenate was subsequently centrifuged at 12000 × *g* for 20 min, and the obtained supernatant was utilized for calculation of the specific activity of the antioxidant enzymes. Spectrophotometric analysis was carried out on a T80 series of UV-visible spectrophotometer.

SOD (EC 1.15.1.1) specific activity was determined in terms of its capacity for inhibition of the photochemical reduction of NBT recorded at 560 nm as previously described by Giannopolites and Ries (1977).[36]

CAT (EC 1.11.1.6) specific activity was quantified by measuring the disappearance rate of H_2O_2 (ε = 39.4 mM^{-1} cm^{-1}) read at 240 nm as previously reported by Aebi (1984).[37]

POD (E.C.1.11.1.7) specific activity was determined based on the rate of tetraguaiacol formation (ε = 26.6 mM^{-1} cm^{-1}) from guaiacol recorded at 470 nm as described by Polle *et al.* (1994).[38]

APX (EC 1.11.1.11) specific activity was estimated based on the decrease in the amount of ascorbate oxidized (ε = 2.8 mM^{-1} cm^{-1}) noted at 290 nm following the method of Jimenez *et al.* (1997).[39] The detailed information for extraction and measurement of the activity of antioxidant enzymes is described in the ESI† (S1.4.).

2.9. Total phenolics and flavonoids content

The methanolic extract of dried shoot samples was used to measure the total phenolics content using the Folin Ciocalteu reagent as previously described by Singleton and Rossi (1965).[40] Total phenolics content was quantified by comparison with the calibration curve of standard gallic acid (50–1000 mg L^{-1}), and the results were expressed as mg gallic acid equivalents per g dry weight. The flavonoids content of leaves was determined using the aluminium chloride colorimetric procedure.[41] Total flavonoids content of the leaves was estimated using a rutin calibration curve, and was expressed as mg rutin equivalents per gram of dry weight. The detailed methods for the extract preparation and measurement of total phenolics and flavonoids content are described in the ESI† (S1.5.).

2.10. Amino acids and phenolic compounds

The pretreatment of leaf samples and the procedure for extraction of amino acids was based on the method described by Lisiewska *et al.* (2008).[42] The amino acid content of barley leaves was characterized using a Biochrom 20 amino acid analyzer (Pharmacia, Biochrom Ltd, UK), according to the protocol of Anjum *et al.* (2005).[43]

The phenolic acid compounds of the samples was quantified by high performance liquid chromatography (HPLC, Shimadzu LC-20A, Japan) technique equipped with a diode array detector (SPD-M20A) and Phenomenex C18 110A column (5 μm, 150 × 4.6 mm) as previously described by Chen *et al.* (2016).[44] Further details about the measurement of the amino acids and phenolic compounds are presented in the ESI† (S1.6.).

2.11. Statistical data analyses

This study was carried out in a factorial experiment based on completely randomized design (CRD) with three replications (n = 3). Data were subjected to analysis of variance (ANOVA) using the general linear models (PROC GLM) procedure of the SAS software (Version 6; SAS Institute, Cary, NC). The analysis of mean (ANOM) was performed using the Tukey's Honestly Significant Difference (HSD) test at two probability levels ($p < 0.05$ and $p < 0.01$). The un-weighted pair group method with arithmetic means (UPGMA) was employed to construct a dendrogram through the sequential agglomerative hierarchical non-overlapping (SAHN) clustering program. The principal component analysis (PCA), an ordination method, was used to show patterns and relationships between the employed treatments using the

PAST software. The hierarchical cluster analysis (HCA) was carried out using the Multi Experiment Viewer.

3. Results and discussion

3.1. Characteristics of Si NPs

Characterization results of the Si NPs used in this study are presented in Fig. 1. The Si NPs possessed specific surface area of 180–600 $m^2\ g^{-1}$, particle size of 20–30 nm, purity of 99+%, bulk and true density of $<0.10\ g\ cm^{-3}$ and 2.4 $g\ cm^{-3}$, respectively. The metal content of SiO_2 NPs was detected using the energy dispersive X-ray spectroscopy (EDX) analysis as followings: Na $<$ 50 mg kg^{-1}, Ca $<$ 70 mg kg^{-1}, Fe $<$ 20 mg kg^{-1} and Ti $<$ 120 mg kg^{-1}. The SEM (Fig. 1A) and TEM (Fig. 1B) results showed that the Si NPs were aggregates with spherical morphology in the range of 20–30 nm. FTIR results (Fig. 1C) showed that the presence of characteristic peaks in the absorption spectra at 1109 and 474 cm^{-1} corresponded to Si–O–Si and Si–O functional groups, respectively.[45] The XRD spectrum of the examined Si NPs (Fig. 1D) showed a broad peak with reflection at 2θ = 23°, confirming that Si NPs is composed of SiO_2 and it is amorphous in nature.[18] Based on these observations, the prepared Si NPs were demonstrated to be of high stability and purity, and were therefore, applied in the current study.

3.2. Penetration and accumulation of Si in plant tissues

We employed SEM to localize Si NPs and silicate in barley roots and aerial tissues and to visualize any morphological and anatomical alterations in drought-treated plants undergoing the recovery treatments compared to the respective controls. As shown in Fig. 2, the outer layer of root and leaf tissues showed the most significant changes following the employed treatments. The well-watered plants had larger lateral root systems and well-developed branching patterns with more root hairs (Fig. 2A) compared to the stressed-plants (Fig. 2B). Interestingly, SEM images of the seedlings exposed to Si NPs at 125 mg L^{-1} recovery treatment showed a wider deposition of Si on both root (Fig. 2D) and leaf (Fig. 2J) tissues, which was also confirmed through the atomic absorption technique presented in Table 1. The formation of cell-Si NP aggregates in the high Si NPs treatment (250 mg L^{-1}) which apparently led to formation of large pores in roots was also verified by SEM images (Fig. 2F) and also led to rapid stomata closure in leaves (Fig. 2L).

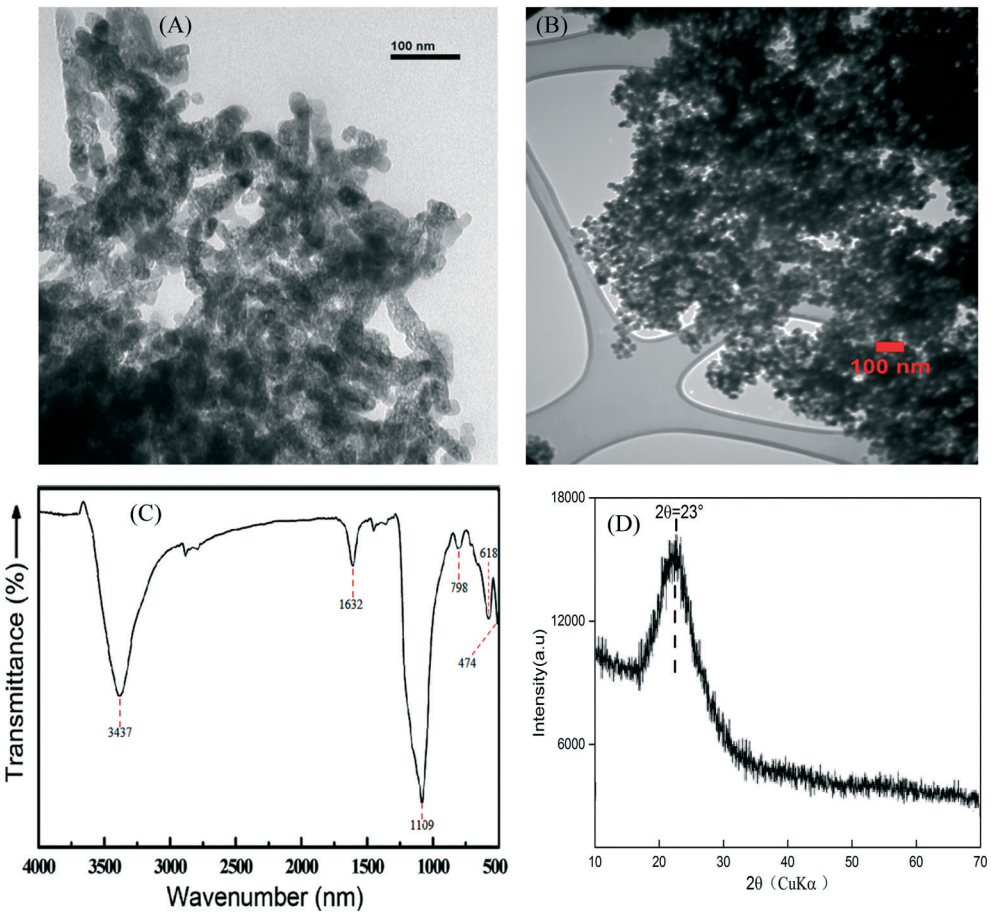

Fig. 1 Characterization results of the silica nanoparticles (Si NPs) used in the present study. (A) Scanning electron microscopy (SEM), (B) transmission electron microscopy (TEM), (C) Fourier-transform infrared spectroscopy (FTIR), and (D) X-ray diffraction (XRD).

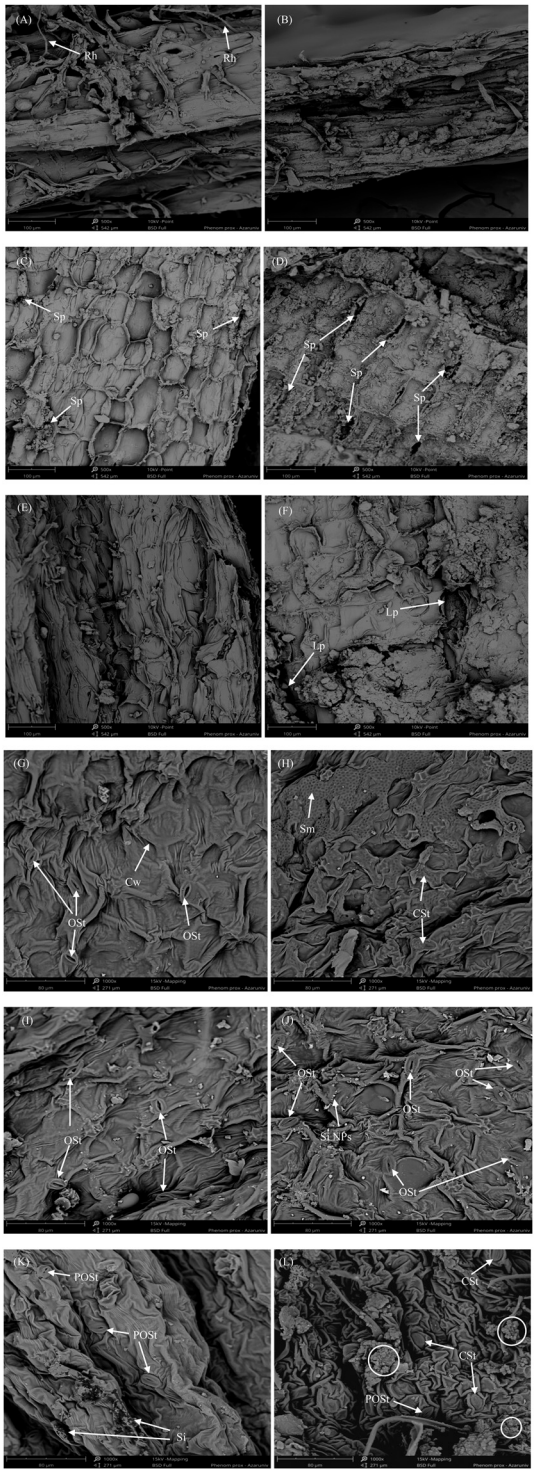

Fig. 2 SEM micrographs of non-stressed and non-recovered plant roots (Fig. 2A) and leaf (Fig. 2G), the topography of root (Fig. 2B) and leaf (Fig. 2H) subjected to severe drought stress and non-recovery, changes in root (Fig. 2C and D) and leaf (Fig. 2I and J) morphology upon recovery with 125 mg L^{-1} Si NPs under mild and moderate drought stress, respectively. Images of the barley root (Fig. 2E and F) and leaf (Fig. 2K and L) tissues under severe drought stress and post-stress recovery treatment with silicate and Si NPs (at 250 mg L^{-1}), respectively. Deposition of Si (K) and small aggregation of Si NPs (L) on leaf surface (circled). Abbreviations: Rh: root hair, Sp: small pores, Lp: large pores, Cw: cell wall, Sm: spongy mesophyll, OSt: open stomata, CSt: closed stomata, POSt: partially open stomata.

According to Hatami *et al.* (2019) presence of a regular porosity pattern (more frequent pores of small size) in *Salvia mirzayanii* seedlings treated by graphene oxide (GO)/polyaniline (PANI) nanocomposite, compared to the large pores around the root tip of GO-treated seedlings may accelerate the water absorption by roots,[46] which could possibly be due to reduced root respiration through decreasing living cortical area, and may also cause an increase in plant biomass.[46,47] However, the mechanisms behind pore formation in the root tissues upon exposure to NPs still remain unknown and remain to be explored. We propose two possibilities that could explain the formation of pores in roots of NP-treated plants: i) the size of pores increase with the NP application dose, and the chance of NP aggregate formation is higher at higher NP concentration, so pore formation could be the outcome of a physical encounter between roots and NP aggregates *i.e.* smaller aggregates (in low NP dose) cause smaller pores and larger aggregates (in high NP dose) cause larger pores, and ii) pore formation in roots is a plant response to NPs as an external stressors, which is obviously correlated with the NPs concentration.

Data associated with Si accumulation rates in barley leaves under various intensities of drought stress and the subsequent recovery treatments are presented in Table 1. Results indicated that the Si content of tissues varied significantly ($p < 0.01$, Turkey's test) under the employed treatments. Drought stress progressively decreased Si accumulation in the leaf, and such a decrease was more pronounced (by 68.1%) in plants under severe stress (1442 mg kg^{-1} DW) compared to the well-watered control plants (4527 mg kg^{-1} DW). In contrast, the Si content of plants was significantly enhanced by recovery treatments (up to 3727 and 3173 mg kg^{-1} DW, respectively) by addition of Si NPs (125 mg L^{-1}) and or silicate (250 mg L^{-1}) compared to the respective controls, suggesting that availability of Si NPs to barley seedlings is greater than silicate (Table 2). Previous studies showed that Si addition markedly enhanced the Si content of leaves in *Glycyrrhiza uralensis*, while it diminished the Si accumulation in both root and stem tissues under the drought stress conditions.[48] In barley plants subjected to drought and salinity stresses, high level of Si in leaves treated with Si is probably due to deposition of Si on epidermal cell wall, which may potentially contribute to reduced transportation of ions in cells and tissues, or tolerance against drought stress, respectively.[49]

3.3. Morpho-physiological traits

The impacts of drought stress and the subsequent recovery treatments on shoot biomass, RWC, total chlorophyll and carotenoid contents of barley plants are shown in Table 1. There were 8.9%, 22.7% and 44.6% decreases in dry shoot biomass in mild, moderate and severe drought-stressed plants, respectively, as compared to the control (non-stressed plants). Upon recovery from all the employed drought stress treatments, shoot biomass increased significantly ($p < 0.05$;

Table 1 Leaf silicone (Si) content, shoot biomass (SB), leaf relative water content (RWC), total chlorophyll (TCHL) and total carotenoid (TCAR) contents in barley seedlings subjected to various drought stress intensities and post-stress recovery treatments with silicone nanoparticles (Si NPs) and silicate (125 and 250 mg L^{-1}) compared to the non-recovery (NR) and recovery with distilled water (RW, rewatering) as control groups. For drought conditions, plants were maintained under 75% FC (mild drought stress, MIDS), 50% FC (moderate drought stress, MODS), and 25% FC (severe drought stress, SDS). Control plants (NDS, no drought stress) were maintained in optimal water availability conditions (*i.e.*, 100% FC). Means labeled by different letters are significantly different at $p < 0.05$ using the Tukey's test

Treatments		Morpho-physiological traits				
Drought stress	Stress recovery	Leaf Si (mg kg^{-1} DW)	Shoot dry biomass (mg)	RWC (%)	Total chlorophyll (mg g^{-1} FW)	Total carotenoid (mg g^{-1} FW)
NDS	NR	4527^{e}	238.6^{e}	84.3^{a}	1.741^{c}	3.264^{c}
	RW	4731^{d}	254.3^{d}	85.6^{a}	1.752^{c}	3.258^{c}
	SiNPs 125	6402^{a}	293.7^{a}	86.1^{a}	2.011^{a}	3.524^{b}
	SiNPs 250	6429^{a}	286.2^{a}	84.7^{a}	1.973^{a}	3.725^{b}
	Si 125	6193^{c}	269.1^{c}	84.6^{a}	1.826^{b}	4.012^{a}
	Si 250	6238^{b}	277.4^{b}	86.5^{a}	1.942^{a}	4.183^{a}
MIDS	NR	3376^{d}	217.3^{d}	76.4^{c}	1.738^{d}	2.875^{b}
	RW	3391^{d}	231.4^{c}	78.6^{b}	1.763^{c}	2.951^{b}
	SiNPs 125	5641^{a}	274.5^{a}	84.4^{a}	1.845^{a}	3.251^{a}
	SiNPs 250	5593^{a}	258.3^{b}	82.2^{a}	1.792^{bc}	3.067^{a}
	Si 125	5128^{c}	242.6^{bc}	79.6^{b}	1.764^{c}	3.092^{a}
	Si 250	5322^{b}	253.2^{b}	81.4^{ab}	1.802^{ab}	3.175^{a}
MODS	NR	2895^{f}	184.5^{f}	71.3^{d}	1.542^{d}	2.215^{d}
	RW	2964^{e}	202.6^{e}	75.1^{c}	1.565^{cd}	2.342^{c}
	SiNPs 125	5127^{a}	253.9^{a}	81.4^{a}	1.694^{a}	2.921^{a}
	SiNPs 250	4835^{c}	237.7^{b}	78.8^{ab}	1.608^{b}	2.614^{ab}
	Si 125	4322^{d}	211.6^{d}	74.3^{c}	1.579^{c}	2.425^{b}
	Si 250	4921^{b}	225.3^{c}	76.1^{bc}	1.593^{bc}	2.711^{a}
SDS	NR	1442^{e}	132.1^{d}	60.3^{de}	1.021^{d}	1.202^{a}
	RW	1503^{d}	137.3^{d}	62.7^{d}	1.052^{c}	1.208^{a}
	SiNPs 125	3827^{a}	164.7^{a}	76.2^{a}	1.234^{a}	1.279^{a}
	SiNPs 250	3173^{b}	155.3^{b}	70.5^{bc}	1.085^{b}	1.244^{a}
	Si 125	3035^{c}	140.2^{c}	67.4^{c}	1.063^{c}	1.226^{a}
	Si 250	3874^{a}	153.4^{b}	71.8^{ab}	1.108^{b}	1.251^{a}

Table 1) in recovered plants compared to the respective non-recovered controls. However, the maximum increase in shoot biomass (27.3%) was obtained in moderate-stressed plants treated with Si NPs (at 125 mg L^{-1}), while the recovery with water caused a relatively smaller increase (8.9%) in biomass as compared to the non-recovered control plants. The positive effects of Si NPs application on biomass increment of barley seedlings may also be linked to an increased water content and Si accumulation in leaf tissues.[18,50] Moreover, Si NPs appeared to be more efficient in recovery process than silicate, which is to a certain extent related to its higher accessibility to barley seedlings than silicate as presented by the Si content dataset (Table 1).

Leaf relative water content showed a significant ($p < 0.01$) decrease by 9.4%, 15.4% and 28.5% upon mild, moderate and severe drought stress treatments, respectively (Table 1). When the stressed plants recovered from drought with water, the leaf relative water content under the mild and moderate stress treatments increased (by 2.8% and 5.0%), respectively, whereas a significant difference was not found ($p > 0.05$) in leaf relative water content of plants subjected to the severe stress compared to the respective control. Following recovery with Si NPs (at 125 mg L^{-1}) in plants subjected to mild, moderate and severe water stress levels, however, the leaf relative water content increased by 9.5%, 12.4% and 20.9%, respectively (Table 1). It possibly means that recovery by Si NPs and silicate is advantageous to the RWC under all the employed drought levels. Furthermore, compared to the recovery with both types and doses of Si, RWC, shoot biomass, and total carotenoid content of plants were not recovered from the severe drought stress by rewatering alone. Root porosity was shown to reduce the xylem sap flow rate and/or root hydraulic conductivity in plants under drought stress.[51] Moreover, decreased root hydraulic conductivity and NP-mediated suppression of root nutrient uptake in drought-stressed plants resulted in reduced shoot macronutrient concentrations and leaf chlorophyll content.[15] However, application of Si was shown to enhance the root hydraulic conductance through regulating aquaporin activities and improving the root osmoregulatory capacities, which can contribute to an increase in water uptake and transport.[47] Although drought stress can lead to significant reductions in leaf RWC and water potential,[4,52] this dehydration is often reversible.[53]

As shown in Table 1, the total chlorophyll and carotenoid contents of leaves decreased significantly ($p < 0.05$) under moderate (by 11.43% and 41.35%) and severe drought (by 32.13% and 63.17%) conditions, respectively. The recovery (with both Si sources and doses) after all drought stress intensities caused a significant increase in total chlorophyll and carotenoid contents of leaves except for the carotenoid content under the severe drought stress (Table 1), which did

Table 2 Soluble protein (SP), soluble sugar (SS), total phenolics (TPHC) and total flavonoids (TFD) contents of barley seedlings subjected to various drought stress intensities and post-stress recovery treatments with silicone nanoparticles (Si NPs) and silicate (125 and 250 mg L^{-1}) compared to the non-recovery (NR) and recovery with distilled water (RW, rewatering) as control groups. For drought conditions, plants were maintained under 75% FC (mild drought stress, MIDS), 50% FC (moderate drought stress, MODS), and 25% FC (severe drought stress, SDS). Control plants (NDS, no drought stress) were maintained in optimal water availability conditions (*i.e.*, 100% FC). Means labeled by different letters are significantly different at $p < 0.05$ using the Tukey's test

Treatments		Osmolytes and metabolites			
Drought stress	Stress recovery	Soluble protein (mg g^{-1} FW)	Soluble sugar (mg g^{-1} FW)	Total phenolics (mg GAE g^{-1} DW)	Total flavonoids (mg RE g^{-1} DW)
NDS	NR	14.3^{e}	21.3^{e}	8.1^{a}	3.4^{a}
	RW	15.5^{de}	24.5^{d}	8.52^{a}	3.1^{a}
	SiNPs 125	17.8^{a}	29.4^{bc}	7.4^{a}	2.8^{a}
	SiNPs 250	16.4^{bc}	27.1^{c}	7.8^{a}	3.0^{a}
	Si 125	15.9^{cd}	31.7^{ab}	8.2^{a}	3.1^{a}
	Si 250	17.1^{ab}	36.5^{a}	7.6^{a}	3.5^{a}
MIDS	NR	10.4^{d}	26.2^{d}	13.4^{a}	5.7^{d}
	RW	13.2^{c}	31.5^{c}	14.1^{a}	6.9^{c}
	SiNPs 125	15.7^{a}	38.7^{a}	14.8^{a}	8.5^{a}
	SiNPs 250	14.3^{b}	35.3^{b}	14.3^{a}	7.8^{ab}
	Si 125	14.5^{b}	32.3^{c}	14.2^{a}	7.3^{bc}
	Si 250	15.1^{a}	34.6^{b}	15.5^{a}	8.3^{a}
MODS	NR	16.8^{a}	35.2^{e}	21.7^{c}	12.3^{e}
	RW	11.7^{e}	39.1^{d}	22.6^{c}	17.5^{d}
	SiNPs 125	13.4^{c}	46.5^{a}	25.6^{a}	22.4^{a}
	SiNPs 250	12.5^{d}	42.4^{bc}	23.7^{b}	18.1^{c}
	Si 125	12.3^{d}	40.2^{cd}	24.1^{b}	19.0^{c}
	Si 250	14.6^{b}	44.7^{ab}	25.3^{a}	20.5^{b}
SDS	NR	19.1^{a}	41.3^{d}	25.6^{d}	15.7^{a}
	RW	7.6^{f}	44.5^{c}	28.4^{c}	10.1^{e}
	SiNPs 125	11.5^{b}	49.6^{a}	34.3^{a}	11.7^{d}
	SiNPs 250	9.2^{d}	46.2^{bc}	31.5^{b}	13.6^{b}
	Si 125	8.8^{e}	46.4^{bc}	30.2^{b}	12.4^{c}
	Si 250	10.4^{c}	47.3^{ab}	33.1^{a}	11.9^{d}

not differ significantly compared to the respective untreated control. Chlorophyll concentration is one of the key factors in determining intensity of photosynthesis and biomass accumulation.[54] The increase in chlorophyll content of drought-treated barley plants after rewatering is in accordance with earlier observations made on maize.[55] A similar trend has been reported for drought-treated wheat plants exposed to selenium (Se), where the chlorophyll content of plants under the combined treatment of rewatering and Se was higher than those of plants under rewatering treatment alone.[56] However, high concentration of Si NPs was shown to cause a decrease in membrane resistance and disturb the structural integrity of biomembrane that varied with surface functional groups of the silica-core NPs used[57] Differential effects of Si NPs and silicate on growth and physiology of barely seedlings, further emphasizes the fact that interactions between NPs and plant cells is mainly driven by the physicochemical properties of the NPs and the application rates.[9,50]

3.4. Cellular injury and membrane stability indices

The H_2O_2 content of leaves increased by 26.1%, 48.7%, and 60.7% in barley seedlings upon exposure to mild, moderate and severe drought stress, respectively (Fig. 3A). On re-watering, the leaf H_2O_2 content of plants under the mild and moderate drought stress decreased by 18.8% and 38.1%, respectively, while it was not significantly affected at the severe drought stress level compared to the respective control. However, when the severe drought-stressed plants received Si NPs (125 mg L^{-1}) and silicate (250 mg L^{-1}), significant reductions (by 34% and 28%, respectively) in H_2O_2 content were observed in recovered plants compared to the respective controls (Fig. 3A). The MDA content and ELI presented identical response patterns to drought stress and recovery conditions (Fig. 3B and C). Drought stress progressively increased the MDA content and ELI, and such increase was more pronounced (by 62.9% and 70.8%, respectively) in the severe drought stress treatment compared to control group. After re-watering, the MDA content and ELI decreased significantly ($p < 0.05$) under mild to moderate drought stress conditions, but no significant difference was observed in these parameters under severe drought stress as compared to the respective controls (Fig. 3B and C). However, across different Si application treatments, significant reductions in MDA and ELI were recorded in plants under severe drought stress compared to the respective controls. There were 11.8%, 26.5% and 47.2% decreases in MSI, respectively, in mild, moderate and severe drought-stressed plants as compared to the controls (Fig. 3D). MSI was significantly ($p < 0.01$) affected by Si treatments under both normal and water shortage conditions (Fig. 3D). Upon

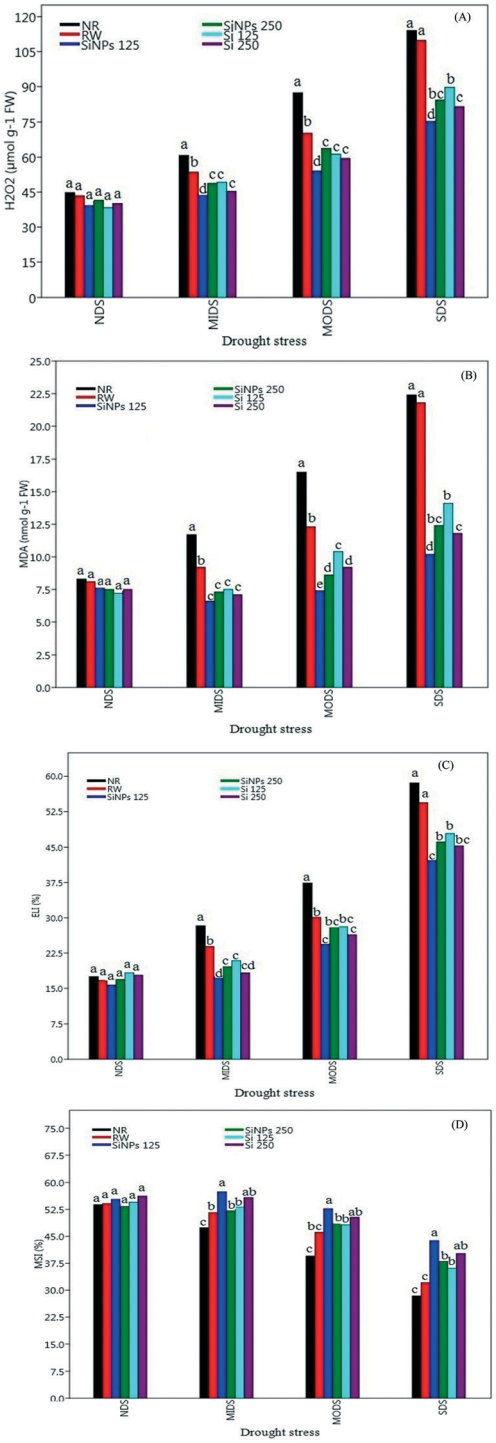

Fig. 3 (A) Hydrogen peroxide (H_2O_2), (B) malondialdehyde (MDA), (C) electrolyte leakage (ELI) and (D) membrane stability index (MSI) in barley seedlings subjected to various drought stress intensities and post-stress recovery treatments with silicone nanoparticles (Si NPs) and silicate (125 and 250 mg L^{-1}) compared to the non-recovery (NR) and recovery with distilled water (RW, rewatering) as control groups. For drought conditions, plants were maintained under 75% FC (mild drought stress, MIDS), 50% FC (moderate drought stress, MODS), and 25% FC (severe drought stress, SDS). Control plants (NDS, no drought stress) were maintained in optimal water availability conditions (*i.e.*, 100% FC). The values are mean ± standard deviations (SD). Means labeled by different letters are significantly different at $p < 0.05$ using the Tukey's test.

recovery from all the drought stress levels, MSI increased significantly, which was more evident in Si treatments compared to the respective controls.

Decline in growth and physiological processes of barley seedlings subjected to drought might be linked to high levels of H_2O_2 that can damage macromolecules such as proteins and lipids and disrupt their function, and also led to fluctuations in the metabolites level of plants. The enhanced level of H_2O_2 following severe drought stress was accompanied by enhanced levels of MDA and ELI, which indicates a severe oxidative stress in barley seedlings under water deficit. Corroborating our results, several studies have also reported a significant accumulation of H_2O_2 in leaves of various plants under drought stress including *Zea mays*,[58] *Tagetes erecta*,[59] *Triticum aestivum*,[60] and *Hyoscyamus niger*.[61] The decrease in H_2O_2 content of plants under the Si NPs treatment at low concentration was more than that under non-recovery and recovery with water and silicate, indicating that Si NPs (at 125 mg L^{-1}) was superior in alleviating the oxidative stress of barley seedlings at recovery after all drought levels. Similar to the Si NPs effect at 125 mg L^{-1}, silicate (at 250 mg L^{-1}) efficiently regulated the levels of H_2O_2, which was also accompanied by lower levels of MDA and ELI, and higher value of MSI. Moreover, our findings suggest that Si NPs and silicate (at an appropriate application dose) might play a key role in promoting the protective strategies of plants against oxidative stress including a decline in membrane lipid peroxidation and electrolyte leakage by preserving the plasma membrane integrity, and stimulation of both enzymatic and non-enzymatic antioxidant defense systems.[62,63] The high Si content of leaves in plants fortified with Si under drought possibly resulted from translocation and deposition of Si on cell walls, which could contribute to strengthened cell membranes and changing their permeability to drought stress.[64]

3.5. Antioxidant enzymes

The specific activity of SOD increased with increasing drought stress intensity up to the moderate level and thereafter declined. However, the specific activity of SOD under the severe drought treatment was not significantly lower than that of the control (Fig. 4A). Upon recovery from drought stress, Si NPs and silicate treatments (at both concentrations) induced a significant ($p < 0.01$) increase in the activity of SOD with respect to the non-recovery treatment. The CAT activity showed an opposite pattern, decreasing during the drought stress and increasing in the recovery treatments (Fig. 4B). Compared with the non-recovery treatment, the CAT activity recovered by 26.9%, 38.2%, and 51.5% in plants grown in soil amended with 125 mg L^{-1} Si NPs under mild, moderate and severe drought stress, respectively. As shown in Fig. 4C, mild drought stress and subsequent recovery treatments had no significant effect on POD activity. Upon recovery from severe drought, the

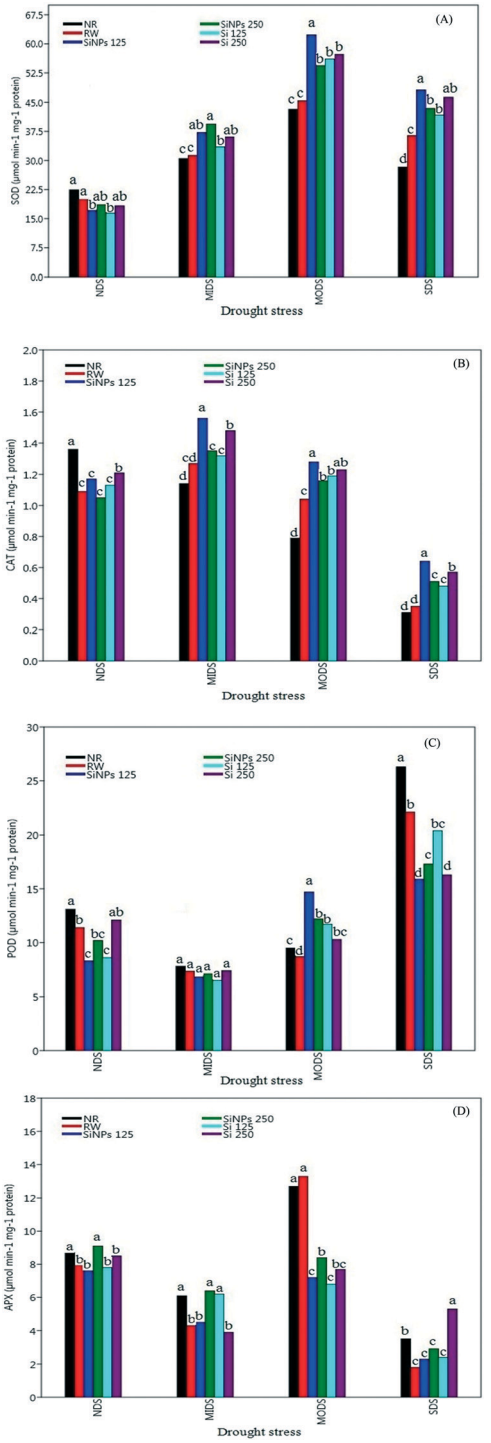

Fig. 4 (A) Superoxide dismutase (SOD), (B) catalase (CAT), (C) peroxidase (POD), and (D) ascorbate peroxidase (APX) activities in barley seedlings subjected to various drought stress intensities and post-stress recovery treatments with silicone nanoparticles (Si NPs) and silicate (125 and 250 mg L^{-1}) compared to the non-recovery (NR) and recovery with distilled water (RW, rewatering) as control groups. For drought conditions, plants were maintained under 75% FC (mild drought stress, MIDS), 50% FC (moderate drought stress, MODS), and 25% FC (severe drought stress, SDS). Control plants (NDS, no drought stress) were maintained in optimal water availability conditions (*i.e.*, 100% FC). The values are mean ± standard deviations (SD). Means labeled by different letters are significantly different at $p < 0.05$ using the Tukey's test.

POD activity decreased further in plants treated with different types and doses of Si. The APX activity in plants exposed to the severe drought stress significantly ($p < 0.05$) decreased compared to the control and other stress intensities (Fig. 4D). After recovery, the APX activity only recovered (by 34%) in plants grown under severe drought stress and silicate treatment of 250 mg L^{-1} compared to the respective control.

To cope with the oxidative damage under stressful conditions, plants need to balance its ROS levels inside the cell that can be achieved *via* a complex enzymatic (*e.g.*, SOD, CAT, POD, GR, and APX) and non-enzymatic antioxidants (*e.g.*, carotenoids, non-protein amino acids, phenolic compounds, *etc.*).[65] Here, the low levels of oxidative injury indices following rehydrating the seedlings with Si NPs and silicate indicate that Si plays essential role in scavenging the excess ROS and activation of antioxidant defense systems in plants under water deficit stress.

There was an opposite expression pattern for CAT activity compared to the other antioxidative enzymes examined, *i.e.* decreasing during the drought stress and increasing during the recovery treatments. High levels of ROS could be behind the declined CAT activity under the stressful environmental conditions.[66] Here, the elevated CAT activity in the seedlings recovered with Si NPs reveals an increased ROS-scavenging ability of treated plants, which was coincided with protection of barley seedlings from the oxidative injuries. Our results are in line with those of Zhang *et al.* (2018), which showed that Si addition increased CAT and APX activities in *Glycyrrhiza uralensis* under drought and salinity stresses.[64] Although the POD activity substantially increased under drought stress, the generation of the H_2O_2 was apparently beyond the plant cell's ability to remove it, resulting in ROS accumulation and oxidative stress in plants. Furthermore, POD mediates the transformation of H_2O_2 into $OH^{\bullet}$ (a most reactive and toxic form of ROS).[67] Therefore, in our present study, the Si-mediated reduction in POD specific activities might have also contributed to reduced production of $OH^{\bullet}$, leading to less oxidative damage to the cellular component of barley seedlings. APX is involved in the ascorbate–glutathione (AsA–GSH) cycle, a major pathway of ROS scavenging, protects plant cells against oxidative stress and lipid peroxidation.[68] Here, we found a substantial reduction in APX activity in barley seedlings subjected to severe drought stress, which was only recovered in the high silicate treatment (250 mg L^{-1}), suggesting that Si upregulated the activity of AsA–GSH cycle.[69] Moreover, the leaf H_2O_2 content was adversely related to CAT and APX activities, and positively related to the activities of SOD and POD under the reference treatments, which probably reflects a superior H_2O_2 scavenging capacity of CAT and APX in the present experimental context. However, activation of SOD, if accompanied by the other ROS-scavenging enzymes, provides a suitable protection mechanism to mitigate the oxidative burst in drought-treated plants.[70]

3.6. Osmolytes and metabolites content

The impacts of drought stress and recovery on osmolytes (*e.g.*, soluble proteins and sugars) and metabolites (*e.g.*, total phenolics and flavonoids) content of barley leaves are shown in Table 2. The content of soluble proteins showed a significant ($p < 0.01$) increase (by 14.8% and 25.1%) in seedlings subjected to moderate and severe drought stress treatments compared to the well-watered controls, respectively. The soluble protein level decreased significantly ($p < 0.01$) under moderate and severe drought inductions in plants recovered with water (30.3% and 60.2%), and with 250 mg L^{-1} Si NPs (by 25.5% and 51.8%) as well as with 125 mg L^{-1} silicate (by 26.7% and 54.0%), respectively, compared to the respective control. Water deficit stress significantly ($p < 0.01$) enhanced the soluble sugar content of plants (Table 2). Compared with the non-recovery treatment, recovery with water and Si NPs (at 125 mg L^{-1}) induced an evident increase in the soluble sugar content under mild (by 16.8%, 9.9%), moderate (by 7.1%, and 32.2%) and severe drought (by 24.3% and 16.7%) induction, respectively.

The average content of total phenolics increased by 39.5%, 62.6%, and 68.3% in seedlings subjected to mild, moderate and severe drought stress compared to the non-stressed controls (Table 2). There were no significant changes in total phenolics content of seedlings under well-watered and mild drought stress

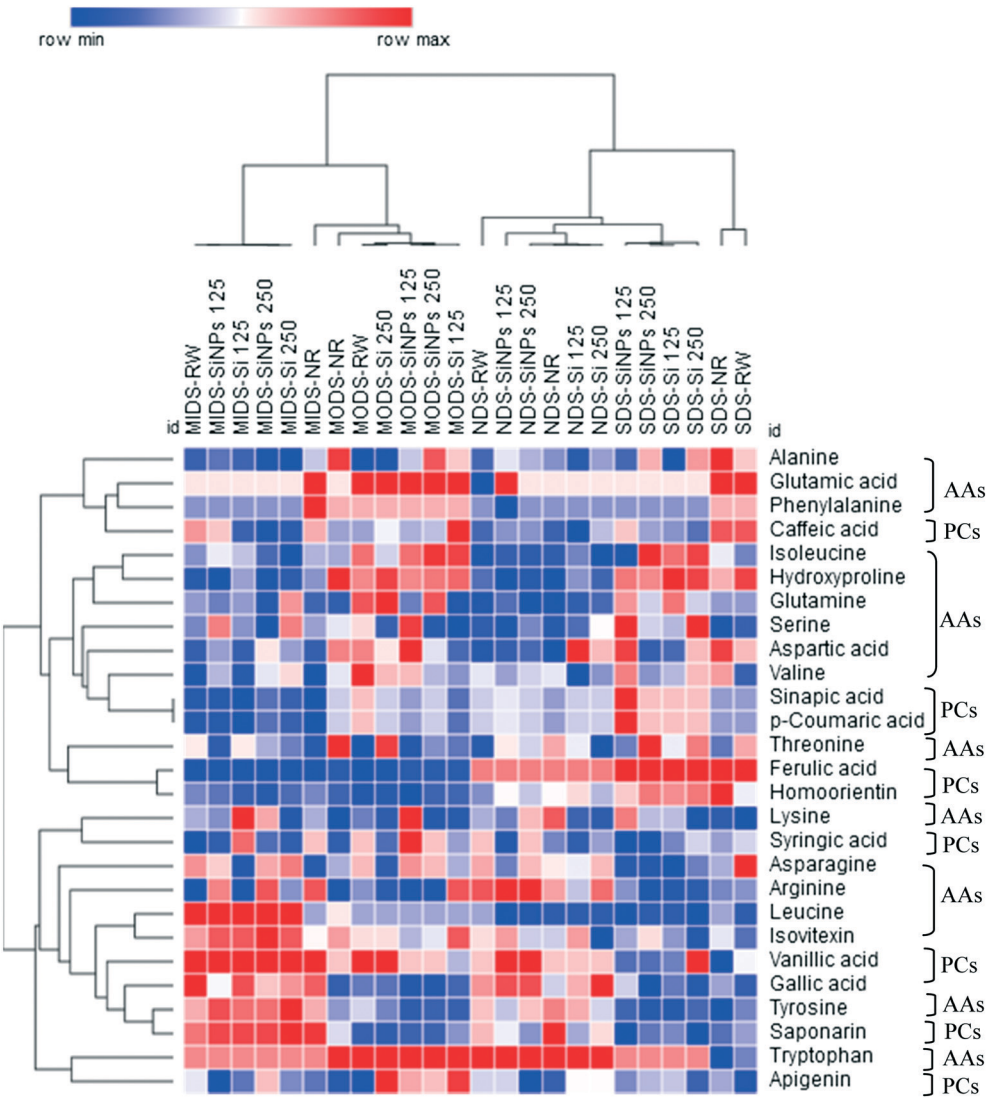

Fig. 5 Hierarchical clustering analysis (HCA) showing clustering of the identified metabolites according to their content in the various drought stress intensities and post-stress recovery treatments with silicone nanoparticles (Si NPs) and silicate (125 and 250 mg L^{-1}) compared to the non-recovery (NR) and recovery with distilled water (RW, rewatering) as control groups. For drought conditions, plants were maintained under 75% FC (mild drought stress, MIDS), 50% FC (moderate drought stress, MODS), and 25% FC (severe drought stress, SDS). Control plants (NDS, no drought stress) were maintained in optimal water availability conditions (*i.e.*, 100% FC). The metabolites grouped together react in a more similar way to the respective treatments than those in other clusters. Red colors represent a higher content than the mean content for the specific compound in all the employed treatments, while blue colors represent a lower content than the mean. AAs: amino acids, and PCs: phenolic compounds.

conditions upon all recovery treatments. The total flavonoids content of leaves did not significantly change in non-stressed seedlings treated with Si NPs and silicate concentrations compared to the controls, while in drought-treated plants, total flavonoids content increased by 1.7-fold, 3.6-fold, and 4.6-fold in mild, moderate and severe stress conditions, respectively (Table 2). Under moderate drought stress, the subsequent recovery with both Si NPs and silicate caused a significant increase (by 1.8-fold and 1.6-fold, respectively) in total flavonoids content over the respective controls. Under severe water deficit, however, the recovery (with all employed treatments) was accompanied by a significant reduction of the total flavonoids content compared to the respective non-recovered control seedlings (Table 2).

To mitigate the destructive effects of ROS, induction of both enzymatic and non-enzymatic antioxidants such as flavonoids and phenolic compounds are actively involved in controlling ROS levels within plant cells.[71] Several studies demonstrated that Si application may enhance plant tolerance against drought and salinity stresses through modifying the content of solutes,[72,73] carbohydrates,[74] polyols, and antioxidant secondary metabolites such as phenolics,[75] soluble sugars, and free amino acids.[76,77] Here, the post-stress recovery with Si NPs and silicate after moderate drought stress caused a significant increase in total flavonoids content of plants.

3.7. Amino acids and phenolic compounds

A specific accumulation pattern of free amino acids and phenolic constituents in relation to drought stress and subsequent recovery treatments was evident from the hierarchical cluster analysis, showing the clustering of the identified metabolites based on their content in the various employed treatments (Fig. 5). The content of major amino acids (tryptophan, phenylalanine, glutamic acid, and leucine) and phenolic compounds (ferulic acid and vanillic acid) are shown in Table 3. The content of phenylalanine and tryptophan in the leaves of barley plants were significantly ($p < 0.05$) different between the control and water stress treatments as well as between water stress and recovery treatments. The levels of phenylalanine, glutamic acid, and leucine considerably enhanced in plants exposed to the mild (by 41.7%, 50%, and 62.9%) and moderate (by 49.3%, 35.4%, and 77.6%) drought stress treatments compared to the well-watered control plants, respectively.

However, the leaf tryptophan content decreased under the mild and severe drought stress (by 16.5% and 83.1%), and slightly increased upon moderate stress (by 2.7%) compared to control, respectively (Table 3). The recovery treatments after the mild drought stress caused a significant increase only in leucine content compared to the respective control, and such an increase (up to 58.6%) was more pronounced in

Table 3 The leaf content of major amino acids including tryptophan (TRTN), phenylalanine (PHAN), glutamic acid (GLUA), and leucine (LECN), and main phenolic compounds such as ferulic acid (FERA) and vanillic acid (VANA) in barley seedlings subjected to various drought stress intensities and post-stress recovery treatments with silicone nanoparticles (Si NPs) and silicate (125 and 250 mg L^{-1}) compared to the non-recovery (NR) and recovery with distilled water (RW, rewatering) as control groups. For drought conditions, plants were maintained under 75% FC (mild drought stress, MIDS), 50% FC (moderate drought stress, MODS), and 25% FC (severe drought stress, SDS). Control plants (NDS, no drought stress) were maintained in optimal water availability conditions (*i.e.*, 100% FC). Means labeled by different letters are significantly different at $p < 0.05$ using the Tukey's test

Treatments		Major amino acids and phenolic compounds					
Drought stress	Stress recovery	Tryptophan (%)	Phenylalanine (%)	Glutamic acid (%)	Leucine (%)	Ferulic acid (%)	Vanillic acid (%)
NDS	NR	25.4^{a}	12.4^{c}	8.2^{e}	4.3^{b}	5.4^{a}	2.8^{b}
	RW	26.8^{a}	13.6^{b}	5.3^{f}	9.5^{a}	3.5^{b}	3.3^{a}
	SiNPs 125	27.3^{a}	14.0^{b}	14.6^{b}	2.7^{d}	2.8^{c}	3.1^{a}
	SiNPs 250	27.5^{a}	15.1^{a}	13.2^{c}	3.1^{c}	3.1^{b}	3.6^{a}
	Si 125	26.2^{a}	13.9^{b}	12.3^{d}	3.6^{c}	2.4^{d}	2.9^{b}
	Si 250	27.9^{a}	15.3^{a}	15.1^{a}	4.1^{b}	2.7^{c}	3.4^{a}
MIDS	NR	21.2^{b}	21.3^{a}	16.4^{a}	11.6^{e}	14.3^{f}	6.3^{a}
	RW	18.5^{c}	14.6^{e}	9.1^{f}	25.1^{d}	15.2^{e}	5.2^{b}
	SiNPs 125	22.8^{b}	17.8^{c}	11.7^{d}	28.0^{a}	18.4^{c}	4.9^{c}
	SiNPs 250	24.6^{a}	16.2^{c}	13.2^{b}	26.7^{c}	17.7^{d}	5.7^{b}
	Si 125	22.3^{b}	19.3^{b}	10.5^{e}	27.1^{b}	21.9^{a}	4.3^{c}
	Si 250	25.7^{a}	15.4^{d}	12.6^{c}	27.5^{b}	19.3^{b}	6.1^{a}
MODS	NR	26.1^{d}	24.5^{d}	12.7^{e}	19.2^{a}	16.5^{f}	6.5^{a}
	RW	31.6^{cd}	23.8^{e}	12.5^{e}	7.2^{c}	18.4^{e}	3.1^{cd}
	SiNPs 125	35.5^{a}	26.0^{b}	16.3^{c}	7.5^{c}	20.7^{c}	3.9^{c}
	SiNPs 250	35.9^{a}	27.3^{a}	18.2^{a}	6.3^{d}	22.6^{a}	3.5^{c}
	Si 125	32.2^{bc}	25.2^{c}	14.7^{d}	9.1^{b}	19.2^{d}	2.8^{d}
	Si 250	35.6^{ab}	24.1^{d}	17.2^{b}	7.4^{c}	21.4^{b}	4.1^{b}
SDS	NR	4.3^{d}	9.4^{e}	6.7^{e}	5.3^{a}	7.5^{a}	1.4^{d}
	RW	7.2^{c}	12.1^{d}	8.2^{d}	2.7^{d}	2.4^{e}	2.5^{bc}
	SiNPs 125	19.2^{ab}	14.3^{c}	11.7^{b}	3.5^{c}	5.6^{c}	2.6^{b}
	SiNPs 250	21.5^{a}	15.8^{b}	8.3^{d}	4.4^{b}	4.3^{d}	2.3^{c}
	Si 125	18.6^{bc}	12.0^{d}	9.1^{c}	3.9^{c}	6.1^{b}	2.7^{b}
	Si 250	20.3^{a}	17.9^{a}	12.2^{a}	2.2^{d}	2.9^{e}	3.9^{a}

Si NPs treatment at 125 mg L^{-1}. Upon recovery from the severe drought stress, the content of tryptophan, phenylalanine, and glutamic acid increased (by 27.2%, 10.3%, and 30.2%, respectively) and the increases were more pronounced when stressed plants re-watered with Si NPs and silicate at 250 mg L^{-1} (Table 3). The content of ferulic acid showed a significant ($p < 0.05$) increase (by 62.2%, 67.3%, and 28%) in plants exposed to mild, moderate and severe drought stress when compared to the non-stressed control seedlings, respectively (Table 3). After recovery with water (RW) and silicate (at 250 mg L^{-1}), the vanillic acid content increased (by 44% and 64.1%) under severe drought stress, but it significantly decreased through the recovery treatments under mild and moderate stress compared to the respective control groups.

Mechanisms underpinning the elicitation effects of Si NPs and silicate on amino acids and phenolic constituents in plants under drought stress are not clear and need to be explored further. However, Si treatment can stimulate the accumulation of defensive secondary metabolites including phenolics, phytoalexins, and momilactones[78,79] by altering the expression of defense-related genes.[80–82] Cell wall fortification by Si could also be linked with biosynthesis of phenolic compounds.[83] Our results are in line with those of a study by Večeřová *et al.* (2016), demonstrating that phenylalanine, tryptophan, leucine, asparagine, valine, and tyrosine contents were significantly affected by cadmium oxide (CdO) NP treatments in both roots and leaves of barley plants.[84] Likewise, levels of tryptophan and phenylalanine were also shown to increase in maize plants exposed to drought stress.[85] As tryptophan is a potential oxidation target, the free tryptophan may provide a buffer between ROS and major functional proteins in the chloroplast and maintain normal cell function and plant growth.[86] The enhanced phenolic compounds in plant cells possibly reflect the induction of protective mechanisms[64] by addition of Si, presumably *via* activation of defensive genes.[78,81,87] A schematic model of possible mechanisms behind the changes induced in the physiological, biochemical and metabolic attributes of barley seedlings during drought stress and post-Stress recovery with Si NPs and silicate is summarized in Fig. 8.

3.8. Correlation matrix and multivariate analyses

A general overview of the correlation analysis among the examined traits is presented in Fig. 6. As shown, there was a significant positive correlation between shoot biomass and MSI ($r_{0.01}$ = 0.94), soluble protein ($r_{0.01}$ = 0.86), Si accumulation ($r_{0.01}$ = 0.97), and total chlorophyll content ($r_{0.01}$ = 0.97) under the experimental treatments. Furthermore, a significant negative correlation was found between RWC and H_2O_2 ($r_{0.01}$ = −0.97), total flavonoids content ($r_{0.05}$ = −0.51), and POD ($r_{0.01}$ = −0.77) in plants under the reference treatments. Leaf tryptophan content was negatively correlated with H_2O_2 and MDA ($r_{0.05}$ = 0.62 and

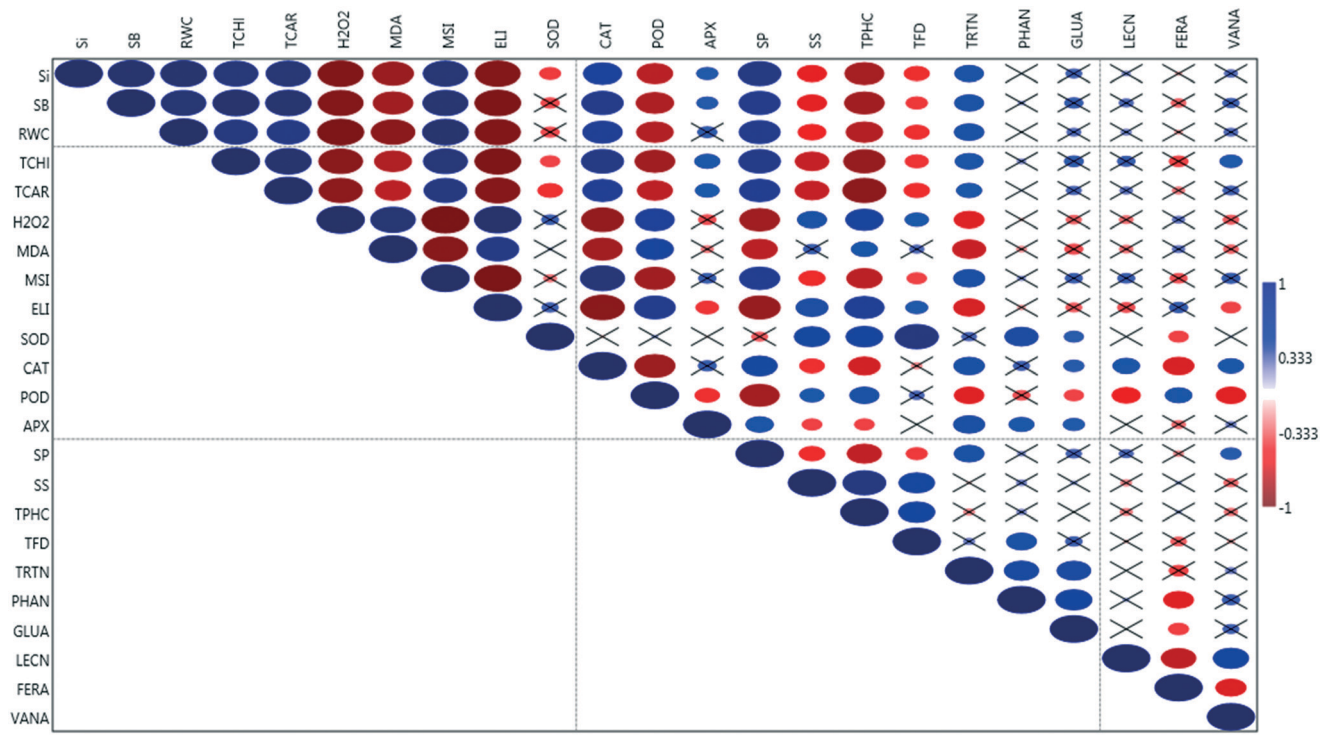

Fig. 6 Pearson's correlation general overview among the examined traits. The values with significance level $p > 0.05$ are crossed out. Positive correlations are displayed in blue and negative correlations in red color. Color intensity and the size of the circle are proportional to the correlation coefficients. In the right side of the correlogram, the legend color shows the correlation coefficients and the corresponding colors.

$r_{0.01}$ = 0.69) contents under the employed treatments, respectively (Fig. 6).

As shown, a clear separation among the control and treatment groups in barley seedlings was unearthed using the multivariate statistical analyses (Fig. 7A and B). Principal component 1 (PC1) explained 86.4% of the variance, however, principal component 2 (PC2) indicated 7.2% of the variance in the data set (Fig. 7A). Also, the severe drought stress and subsequent stress recovery with water (SDS-RW) and without recovery (SDS-NR) scores were substantially farther from the

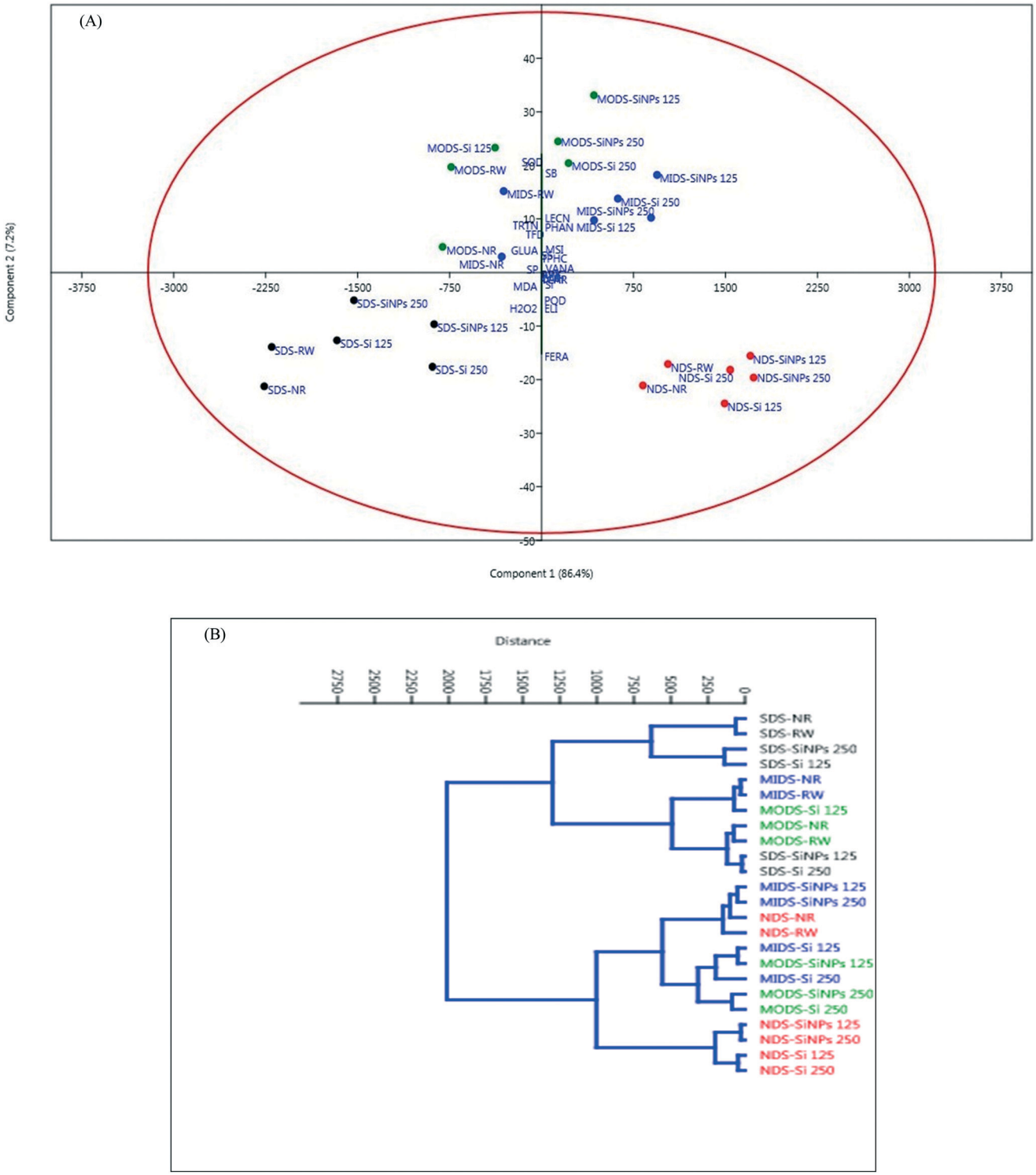

Fig. 7 (A) Biplot of the first two principal components (PC1 and PC2), and (B) dendrogram of cluster analysis (UPGMA) based on the examined traits in barley seedlings under various drought stress intensities and post-stress recovery treatments.

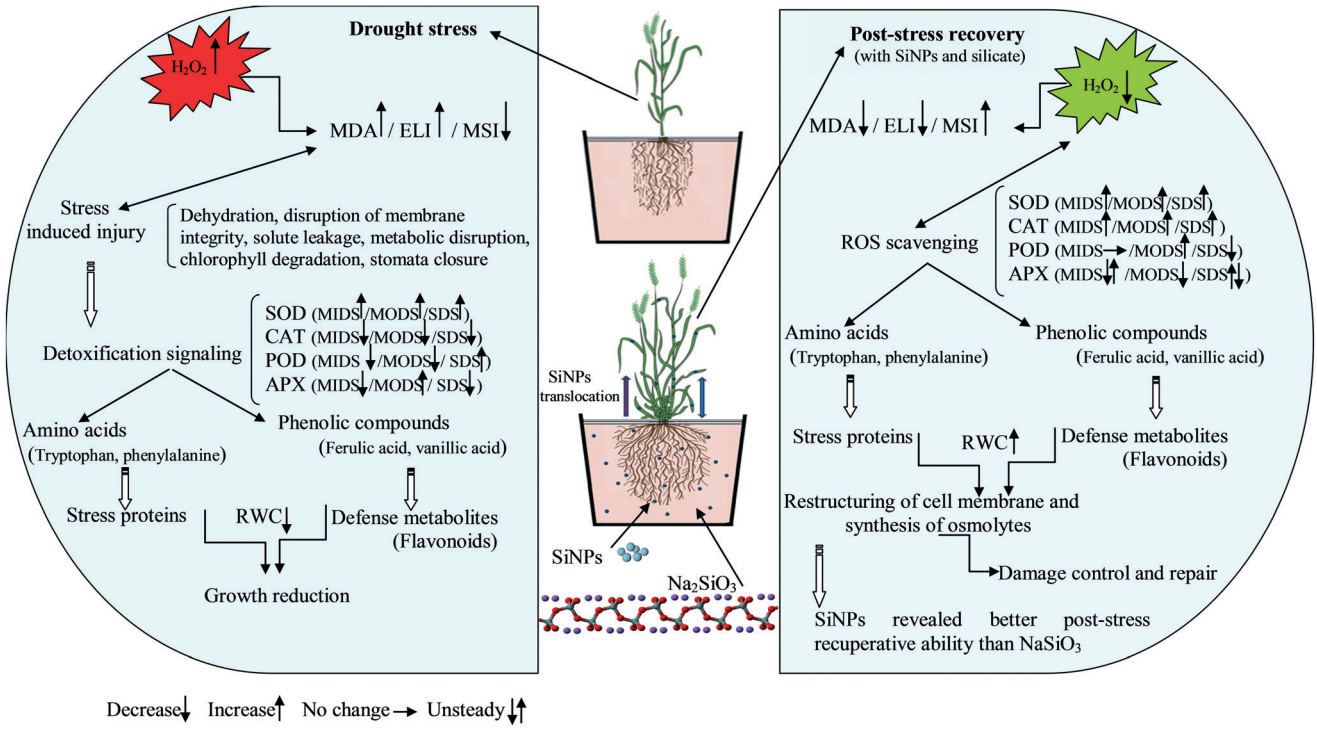

Fig. 8 A schematic model of possible mechanisms behind the changes induced in the physiological, biochemical and metabolic attributes of barley seedlings during drought stress and post-stress recovery with Si NPs. Abbreviations: silicone nanoparticles (Si NPs), sodium silicate (Na_2SiO_3), mild drought stress (MIDS), moderate drought stress (MODS), severe drought stress (SDS), control plants (NDS, no drought stress), leaf silicone (Si) content, shoot biomass (SB), leaf relative water content (RWC), total chlorophyll (TCHL), total carotenoid (TCAR), soluble protein (SP), soluble sugar (SS), total phenolics (TPHC), total flavonoids (TFD), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), electrolyte leakage index (ELI), membrane stability index (MSI), non-recovery (NR), recovery with distilled water (RW), superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), tryptophan (TRTN), phenylalanine (PHAN), glutamic acid (GLUA), and leucine (LECN), ferulic acid (FERA), and vanillic acid (VANA).

control (NDS-NR) mean, located on the down-right quadrant, mainly due to the enhanced cellular injury indices (*i.e.*, H_2O_2, MDA, and ELI) and lower seedling dry weight, soluble protein and amino acid (particularly tryptophan) contents (Fig. 7A).

A UPGMA cluster dendrogram grouped the employed treatments into three main clusters according to the examined traits (Fig. 7B). The second main cluster was formed by the most reference treatments including mild and moderate drought stress and subsequent recovery levels. As shown, plants under the moderate drought stress and without recovery treatment (MODS-NR) were most closely clustered with the severe drought stressed-plants. The UPGMA cluster analysis further supported the results of the biplot analysis in which the employed treatments were clustered into three main groups based on the investigated traits (Fig. 7B).

4. Conclusions

Our study demonstrated that drought stress negatively influenced barely seedlings growth through interference in both primary and secondary metabolism due to increased levels of H_2O_2, MDA, and ELI, and decreased levels of Si, RWC, MSI, and photosynthetic pigments. Following the drought stress cessation, recovery with water did not restore the maximum level of growth and performance in barley seedlings. However, post-drought recovery with Si NPs and silicate was more effective, which was linked to modifications in the plant chlorophyll content, osmolyte and metabolite profile, cellular injury and membrane stability indices, and the activity of antioxidant enzymes (SOD, CAT, POD, and APX). The recovery capacity of Si treatments varied according to the drought stress severity; however, our data indicate that even barley plants subjected to severe drought stress can be recuperated after seven days upon recovery with Si NPs at low dose, which can be attributed to greater availability of Si NPs to seedlings and more uniform deposition of Si in plant cells, reduced oxidative stress, protection of cell membrane, and synthesis of specific defense-related metabolites. Results of this work could be used to develop further knowledge on crops responses to different intensities of drought stress and post-drought recovery by Si-based materials (nanoscale and bulk types), which is of major significance for agriculture in arid and semi-arid environments. However, the present study only dealt with the barley response to drought at a vegetative stage, and the effects of the given Si treatments on crops grown to maturity (plant biomass and grain yield) need to be investigated under controlled environment, field or field-like

conditions in order to achieve a more realistic knowledge of the drought-recovery potential of Si NPs and silicate for crops subjected to drought stress.

Conflicts of interest

There are no conflict of declare.

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