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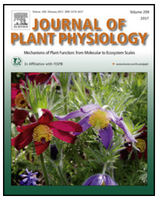

H_2O_2 seed priming improves tolerance to salinity; drought and their combined effect more than mannitol in *Cakile maritima* when compared to *Eutrema salsugineum*

Hasna Ellouzi*, Souhir Sghayar, Chedly Abdelly

Laboratoire des Plantes Extrêmophiles, Centre de Biotechnologie de Borj Cedria (CBBC), BP 901, Hammam Lif 2050, Tunis, Tunisie

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ABSTRACT

The effect of H_2O_2 and mannitol seed priming was investigated on plant growth, oxidative stress biomarkers and activities of antioxidant enzymes in leaves of *Cakile maritima* and *Eutrema salsugineum*, when exposed to drought and salt stress, either separately applied or combined. Under unprimed conditions, drought severely restricted growth (40% as compared to the control) and redox balance of *C. maritima* seedlings, whereas *E. salsugineum* showed these drastic effects under individual salinity (33% as compared to the control). Combined salinity and drought maintained and even stimulated the antioxidant defense of both plants from unprimed seeds. Both priming agents (mannitol and H_2O_2) significantly ameliorated growth and antioxidant defense of both species grown under salinity, drought and their combined effect. However, H_2O_2 priming appeared to be more beneficial in *C. maritima* seedlings. Indeed, oxidative injuries were significantly reduced, together with significantly higher concentrations of ascorbic acid (36%), glutathione (2-fold) and proline production (2-fold), leading to a greater redox balance that was closely associated with enhanced antioxidant enzyme activities, specifically under salt stress. Overall, our results indicate that it is very likely that H_2O_2 priming, due to its signal role, improves *C. maritima* tolerance to both osmotic stresses and enables the plant to memorize and to decode early signals that are rapidly activated when plants are later exposed to stress.

1. Introduction

Due to their sessile life style and because they are constantly confronted with biotic and abiotic stressors, plants have developed sophisticated strategies to decode external signals allowing them to respond appropriately to these environmental cues. Thus, responses to specific stresses vary from species to species.

In nature, plants are normally subjected to the action of multiple stressors that often occur simultaneously. Plant responses to abiotic changes have been intensely investigated with much emphasis on tolerance mechanisms related to individual stresses, but how plants respond to a combination of these is not well documented. Recent investigations suggest that, under combined stress factors, plants respond in a complex manner and exhibit novel metabolic behavior that differs from that for single stressors and is not additive (Miller et al., 2010; Pasch and Sonnewald, 2015). Many studies have suggested that some abiotic stresses could actually improve the tolerance of plants to another abiotic or biotic stress when the two stresses are combined (Foyer and Noctor, 2016). In this context, Rivero et al. (2014) reported that the submission of tomato plants to a combination of salinity and high temperature leads to a better maintenance of water status and photosynthesis, which is strongly related to the important accumulation of osmolytes as well as to the high concentration of K^+, as compared with single salt stress. Moreover, the response of *Arabidopsis thaliana* subjected to drought and heat stress was different from that of plants exposed separately to drought or heat, showing new profiles of gene expression that are not found during individual stresses (Rizhsky et al., 2004). In the same context, the interaction between drought and heat resulted in a novel protein identification that is specifically detected under combined stresses and conferred to barely plants particular tolerance to drought (Ashoub et al., 2015).

Abbreviations: ABA, abscisic acid; AsA, ascorbic acid; BABA, β-amino butyric acid; CAT, catalase; DAB, 3,3′-diaminobenzidine; Fv/Fm, maximum quantum efficiency of photosystem II; GA, gibberellin; GSH, glutathione; GPX, guaiacol peroxidase; H_2O_2, hydrogen peroxide; -OH, hydroxyl radicals; MDA, malondialdehyde; NBT, nitrobluetetrazolium; NO, nitric oxide; PEG, polyethylene glycol; PS, primed seeds; ROS, reactive oxygen species; SOD, superoxide dismutase; $O2^{\cdot -}$, superoxide radicals; UPS, unprimed seeds.

* Corresponding author.
E-mail address: ellouzihasnaa@gmail.com (H. Ellouzi).

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Drought and salinity are the most important constraints affecting plant growth and are good examples of stresses that may occur simultaneously in the field (Forieri et al., 2016). These two constraints are strongly related in many physiological aspects and sometimes overlap in various tolerance mechanisms. However, although these two stresses are physiologically associated, other mechanisms of plant metabolism may vary if the plant is exposed to individual salt stress or to both stresses simultaneously (Sucre and Suarez, 2011). It was previously thought that the deleterious impact of salinity could be amplified when combined with drought. Slama et al. (2008) demonstrated that the interaction between water deficit and salinity may contribute to growth inhibition and nutrient deficiency. Similar to these findings, Sun et al. (2015) reported that, when applied individually, drought and salinity did not affect photosynthesis in maize plants, but their interaction interferes with total carbon reduction and may inhibit the water availability in wheat. According to Rizhsky et al. (2004), drought and salinity combine to show detrimental effects on plant growth and productivity in many species such as barely and sorghum.

In contrast to the above examples, the addition of salt to plants exposed to drought stress further improves the ability of these plants to support water deficit caused by single drought (Álvarez and Sánchez-Blanco, 2015). One of the most important phenomena studied under drought and salinity is osmolyte accumulation, such as the accumulation of proline and sugars as a common response to these stress factors. For example, some of these osmoprotectants are induced under combined stresses, while others are suppressed in the case of single stresses (Ahmad et al., 2014). Ivanov et al. (2015) reported that the combination of salinity and drought enhances plant hydration and the carbon balance. Moreover, Choudhury et al. (2016) suggested that salinity may have a protective effect from oxidative damage caused by drought. From the standpoint of reactive oxygen species (ROS) implication for plant acclimation under combined stresses, many studies have shown that ROS production, levels of several antioxidants and activities of ROS-scavenging enzymes exhibit a unique profile when salinity and drought are coupled that is different and higher than that found under each stress when applied individually (Suzuki et al., 2016; Zandalinas et al., 2016). Also, phytohormones such as ABA seem to be excellent regulators that mediate plant acclimation to combined stresses (Zandalinas et al., 2016). For now, stress combination appears to be a key approach for improving plant response to single stresses.

Priming is considered an important approach for enhancing plant defense against both biotic and abiotic stresses (Hossain et al., 2015). It is defined as the pre-exposure of seeds or young seedlings to chemical agents or to abiotic stressors (such as salinity, drought, cold, etc.) making them more resistant to subsequent stresses and more able to rapidly detect second signals (Borges et al., 2014). Many efficient priming compounds are used to broaden the application of this technique, especially in agricultural areas. For example, it has been reported that pretreatment of seeds with GA (hormonal priming) improved germination and enhanced tolerance in trifolium plants grown in heavy metal contaminated soil (Galhaut et al., 2014). Also, Ouhibi et al. (2014) demonstrated that the exposure of lettuce seeds to UV radiation amplifies the resistance of this species to subsequent salt stress. It has been found that osmopriming (using mannitol or PEG) is a beneficial pretreatment for cotton seeds and confers to this species subsequent oxidative stress tolerance (Santhy et al., 2014).

Elevated generation of reactive oxygen species (ROS) such as superoxide radicals (O2$^{\cdot-}$), hydroxyl radicals ($^{-}$OH) and hydrogen peroxide (H_2O_2), generally occur under several environmental stresses. At high concentrations, these ROS interfere with other cellular structures, resulting in oxidative damages like lipid peroxidation, protein, DNA damage etc. Although being toxic, ROS may serve as crucial components in stress signaling pathways and recently have been considered key molecules in priming as well as in seed germination (Hossain et al., 2015). Interestingly H_2O_2 is widely noted as the most important signal molecule, due to its high mobility. Many investigations support the idea that H_2O_2 is an effective seed priming agent and possesses an ameliorative effect on the plant response to various abiotic stresses (Christou et al., 2014). Wahid et al. (2007) reported that pretreatment of seeds with H_2O_2 alleviated oxidative damage and stimulated the expression of stress proteins in wheat plants when grown under salinity. In line with these findings, H_2O_2 priming has also been associated with increased glutathione levels and enhanced activities of antioxidant enzymes in both seeds and seedlings of *mung bean* plants when subsequently submitted to severe salt stress (Murphy et al., 2002). These findings were also supported by Ahmad et al. (2011), who showed improved cold tolerance in maize seedling as result of seed primed with H_2O_2. Pretreatment of plants with signal molecules, has been also largely explored in many species. In this context, Uchida et al. (2002) reported that nitric oxide (NO) and H_2O_2 are good priming factors to induce a cross-resistance against both salt and heat stresses in rice plants. Several studies demonstrated that H_2O_2 is greatly implicated in the phenomena of cross-tolerance. Furthermore, exogenous application of H_2O_2 activated many transcription factors and signaling proteins resulting in a tolerance to a major of abiotic stresses (Hossain et al., 2015). Based on these findings, Pastor et al. (2013) stated that “primed plants do not forget” and that priming is strongly related to an adaptive plant immune behavior. This is an indicator, on one hand, that both priming strategies were considered as an initial stress exposure that may leave seeds or plants with a stress imprint or memory, and on the other hand that the post-priming adaptation can be explained by a cross-tolerance which is achieved by priming (Chen and Arora, 2013).

Considering that priming may be a helpful approach to improve the plant response to abiotic stresses, here we investigated the effect of priming with mannitol or H_2O_2 on *Eutrema salsugineum* (*Thellungiella salsuginea* a plant model) and *C. maritima* (a local halophyte) mainly based on the plant growth and oxidative status when exposed to single drought and salinity or to a combination of both stresses.

2. Material and methods

2.1. Seed pretreatments

Seeds of *Cakile maritima* (L.) Karst. (local halophyte), and *Eutrema salsugienum* (Pall.) O.E. Schulz (Shandong ecotype) were soaked separately for 12 h either in H_2O_2 solution (120 μM) or mannitol solution (2%). Then, seeds were washed three times with distilled water and dried back at room temperature for 48 h to the original seed moisture content.

2.2. Plant growth in a greenhouse: emergence in inert sand

Unprimed and primed seeds (UPS and PS, respectively) were germinated in pots filled with inert sand in a greenhouse and irrigated daily with distilled water during 1 week until germination; photoperiod: 8–16 h (day: night), temperature: 20–25 °C and relative humidity: 65–75%. After germination, seedlings were watered every 2 days with 50% Hoagland solution during 3 weeks. The experimental design is illustrated in Fig. 1. Seedlings (4-week-old) were randomly divided into four groups. Each group contained 32 plants and 4 replicates per treatment. The first group was corresponded to the control plants (irrigated every 2 days with nutrient solution), the second one to salt-treated plants (at 300 mM NaCl), the third one to drought stressed plants (without irrigation) and in