South African Journal of Botany 106 (2016) 23–28

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Exogenous application of hydrogen peroxide alleviates drought stress in cucumber seedlings

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ARTICLE INFO

Article history:
Received 8 November 2015
Received in revised form 3 May 2016
Accepted 13 May 2016
Available online 4 June 2016

Edited by AO Aremu

Keywords:
Antioxidants
Cucumber
Drought
Hydrogen peroxide
Osmotic adjustment

ABSTRACT

Drought limits plant growth and crop yield worldwide and more frequent occurrences of water shortages are expected according to climate change projections. However, relatively little is known about whether the spraying of plants leaves with hydrogen peroxide (H_2O_2) would alleviate the symptoms of drought stress. In this study, 3-week-old cucumber seedlings exposed to different soil water contents (non-limiting soil water conditions, medium drought, and severe drought) were foliar-sprayed with 1.5 mM H_2O_2. Growth, photosynthesis and oxidative defense system were determined after one week of treatment. Results showed that exogenous H_2O_2 significantly increased biomass, leaf relative water content (RWC) and chlorophyll content, coupled with increased net photosynthetic rate (P_n), especially under medium drought. Meanwhile, superoxide anion radicals (O_2^-), electrolyte leakage and malondialdehyde (MDA) levels decreased whereas the activities of key antioxidant enzymes superoxide dismutase (SOD) and peroxidase (POD) as well as soluble sugar and proline contents increased in H_2O_2-treated plants. Hence, exogenously applied H_2O_2 considerably improved drought tolerance of cucumber plants by increasing the plant antioxidative defense system and the capacity for osmotic adjustment to alleviate membrane lipid peroxidation and to reestablish cell turgor, and thereby increasing photosynthesis, particularly under medium drought.

1. Introduction

Under global climate change, the frequency, intensity and duration of drought are expected to increase across the arid and semi-arid areas (Boyer, 1982; Chaves et al., 2003). Thus, tolerance and acclimation to low soil water availability are crucial factors affecting plant growth. In response to water stress, a decrease in photosynthesis is generally observed under mild or moderate drought, which can be the result of CO_2 diffusion from the atmosphere to the site of carboxylation caused by stomata closure (Cornic, 2000; Flexas et al., 2004; Chaves et al., 2009). However, with prolonged drought, metabolic impairment such as the degradation of photosynthetic pigment and the inhibition of key photosynthetic enzyme activity may further reduce the photosynthetic capacity (Bota et al., 2004; Flexas et al., 2006; Gallé and Feller, 2007; Galmés et al., 2011).

Drought limits photosynthesis in most plants mainly due to an imbalance between light capture and its utilization (Reddy et al., 2004). If light energy absorbed by chlorophyll cannot be dissipated, reactive oxygen species (ROS) will burst within the plant (Larson, 1988; Smirnoff, 1993). However, the excessive production of ROS, such as $^1O_2$, H_2O_2, O_2^- and HO·, is potentially harmful to proteins, DNA and lipids (Apel and Hirt, 2004), leading to impairment of membrane integrity, enzyme inhibition, chlorophyll degradation (Scandalios, 2005; Miller et al., 2010). In order to cope with the toxicity of ROS, plants developed a highly efficient antioxidative defense system, including both nonenzymatic and antioxidant enzymatic systems such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which permit plants continuous growth and survival (Asada, 2006; Gong et al., 2006; Talbi et al., 2015).

Although ROS are toxic molecules causing oxidative damage to plant tissues exposed to environmental stress, increasing evidence indicates that ROS play important role in the regulation of stomatal behavior to optimize water use efficiency (Pei et al., 2000; Huang et al., 2009). The H_2O_2 belongs to non-radical ROS and a by-product of oxidative stress metabolism, being the only ROS species that is stable in solution due to no net charge (Matilla-Vázquez and Matilla, 2012). Emerging evidence suggests that H_2O_2 ameliorated the stress-induced adverse effects via rapid stomatal closure (McAinsh et al., 1996; Pei et al., 2000; Kolla et al., 2007; Quan et al., 2008; Wang and Song, 2008) or by the promotion of oligosaccharide biosynthesis to maintain leaf water content (Ishibashi et al., 2011). In addition to oligosaccharide, the accumulation

Abbreviations: H_2O, Hydrogen peroxide; RWC, Relative water content; FW, Fresh weigh; DW, Dry weight; TW, Saturated weight; P_n, Net photosynthesis rate; O_2^-, Superoxide anion radicals; EL, Electrolyte leakage; MDA, Malondialdehyde; ROS, Reactive oxygen species; SOD, Superoxide dismutase; POD, Peroxidase; CAT, Catalase.

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http://dx.doi.org/10.1016/j.sajb.2016.05.008
0254-6299/

of compatible osmolytes such as sucrose, proline and other low molecular weight molecules could also ameliorate the adverse effects of drought stress (Ashraf, 2009). Hence, different strategies are being used to induce plants stress tolerance via enhancing the concentration of these osmolytes, including gene engineering and exogenously spraying on drought stressed leaves (Jubany-Marí et al., 2009; Farooq et al., 2010; Liu et al., 2010). Evidence suggest that H_2O_2 spraying could increase the drought tolerance of plants (Ozaki et al., 2009; Liu et al., 2010; Ishibashi et al., 2011), however, the precise physiological mechanisms underlying the induction of plant drought tolerance are still under discussion.

Cucumber (*Cucumis sativus* L.), one of the most important horticultural crops, is known to be susceptible to drought due to their high water requirement and unfavorable soil water conditions may limit the quality and thus yield parameters. Although different strategies are being used to induce plants stress tolerance, it remained to be known whether H_2O_2 spraying could enhance the resistance of cucumber to drought. Hence, we performed the study to observe the role of foliar exogenous application of H_2O_2 in alleviating the adverse effects of drought on growth, photosynthesis and antioxidant system in cucumber seedlings. This might provide a scientific basis for the large-scale cucumber culture under global climate change.

2. Materials and methods

2.1. Materials and treatments

Potted experiments were performed outdoors from March to April, 2015 in the Botany Institute of Northwest of Hubei Province, Hubei University of Arts and Science, Xiangyang, China. Throughout the experiments, the air temperature was about 22–27 °C during daytime and 14–18 °C during nighttime with a relative humidity of 70 ± 5% and an average daily photosynthetic photon flux density (PPFD) of 560 μmol m^{-2} s^{-1}. Seeds of the popular cucumber cultivar (*C. sativus* L. var. E no. 4) were sown individually in plastic, cylindrical pots (top and bottom diameter were 34 cm and 24.5 cm, respectively, and 31 cm in height, volume ≈ 20 L), containing a mixture of yellow–brown soil and sand (2:1, v/v). Before sowing, 4 g compound fertilizer ($N:P_2O_5:K_2O$ = 5:2:6) was added to each pot as base fertilizer. Three weeks after sowing, cucumber seedlings (the plants had 2 fully expanded leaves) were exposed to three soil water treatments: well-watered, medium drought and severe drought, corresponding to soil water contents between 80 ± 5%, 60 ± 5% and 45 ± 5% of field water capacity. One week after the water treatment, seedlings were divided into six groups. Among the groups, three groups were sprayed with distilled water and the other three groups were sprayed with freshly prepared 1.5 mM H_2O_2 at 18:00 (the optimum concentration was based on our earlier preliminary experiment, 100 ml/pot), and were characterized as CW, MW, SW, and CH, MH, SH, respectively. The soil water contents were maintained by weighing the pots and compensating water daily. During the experiment phase, a transparent plastic sheeting was covered on the pots approximately 10 cm above the soil to avoid the effect of rainfall on soil water content. After one week of foliar application, plants (n = 6) were harvested and weighed separately, and then dried at 80 °C to constant weight to determine biomass. The other samples were frozen immediately in liquid nitrogen and stored at −80 °C until further processing. The following measurements of all parameters consisted of six replicates. Before sampling, leaf P_n and relative water content (RWC) were determined.

2.2. Determination of RWC

The second fully expanded leaf from the apex was collected and fresh weight (FW) of the leaf was measured immediately. Dry weight (DW) was determined via drying the sample at 80 °C to constant weight, and turgid weight (TW) was obtained after floating the leaf in distilled water at 4 °C for 48 h. RWC was calculated as $RWC(\%) = (FW - DW)/(TW - DW) \times 100\%$ (Weatherley, 1950).

2.3. Diurnal variations in leaf net photosynthesis rate (P_n)

The measurement of diurnal variations in leaf P_n was conducted on the second fully expanded leaf from the apex using a portable gas exchange system (Licor-6400, Li-COR Biosciences, Lincoln, NE, USA) from 08:00 to 18:00 at two-hour intervals (Kim et al., 2012).

2.4. Estimation of photosynthetic pigments

0.5 g of fresh leaves was ground to a fine powder in liquid nitrogen and then homogenized in 1.8 ml of 80% acetone. The homogenate was centrifuged at 12,000 rmp for 20 min. The chlorophyll content in the extracts was colorimetrically measured according to Porra et al. (1989).

2.5. Determination of leaf membrane permeability and lipid peroxidation

Membrane integrity was evaluated by the leaf relative electrolyte leakage (EL). 20 leaf disks (10 mm diameter) were soaked in 10 ml distilled water and then pumped using a vacuum air pump for 10 min. The initial electrical conductivity was determined by measuring the electrical conductivity of the solution using a conductivity meter (Cole-Parmer 19820, Cole-Parmer Inc., Vernon Hills, IL, USA), whereas the final conductivity was obtained by boiling the same solution for 5 min, and then cooling it to room temperature. EL was calculated as a percent of the initial to the final conductivity.

Lipid peroxidation was estimated by measuring the content of malondialdehyde (MDA) using a modification of the method of Zhou et al. (2007). Aliquots of 50 mg powdered leaves were homogenized with 1 ml of 50 mM phosphate buffer solution (pH 7.8), then centrifuged at 15,000 rpm for 20 min. 600 μl of the supernatants were mixed with 1.2 ml of 0.6% (w/v) thiobarbituric acid (TBA) dissolved in 10% trichloroacetic acid. The mixture was heated at 95 °C for 30 min and then immediately cooled on ice. After centrifugation at 10,000 rpm for 10 min, the absorbance values of the supernatants were measured colorimetrically at 532, 450, and 600 nm. Leaf MDA content (μmol·l^{-1}) was calculated as$6.45 \times (A532 - A600) - 0.56 \times A450$, where, A_{532}, A_{600} and A_{450} represent the absorbance values at 532, 450, and 600 nm using a UV–vis spectrophotometer (Hewlett-Packard 8452A, Diode Array Spectrophotometer, USA), respectively.

2.6. Determination of superoxide anion radicals (O_2^-) and H_2O_2 contents

O_2^- contents in leaves were determined using the modified method of Elstner and Heupel (1976). 200 mg of powdered leaves was homogenized with 1 ml of 50 mM phosphate buffer solution (pH 7.8) containing 2 mM hydroxylamine hydrochloride. After centrifugation at 12,000 rpm for 30 min, 600 μl of the supernatants was mixed with 400 μl of phosphate buffer solution and incubated at 25 °C for 30 min, then 1 ml of 17 mM L-sulfanilic acid and 1 ml of 7 mM L-1-α-naphthylamine were added, and the mixture was shaken at room temperature for 30 min. The absorbance was determined colorimetrically at 530 nm.

The H_2O_2 content was assayed by a modification of the method of Brennan and Frenkel (1977). Aliquots of 50 mg powdered leaves were mixed with 1 ml of cold acetone and incubated at room temperature for 10 min, then centrifuged at 15,000 rpm for 10 min. 500 μl of supernatants was mixed with 50 μl of 5% (w/v) titanous sulfate ($TiSO_4$) and 100 μl of 100% ammonia. After centrifugation at 12,000 rpm for 10 min, the supernatant was discarded and the precipitate solubilized in 2 ml of 2 M H_2SO_4 and washed three times with acetone to remove chlorophyll. The absorbance values of derived titanium-peroxide compound were recorded at 415 nm.