Supporting Information for

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

Mansour Ghorbanpour[1,*], Hamid Mohammadi[2], Khalil Kariman[3]

[1]Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, 38156-8-8349,Arak, Iran.

[2]Faculty of Agriculture, Azarbaijan Shahid Madani University, Tabriz, Iran.

[3]School of Agriculture and Environment, The University of Western Australia, Perth, WA, 6009, Australia.

*Corresponding author

Content

S1.1. Sample preparation for SEM observations

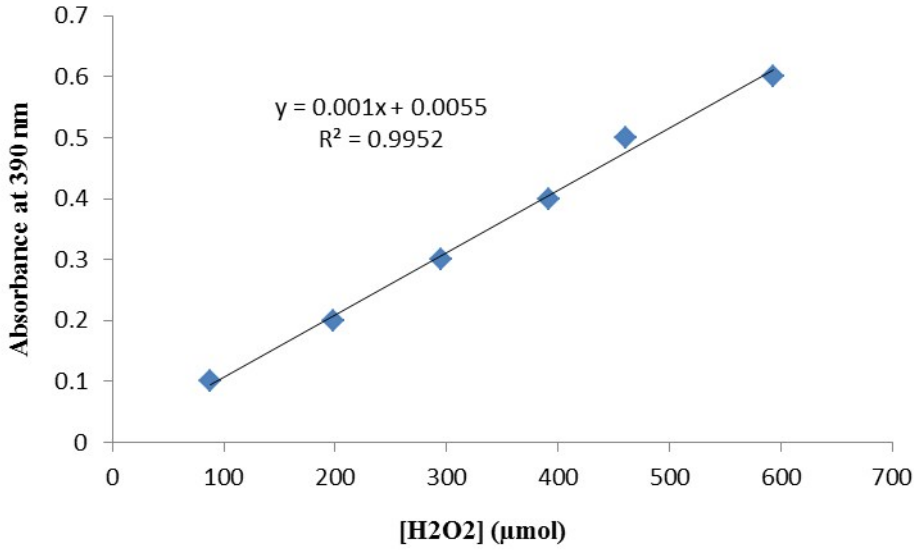
Root and leaf samples were collected and fixed immediately in a chemical fixing solution comprising of2.5% glutaraldehyde in 0.05M potassium phosphate buffer (pH 7.1) for 8 h. The samples were subsequently dehydrated in a graded series of ethanol (10%, 20%, 30%, 50%, 70%, and 95%, for 15 min each).After the ethanol dehydration step, the samples were transferred to tetramethylsilane (TMS) for 20 min prior to air-drying in a desiccator under vacuum, and then coated with a 20-nm thick gold layer. The field emission SEM was used to analyze the surface morphology of leaf and root cells.[1]

S1.2. Si determination of plant tissues

The Si content of leaf samples was determinedly an atomic absorption spectrometer (Shimadzu, model AA-7000F Series, Japan), containing hollow cathode lamp L-2433. The AA-7000F Series automatically optimizes the gas flow rate by calculating the changes in absorbance values between a blank and a standard sample. The gas flow rate with the highest sensitivity will be detected, and automatically set as the gas flow rate value. The detection limit was < 0.01 mg Si L^{-1}. The analysis was conducted following the manufacturer's standard protocols (https://shimadzu.com.au/system/files/AA-7000%20Brochure%20Ver.%20C.pdf).

S1.3. Determination of H_2O_2, MDA, ELI, and MSI

The H_2O_2content of leaf samples was quantified following the established protocols.[2] Briefly, a subsample of fresh tissues (0.5 g) was homogenized in 5 mL of 0.1 % w/v trichloroacetic acid (TCA) and centrifuged (12,000×g for 15 min). An aliquot of the supernatant (0.5 mL) was added to 0.5 mL of potassium phosphate buffer (10 mM, pH 7.0) and 1 mL of potassium iodide (1 M). The H_2O_2 concentration was determined following preparation of acalibration curve. The H_2O_2 content of leaf samples was expressed as μmol g^{-1}FW.



Malondialdehyde (MDA) is an end-product of polyunsaturated fatty acids (PUFA) peroxidation of cellular membrane, commonly known as an oxidative stress biomarker. For MDA quantification, fresh leaf tissues (0.5 g) were ground in10% trichloroacetic acid and centrifuged at 10,000 × *g* for 10 min. Two mL of the supernatant was added to 2 mL of 0.6% thiobarbituric acid. The mixture was subsequently heated at 95 °C for 30 min, cooled on ice, and centrifuged at 4000 × *g* for 20 min. Absorbance of the supernatant was read at 532 nm, and the MDA content of samples was calculated according to its extinction coefficient of $155 mM^{-1}$ cm^{-1}.

To characterize the cell membrane injury and stability, electrolyte leakage index (ELI) and membrane stability index (MSI) of the leaf samples were measured. Briefly, a fresh leaf subsample (50 mg) was rinsed and kept in approximately 15 mL of distilled water at 4 °C for 24 h and the initial electrical conductivity (EC1) was noted. The samples were then fully damaged by boiling at 100 °C for 20 min. The final electrical conductivity (EC2) of the samples was recorded using a conductivity meter. ELI and MSI indices were calculated as:

ELI (%) = (EC1/(EC2) × 100, and MSI= [1 – (EC1/EC2)] × 100

S1.4. Determination of antioxidant enzymes activities

To determine the activity of antioxidant enzymes, fresh leaf subsamples (0.3 g) were ground 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)using a liquid nitrogen-chilled mortar and pestle . The generated homogenate was centrifuged at 12000 × *g* for 20 min, and the supernatant was used to measure the specific activity of different antioxidant enzymes. The spectrophotometric analysis was performed on a T80 series of UV-Visible Spectrophotometer.

The SOD (EC 1.15.1.1) specific activity was quantified by determiningits capacity for inhibition of the photochemical reduction of NBT noted at 560 nm. Briefly, the required cocktail for estimation of SOD activity was prepared by mixing 25 mL of sodium phosphate buffer (pH 6.8), 1 mL of nitroblue tetrazolium (NBT; 75 µM), 1.5 mL of methionine (13mM), 1.5 mL of 0.1 mM EDTA, and 1 mL of 0.002 mM riboflavin. The reaction mixture was subsequently illuminated for 15min using two fluorescent lamps (15 W). A tube without enzyme was kept in the light to serve as the control, and a tube with enzyme extract that was kept in the dark was used as blank.

The SOD activity was determined as the difference in NBT reduction recorded at 560 nm in light in the presence or absence of the enzyme extract. One unit of the SOD activity was considered to bean amount of enzyme required to inhibit 50% initial reduction of NBT under light.
The CAT (EC 1.11.1.6) specific activity was determined by quantifying the disappearance rate of H_2O_2 (ε = 39.4 mM^{-1} cm^{-1}). The reaction mixture contained 50 mM potassium phosphate buffer (pH 6.8), 1 mM EDTA, 15 mM H_2O_2, and 20 µL of the enzyme extract. The CAT activity was calculated by measuring the reduction in sample absorbance at 240 nm for 1 min.
To determine the POD (E.C.1.11.1.7) specific activity, the rate of tetraguaicol formation (ε = 26.6 mM^{-1} cm^{-1}) from guaicol was determined by measuring the sample absorbance at 470 nm.The assay mixture (1 mL) contained40 mM phosphate buffer (pH 6.8), 15 mM guaiacol, and5 mM H_2O_2. The reaction was initiated upon addition of H_2O_2 and the increase in absorbance at 470 nm was measured for 1 min.
The APX (EC 1.11.1.11) specific activity was quantified based on the decrease in the amount of ascorbate oxidized (ε =2.8 mM^{-1} cm^{-1}) as recorded at 290 nm. The soluble protein was extracted by homogenizing the sample in 2 mL of 50 mM sodium phosphate buffer (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid, 250 mM H_2O_2, and an enzyme extract equivalent to 50 µL of protein.

S1.5. Preparation of the extract and measurement of total phenolic and flavonoid contents
The extracts were prepared by addition of 1 mL of 80% methanol to 100 mg of barley leaf tissues. The obtained mixture was shaken with a laboratory rotary shaker in liquid nitrogen under dark conditions at 200×g for 1 h at 25 ◦C, and then sonicated (500 w, 40 °C) for 20 min. The mixture was subsequently centrifuged at 13,000 × g for 20 min at 25 °C. The supernatants were filtered through Whatman No. 1 filter paper and the extraction process was repeated three times. Finally, the combined supernatants were evaporated to dryness under vacuum at 40 ◦C using a rotary vacuum evaporator, and stored at -20 ◦C until analysis.
The total phenolic content (TPC) of leaf samples was measured using Foline-Ciocalteu reagent (FCR) reagent. Briefly, the methanolic extracts (100 µL) were mixed with 2 mL of distilled water and 1 mL of the FCR reagent. After 3 min incubation, the samples were neutralized with sodium carbonate (20%). The mixture was then kept at room temperature for 45 min in the dark. Finally, the samples absorbance was recorded at 765 nm. The TPC values were expressed as mg of gallic acid equivalents per gram of dry weight (mg $GAEg^{-1}$ DW) according to a gallic acid (50-1000 mg L^{-1}) calibration curve ($y = 0.0019x + 0.1265$, R^2=0.98).
To measure the total flavonoid content of leaf samples,1 mL of the methanolic extract was added to 4 mL of distilled water, and then mixed with 300 µL of $NaNO_2$solution (5%). After five min incubation, 300 µL of $AlCl_3$ (10%) and 2 mL of NaOH (1 M) was added to the reaction. The mixture was diluted with 10 mL of distilled water. The extracts were shaken for 30 min at room temperature in the dark. Thereafter, the absorbance was read at 510 nm, and the TFC values were expressed according to a rutin calibration curve ($y = 0.0013x + 0.0806$, R^2=0.96) as mg of rutin per gram of dry weight of sample.

S1.6. Amino acids and phenolic compounds analysis
The amino acid content of barley leaves was determined using a Biochrom 20 amino acid analyzer (Pharmacia LKB, Biochrom Ltd, Cambridge) based on manufacturers protocol. Ten mL of 6N hydrochloric acid (HCl; 1:1) was added to a test tube containing a leaf subsample (0.1 g).

The test tube was evacuated with dry nitrogen, sealed and then incubated in an oven (110 °C, 22 h). The test tube was then allowed to cool down at room temperature to within about 30 min.
In order to remove HCl, the hydrolyzate was subsequently evaporated under vacuum at 60 °C for 10 min. Afterwards, the hydrolyzate was dissolved in 5 mL of 0.02 N HCl, centrifuged at 1000 rpm and filtered (Whatman No. 1) to remove the debris. A 20 µL volume of the supernatant was injected into an amino acid analyzer to identify/quantify the amino acid compositions of each sample. An amino acid score was considered for each sample according to FAO/WHO (1973) reports using the following equation:[3,4]
The individual amino acid score= milligram of amino acid per gram of protein / milligram of amino acid per gram of protein in reference protein×100:
The amino acid with the lowest proportion was termed as the limiting amino acid.
The content of phenolic acid compounds in barley leaf samples was estimated by high performance liquid chromatography (HPLC, Shimadzu LC-20A, Kyoto, Japan) method coupled with SPD-M20A photodiode array detector, and Phenomenex Gemini C18 110A 5 µm column (150 × 4.6 mm). The detection wavelength was set at 280 nm using aforesaid diode array detector. The time of separation of phenolic acids was within 75 min at a constant temperature of 35 °C with a linear gradient flow of 0.9 mL/min. Identification of phenolic compounds was performed by comparing the retention times of analytes with those of the reference standards, and their maximum UV spectrum. Quantification of the phenolic acids was based on generated external calibration curves with a linear range for the two major compounds found in barley leaf samples analyzed.[5] The samples were analyzed in triplicates (*n=3*).

References

1 A.K .Pathan, J. Bond, R.E. Gaskin, Sample preparation for SEM of plant surfaces, Materials today, 2010, 12, 32-34.

2 I. Sergiev, V.Alexieva and E. Karanov, Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants, Compt. Rend. Acad. Bulg. Sci., 1997, 51, 121–124.

3 M. Rubin, D. R. Schoonouerand E. H. Bossard, Amino acid profiles of corn and soybean meal by a modified analysis technique. Poultry Science Journal., 1975, 54 (5), 1811.

4 FAO/WHO, 1973. Energy and protein requirements. Technical Report Series, No. 522, World Health Organization, Rome.

5 M.E. Hefni, L.S. Amann and C.M. Witthöft, A HPLC-UV Method for the Quantification of Phenolic Acids in Cereals. Food Anal. Methods., 2019, 12**,** 2802–2812.