Supplementary Materials

S1: Total ascorbic acid assay

Plant tissue (5 g) was homogenized with 50 ml of 2% oxalic acid and centrifuged at 10,000×g for 30 min. The supernatant was subjected to ascorbic acid analysis using titrimetric method with 2,6-dichlorophenolindophenol. Briefly, 10 ml of the extract was immediately titrated with 2,6-dichlorophenolindophenol (Sigma-Aldrich, Steinheim, Germany) at the concentration 25 mg in 100 ml [1]. Vitamin C content was calculated based on a calibration curve prepared by titration of a series standards of ascorbic acid at the concentration of 0.1, 0.25, 0.5 and 1 mg/ml.

S2: ROS generation assay

A 2 g of the plant tissue was homogenized with 6 ml of 50 mM sodium phosphate buffer (pH 7.4) and centrifuged at 7500×*g* for 30 min at 4 °C. The level of ROS in supernatant was determined with 2',7' –dichlorofluorescin diacetate (H_2DCFDA, Sigma-Aldrich, Steinheim, Germany) as a fluorescent probe. For this purpose, 100 µl of the extract was mixed with 1 ml of phosphate buffer and 25 µl of 5 mM H_2DCFDA (ethanol solution). After 30 min of incubation at 35 °C, the fluorescence of the solution was measured at a wavelength of excitation of 504 nm and emission of 529 nm, respectively using Hitachi F7000 spectrofluorometer [1].

S3: SOD* and *CAT activities assay

Frozen (−67 °C) and powdered plant tissue (2.5 g) was homogenized with 7 ml of chilled 0.9% NaCl containing 0.5% Triton X-100 and protease inhibitors mixture (Sigma-Aldrich, Steinheim, Germany). The homogenate was centrifuged at 10,000×g for 30 min at 4 °C. The SOD activity was determined in the supernatant using colorimetric method which based on the measurement of the inhibition of epinephrine autoxidation by SOD. Briefly, 10 µl of enzyme extract was mixed with 95 µl of 50 mM carbonate buffer (pH 10.2) and 10 µl 50 mM epinephrine (Sigma-Aldrich, Steinheim, Germany). The kinetic of absorbance increase was measured for 10 min at 490 nm [2]. The CAT activity was assayed using the protocol presented by Piechowiak et al. [3]. In this method, the level of H_2O_2 in the mixture after enzymatic reaction was analysed colorimetrically with ammonium metavanadate at 452 nm. A 10 µl of enzyme extract and 50 µl of 50 mM sodium phosphate buffer (pH 7.4) were added into a 96-well plate. The reaction was initiated by adding 20 µl of 10 mM H_2O_2 per well and stopped after 5 min of incubation at 35 °C by adding 50 µl of 50 mM ammonium metavanadate solution (Chempur, Piekary Śląskie, Poland). The absorbance was measured after 10 min of incubation. Total protein content in the extract was measured using Bradford methodology [4].

References

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