Environmental and Experimental Botany 107 (2014) 46–54

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Systemic mitigation of salt stress by hydrogen peroxide and sodium nitroprusside in strawberry plants via transcriptional regulation of enzymatic and non-enzymatic antioxidants

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ARTICLE INFO

Article history:
Received 9 March 2014
Received in revised form 29 April 2014
Accepted 22 May 2014
Available online 27 May 2014

Keywords:
Ascorbate
Fragaria × *ananassa*
Glutathione
Nitrosative stress
Oxidative stress
Priming

ABSTRACT

Nitric oxide (NO) and hydrogen peroxide (H_2O_2) have a pivotal role in plant development and stress responses, thus rendering them as key molecules for priming approaches. In this study, a hydroponic experiment was employed in order to investigate the effects of NO donor, sodium nitroprusside (SNP; 100 μM), or H_2O_2 (10 mM) root pretreatment in major components of redox homeostasis and signaling of strawberry plants (*Fragaria* × *ananassa* cv. 'Camarosa') exposed immediately, or 7 d after root pretreatment, to salt stress (100 mM NaCl, 8 d). Plants stressed immediately after root pretreatment with either reactive species demonstrated increased chlorophyll fluorescence, photosynthetic pigment content, leaf relative water content as well as lower lipid peroxidation and electrolyte leakage levels in comparison with plants directly subjected to salt stress, suggesting a systemic mitigating effect of NO/H_2O_2 pretreatment to cellular damage derived from abiotic stress factors. In addition, primed plants managed to mitigate the oxidative and nitrosative secondary stress and redox homeostasis disturbances, since H_2O_2 and NO were quantified in lower levels, whereas ascorbate and glutathione redox states in leaves were sustained at higher rates, compared with NaCl treatment. Gene expression analysis revealed that priming effects of both H_2O_2 and NO root pretreatment correlated with increased transcript levels of enzymatic antioxidants (*cAPX*, *CAT*, *GR*, *MnSOD*, *MDHAR* and *DHAR*), as well as ascorbate (*GalUR*, *GLDH*, *GDH*, *MIOX*) and glutathione biosynthesis (*GCS*, *GS*) in leaves, in contrast with the general transcriptional suppression observed in plants stressed without pretreatment, or 7 d after root pretreatment. Overall, pretreated plants displayed redox regulated defense responses leading to systemic tolerance to subsequent salt stress exposure.

1. Introduction

During normal cell metabolism, reactive oxygen species (ROS) are constantly produced by partial reduction of molecular oxygen (De Gara et al., 2010). It has been estimated that 1–2% of O_2 consumption leads to the formation of ROS in organelles with high metabolic activity or intense electron transport rate, such as chloroplasts, mitochondria and peroxisomes (Gill and Tuteja, 2010). In turn, ROS are overproduced during exposure to abiotic stress factors, ultimately causing lipid, protein and DNA damage (Apel and Hirt, 2004). In parallel to the generation of ROS, reactive nitrogen species (RNS) are now well recognized plant by-products under stress conditions (Valderrama et al., 2007). As a result, plants have developed a well-organized and sophisticated antioxidant arsenal, in order to control both ROS and RNS production and accumulation (Corpas and Barroso, 2013).

Accumulating evidence during the last decade has shown that, besides exacerbating cellular damage, ROS and RNS accumulation might represent a necessary signal for the control and regulation of a range of biological processes, such as growth, cell cycle, responses to biotic and abiotic stress factors and programmed cell death (PCD) (Besson-Bard et al., 2008; Molassiotis and Fotopoulos, 2011). Within this concept of cellular redox signaling regulation, low

Abbreviations: cAPX, cytosolic ascorbate peroxidase; CAT, catalase; DHAR, dehydroascorbate reductase; GalUR, D-galacturonate reductase; GCS, gamma-glutamylcysteine synthase; GDH, L-galactose dehydrogenase; GLDH, L-galactono-1,4-lactone dehydrogenase; GR, glutathione reductase; GS, glutathione synthetase; MDHAR, monodehydroascorbate reductase; MIOX, myo-inositol oxygenase; MnSOD, manganese superoxide dismutase; NR, nitrate reductase; RNS, reactive nitrogen species; ROS, reactive oxygen species.

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http://dx.doi.org/10.1016/j.envexpbot.2014.05.009
0098-8472/

molecular weight antioxidants such as ascorbate and glutathione may act not only as ROS scavengers, but also as signaling buffers for the regulation of gene expression (Fotopoulos et al., 2010). Cellular redox homeostasis is comprised of a series of interconnecting organellar redox buffers, such as mitochondria (Giraud et al., 2008), chloroplasts (Tseng et al., 2007) and peroxisomes (Kamada-Nobusada et al., 2008), where elaborate feed-forward/feedback loops between oxidants and antioxidants seem to play a crucial role in redox homeostasis and signaling (Gadjev et al., 2006). The observed cross-talk between ROS and RNS has resulted in the speculation of a connection between oxidative and nitrosative signaling networks in plants in response to environmental stimuli (Molassiotis and Fotopoulos, 2011; Corpas and Barroso, 2013).

It is widely accepted that H_2O_2 is one of the most prominent signaling molecules due to its high relative stability and mobility (Moller et al., 2007). As a result, H_2O_2 can migrate from sites of synthesis to adjacent compartments and even neighboring cells (Bienert et al., 2006), orchestrating multiple plant physiological processes (Neill et al., 2002). Recently, accumulating evidence also supports the role of NO as an important signaling molecule with diverse physiological functions in plants (Mur et al., 2013; Filippou et al., 2014); NO actively participates in seed germination and dormancy, root growth, stomatal closure, PCD among others (Zago et al., 2006; Besson-Bard et al., 2008).

The role of both H_2O_2 and NO signaling in plant responses to abiotic stress has been well illustrated. Hydrogen peroxide root pretreatment was found to induce salt tolerance in maize and wheat seedlings (de Azevedo Neto et al., 2005; Wahid et al., 2007), drought tolerance in soybean plants (Ishibashi et al., 2011) and heat stress tolerance in cucumber leaves (Gao et al., 2010), mainly through the mitigation of lipid peroxidation and the regulation of the activity of antioxidant enzymes. The mitigating effects of nitric oxide on drought stress in tomato and wheat (García-Mata and Lamattina, 2001; Nasibi and Kalantari, 2009) and salt stress in maize seedlings (Zhang et al., 2006) have also been reported. Furthermore, Uchida et al. (2002) demonstrated the ameliorative effects of both H_2O_2 and NO on salt and heat stress tolerance in rice, while Tanou et al. (2009) showed that H_2O_2 and NO-induced systemic antioxidant activity confers tolerance to salt-stressed citrus plants. In addition, the employment of 'omics' approaches supported the previously reported cross-talk between H_2O_2 and NO signaling upon abiotic stress or stress-free conditions (Rodriguez-Serrano et al., 2009; Tanou et al., 2012).

To completely unravel the oxidative and nitrosative signaling and redox regulation of transcript expression, knowledge of the activated transduction pathways in response to H_2O_2 and NO treatments during environmental stimuli is essential. The scope of the present work was to shed light on ROS and RNS-mediated redox regulation and homeostasis of primed and subsequently salt-stressed strawberry plants.

2. Materials and methods

2.1. Plant material and stress treatments

Seventy-two 6-month-old strawberry (*Fragaria* × *ananassa* cv. 'Camarosa') plants of uniform size were transferred in a hydroponic system into a growing room, until the commencement of the experimental treatments, as previously described (Christou et al., 2013). Initially, roots of 18 plants were incubated in deionized water containing either 100 μM SNP for 48 h (changed every 12 h) or 10 mM H_2O_2 for 8 h (9 plants for each treatment, completed at the same time), and then transferred to ½-strengthHoagland nutrient solution for 7 d, serving as an acclimation period. Five days after the initiation of the incubation, 36 more plants were incubated with SNP or H_2O_2 (18 for each treatment), as previously described, with no acclimation period. As a result, all strawberry plants were transferred simultaneously (0 d time-point) to nutrient solution containing or not, 100 mM NaCl (EC ~11.6 mS cm^{-1}), for 8 d. In addition, 9 plants were grown in Hoagland solution acting as controls and 9 plants were grown in NaCl acting as positive controls. Overall, strawberry plants were subjected to 8 treatments, schematically described in Supplementary Fig. 1. The experimental set up was largely based on the recent studies of Tanou et al. (2009, 2012). Each treatment was independently run in triplicate, and each replicate consisted of 3 individual plants. Fully expanded leaves were sampled immediately after the imposition of salt stress (0 d) and after 8 d of stress exposure. Leaves were flash-frozen in liquid nitrogen and stored at −80 °C, until needed.

2.2. Physiological parameters and photosynthetic pigment content

Photosynthetic leaf pigments (Chl a, Chl b, carotenoids) extraction was carried out using 1 cm-diameter leaf disks as described by Richardson et al. (2002), while carotenoids and chlorophyll concentrations were estimated using the equations proposed by Sims and Gamon (2002). Chlorophyll fluorescence parameters (Fv/Fm) of leaves representing the maximum photochemical efficiency of photosystem II (PSII) were determined using a portable chlorophyll fluorometer (OptiSci OS-30p Chlorophyll Fluorometer, Opti-Sciences Inc., USA). Leaves were incubated in dark for 1 h prior to fluorescence measurements.

2.3. Leaf water status

Fully expanded leaves were used for the estimation of leaf water potential (MPa), using the WP4-T Dewpoint Potential Meter (Decagon Devices Inc., USA). Measurements were performed at 25 °C. Leaf relative water content (LRWC) was determined following the method of Yamasaki and Dillemburg (1999).

2.4. Cellular damage indicators

Leaf membrane damage was evaluated by electrolyte leakage (EL), as described by Dionisio-Sese and Tobita (1998). The level of lipid peroxidation in leaf tissue was determined in terms of malondialdehyde (MDA) content, a major thiobarbituric acid reactive specie (TBARS), according to Heath and Packer (1968).

2.5. Hydrogen peroxide and nitric oxide quantification

Leaf H_2O_2 content was determined spectrophotometrically based on the oxidation of iodide to iodine, after the reaction of H_2O_2 with potassium iodide (KI), following the method proposed by Loreto and Velikova (2001). Nitric oxide content was indirectly assayed by measuring nitrite (NO_2^-), a stable and non-volatile breakdown product of NO reduction, via the Griess reaction, as described by Christou et al. (2013).

2.6. ASC and GSH content/redox state

Reduced ascorbate (ASC) and oxidized ascorbate (dehydroascorbate; DHA) were measured according to Foyer et al. (1983). Dehydroascorbate content was estimated as the difference of ASC and total ascorbate, while the redox state (%) of ascorbate was expressed as the percentage of ASC to total ascorbate [(ASC/(ASC + DHA)) × 100].

The levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) were measured according to Griffith (1980). The amount of reduced glutathione was estimated as the difference between total glutathione and GSSG. The glutathione redox state