Plant Physiology and Biochemistry 112 (2017) 19–28

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Implications of terminal oxidase function in regulation of salicylic acid on soybean seedling photosynthetic performance under water stress

Yanping Tang [a, b], Xin Sun [a, **], Tao Wen [a, *], Mingjie Liu [a], Mingyan Yang [a], Xuefei Chen [a]

[a] College of Agronomy, Sichuan Agricultural University, No.211, Huimin Road, Gongping Town, Wenjiang District, Chengdu, 611130, Sichuan, China
[b] Agrotechnical Extension Station, Agricultural Bureau of Dazhou City, No.52, Heye Street, Tongchuan District, Dazhou, 635000, Sichuan, China

ARTICLE INFO

Article history:
Received 18 August 2016
Received in revised form
22 November 2016
Accepted 22 November 2016
Available online 25 November 2016

Keywords:
Salicylic acid
Terminal oxidase
Soybean
Photosynthesis
Water stress

ABSTRACT

The aim of this study is to investigate whether exogenous application of salicylic acid (SA) could modulate the photosynthetic capacity of soybean seedlings in water stress tolerance, and to clarify the potential functions of terminal oxidase (plastid terminal oxidase (PTOX) and alternative oxidase (AOX)) in SA′ s regulation on photosynthesis. The effects of SA and water stress on gas exchange, pigment contents, chlorophyll fluorescence, enzymes (guaiacol peroxidase (POD; EC 1.11.1.7), superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.11) and NADP-malate dehydrogenase (NADP-MDH; EC1.1.1.82)) activity and transcript levels of *PTOX*, *AOX1*, *AOX2a*, *AOX2b* were examined in a hydroponic cultivation system. Results indicate that water stress significantly decreased the photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (E), pigment contents (Chla + b, Chla/b, Car), maximum quantum yield of PSIIphotochemistry (Fv/Fm), efficiency of excitation capture of open PSIIcenter (Fv'/Fm'), quantum efficiency of PSIIphotochemistry (ΦPSII), photochemical quenching (qP), and increased malondialdehyde (MDA) content and the activity of all the enzymes. SA pretreatment led to significant decreases in Ci and MDA content, and increases in Pn, Gs, E, pigment contents, Fv/Fm, Fv'/Fm', ΦPSII, qP, and the activity of all the enzymes. SA treatment and water stress alone significantly up-regulated the expression of *PTOX*, *AOX1* and *AOX2b*. SA pretreatment further increased the transcript levels of *PTOX* and *AOX2b* of soybean seedling under water stress. These results indicate that SA application alleviates the water stress-induced decrease in photosynthesis may mainly through maintaining a lower reactive oxygen species (ROS) level, a greater PSIIefficiency, and an enhanced alternative respiration and chlororespiration. PTOX and AOX may play important roles in SA-mediated resistance to water stress.

1. Introduction

Salicylic acid (ortho-hydroxy benzoic acid; 2-Hydroxybenzoic acid; SA), an endogenous plant growth regulator of phenolic nature that possesses an aromatic ring with a hydroxyl group or its functional derivative, is ubiquitously distributed in the whole plant kingdom. It has long been known as a signal molecule in the induction of protective mechanisms against biotic or abiotic stresses in plants (Hayat et al., 2013; Hayat et al., 2010). Several lines of evidence demonstrate the alleviating role of SA during several abiotic stresses including water stress (Singh and Usha, 2003; Hayat et al., 2013). The results published so far have shown that pre-treatment of plants with SA might have an acclimation-like effect, causing enhanced tolerance towards most kinds of abiotic stress, primarily due to enhanced antioxidative capacity or by inducing various signal transduction pathways (Dot et al., 1998; Janda et al., 2012; Janda et al. 2012).There have been several studies on the effects of exogenously applied SA on photosynthetic processes, and it has been supposed that the regulation of SA on photosynthesis might play an most important role in SA-related

Abbreviations: SA, salicylic acid; PTOX, plastid terminal oxidase; AOX, alternative oxidase; POD, guaiacol peroxidase; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; NADP-MDH, NADP-malate dehydrogenase; Pn, photosynthetic rate; Gs, stomatal conductance; E, transpiration rate; Fv/Fm, maximum quantum yield of PSII photochemistry; Fv'/Fm', efficiency of excitation capture of open PSII center; Φ PSII, quantum efficiency of PSII photochemistry; qP, photochemical quenching; MDA, malondialdehyde; ROS, reactive oxygen species; PEG, polyethylene glycol.

* Corresponding author.
** Corresponding author.
E-mail addresses: typing897@163.com (Y. Tang), sunxin529@163.com (X. Sun), wtlst@163.com (T. Wen).

http://dx.doi.org/10.1016/j.plaphy.2016.11.016
0981-9428/

tolerance (Singh and Usha, 2003; Krantev et al., 2008; Wang et al., 2010). However, little is yet known about SA-related mechanisms that alleviate the decline of photosynthesis.

Recently, metabolic interaction between chloroplasts and mitochondria has been intensively studied. Mitochondrial alternative oxidase (AOX), the unique respiratory terminal oxidase in plants, catalyzes energy wasteful cyanide (CN)-resistant respiration and play an important role in the tolerance to environmental stresses (Sieger et al., 2005; Giraud et al., 2008; Watanabe et al., 2008; Keunen et al., 2013). It has been suggested that alternative respiration also acts as an important process in dissipation of the chloroplast reducing equivalent and might have a particular role in relieving the over-reduction of chloroplast (Padmasree et al., 2002; Zhang et al., 2010; Yoshida et al., 2011; Xu et al., 2011; FLOREZ-SARASA et al., 2011). Cooperating with the malate (Mal)/oxaloacetate (OAA) shuttle, it consumes the excessive chloroplast NADPH and NADH and so keeps up the recycling rates of $NADP^+$ and NAD^+ (Noguchi and Yoshida, 2008; Vishwakarma et al., 2014). Its role in re-oxidizing the UQ pool and at the same time of the plastoquinone pool for reducing the generation of ROS is helpful for keeping up efficient photosynthetic performance (Watanabe et al., 2008; Zhang et al., 2011; Keunen et al., 2013; Vishwakarma et al., 2014). So induced level of AOX has a potential ability to protect photosynthesis under environmental stresses.

Also, recent researches have focused on a new photosynthetic electron transport chain (chlororespiration) in chloroplast similar to alternative respiration, and its terminal oxidase (plastid terminal oxidase, PTOX) display high homologous with AOX in structure and sequences, all belonging to quinone alcohol oxidase and sensitive to same inhibitors, e.g. N-propyl gallate (nPG) and salicylhydroxamic acid (SHAM) (Aluru and Rodermel, 2004; McDonald and Vanlerberghe, 2005, 2006; Fu et al., 2009). Binding strongly to the stromal side of thylakoid membranes in their nonappressed regions, PTOX transfers electrons from reduced PQ to molecular oxygen with the formation of H_2O (Josse et al., 2000; Carol and Kuntz, 2001; Joët et al., 2002; Josse et al., 2003). Recent researches have shown that PTOX plays an essential role in avoiding the excessive reduction of PQ pool and keeping harmonization in photosynthetic electron transport (Sun and Wen, 2011; McDonald et al., 2011). It protects plants from oxidative damage and improves the plants' adaptive capacity to environmental stresses. In addition, PTOX also indicated to be a co-factor of carotenoid bio-synthesis. Carotenoids, serving as accessory pigments of photosynthesis, are also known as photoprotective agents against photo-oxidation in plants (Carol and Kuntz, 2001; Aluru et al., 2006). Therefore, PTOX have been suggested to play a beneficial role for plants under environmental stresses.

Increases in AOX expression and activity have been observed in plants after exposure to a range of stresses and SA treatment (Raskin et al., 1989; Bartoli et al., 2005; Clifton et al, 2006; Fung et al., 2006; Feng et al., 2008a,b; Zhang et al., 2010). Low concentrations of exogenous SA would enhance the cyanide resistant respiration activity and AOX protein level (Lei et al., 2008; Matos et al., 2009). PTOX, suggested to be functionally analogous to AOX, is also induced by a lot of environmental stresses (Streb et al., 2005; Wang et al., 2009; McDonald et al., 2011) and might responses to SA the same as AOX. We hypothesized that SA-related protection on photosynthesis of plant under obiotic stresses is related to its regulation on terminal oxidase (AOX and PTOX). The present study was conducted to assess terminal oxidase function in regulation of SA on photosynthetic performance.

Soybean, as an important source of quality protein and edible oil, is wildly cultivated all over the world. It is most particularly vulnerable to water stress in leguminosae. Water stress caused by drought and other natural disasters has a serious effect on soybean production. In this work, a widely cultivated cultivar of soybean in Sichuan China was selected to study SA's effects on soybean seedlings under water stress. The transcript levels of *PTOX* and *AOX* under water stress and SA pre-treatment were determined to clarify the relationship between terminal oxidase and photosynthetic performance, and potential functions of terminal oxidase in SA's regulation on photosynthesis.

2. Materials and methods

2.1. Plant materials and growth conditions

Seeds of soybean(Glycine max L. evar Nandou 12) were surface sterilized with 3% H_2O_2 for 30 min, rinsed with distilled water, imbibed in the darkness for 12 h in room temperature, and then directly seeded in basins with organic nutritional soil and sand (1:1) mixed matrix. The plants were grown in a self-regulation culture room (greenhouse), under 100 μmol m^{-2} s^{-1} light intensity and at 14/10 day/night photoperiod. The temperature was maintained at 25 °C and the relative humidity was around 50%.

The 20-days-old seedlings were transferred to hydroponics medium(1/2 Hoagland's nutrient solution) (pH 6.8) for two days of adaptive cultivation. Roots were gently washed and planted in 1/2 Hoagland's nutrient solution containing 0 or 500 μM SA (Xilong Chemical Co., Ltd) (selected based on our preliminary experiment). The mediums were continuously aerated. After 10 h of initial treatment, plants of each treatment were transferred to 0 or 8% (W/V) polyethylene glycol (PEG 6000) (Xilong Chemical Co., Ltd) aqueous solution to achieve osmotic potential of 0 MPa and −1.03 MPa. At this time, the following treatments were imposed: control (without SA and PEG); SA(0.5 mM SA); PEG (8% PEG); PEG + SA (8%PEG + 0.5 mM SA). After 8 h of root osmotic stress, the fully expanded first euphylla were used for experiments.

2.2. Gas exchange

Net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (E), and intercellular CO_2 concentration (Ci) were recorded on the fully expanded first euphylla in an open system under ambient temperature and CO_2 partial pressure, using a portable photosynthesis system (LI-6400, Li-COR Inc.,USA) and an LED light source (6400-40 LCF) following the manufacturer's instructions. The light intensity inside the leaf chamber were kept constant at 200 μmol m^{-2} s^{-1}. The measurement was done once for each leaf and for nine leaves per treatment.

2.3. Chlorophyll fluorescence measurements

Chlorophyll fluorescence parameters were performed using a portable photosynthesis system(LI-6400, Li-COR Inc., USA) and an LED −based fluorescence source (6400-40 LCF). Following 30 min of dark adaption, the minimal fluorescence of dark-adapted state (Fo) was determined by a weak modulated light. A brief period saturating light (>7000 μmol m^{-2} s^{-1}) was used on dark-adapted state (Fm). Then, the leaf was illuminated with actinic light of 200 μmol m^{-2} s^{-1}. When the leaf reached steady-state photosynthesis, the steady-state fluorescence (Fs) was recorded, and a second brief period saturating light (>7000 μmol m^{-2} s^{-1}) was applied to determine the maximal fluorescence of light-adapted state (Fm'). The actinic light was turned off, the minimal fluorescence of light-adapted state (Fo') was determined by the illumination with a far-red light. The maximum quantum yield of PSIIphotochemistry (Fv/Fm), quantum efficiency of PSIIphotochemistry (ΦPSII), efficiency of excitation capture of open PSIIcenter (Fv'/Fm'), and photochemical quenching coefficient (qP)