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Treating seeds in menadione sodium bisulphite primes salt tolerance in Arabidopsis by inducing an earlier plant adaptation

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ABSTRACT

For the majority of crops, salinity is one of the most important abiotic stresses, since about 20% of irrigated agricultural land is adversely affected by it. Menadione sodium bisulphite (MSB), a water-soluble vitamin K_3 o menadione derivative, has been previously reported as a plant defence activator against several pathogens in a number of species. We have further explored the MSB effects on salt tolerance. In this study, *Arabidopsis thaliana* wild ecotype Col-0 plants were exposed to prolonged salt (50 mM) stress. Salt treatment resulted in severe growth inhibition. This detrimental effect was lower in terms of relative growth rate (RGR) in plants from seeds soaked in 20 mM of MSB. In these plants, the drop in RGR was nearly 30% lower than untreated plants after 7 days in salt. Furthermore, we found that the salt stress imposed was not enough to disturb photosystem II or induce the expression of several detoxification genes. These functional impairments are characteristic of ionic injuries due to high levels of reactive oxygen species (ROS). At the end of the second week of the experiment, salt-treated plants recover RGR levels close to those of the control. Under our experimental conditions plants seem to be challenged by an osmotic stress with a minimum ionic imbalance. Those from MSB-treated seeds were primed to induce an earlier proline accumulation. Although no significant expression of ROS detoxification genes was found, several transcription factors involved in ROS signalling were detected after salt addition. In this context, MSB treatment was able to prime these transcription factors, resulting in an early adaptation of plants in response to salt stress.

1. Introduction

Throughout evolution, plants have developed numerous defence mechanisms to endure environmental abiotic stresses such as drought, water excess, salinity, heat, cold and wounding, and to combat challenges arising from biotic stress (Bohnert et al., 2006). Abiotic stresses are considered to be the main cause of decreased agricultural yield (Ashraf et al., 2008). Drought and soil salinity are two major environmental stresses that limit crop production, affecting nearly 37% of the total agricultural land in the world (Munns and Tester, 2008; Reynolds and Tuberosa, 2008). Plants exposed to a mild chronic stress are able to induce specific morphogenic stress responses. These responses are characterized by a blockage of cell division in the main meristem tissues, an inhibition of cell elongation, and a redirected development of lateral organs (Potters et al., 2007). These stress induced morphogenic responses are part of a general adaptation strategy, whereby plants redirect their growth when exposed to stress. These stress responses are also characterized by the presence of antioxidants that prevent damage caused by reactive oxygen species (ROS). For instance, the accumulation of foliar anthocyanin acts as a modulator of stress signals. Another strategy is the accumulation of compatible low molecular weight osmolytes, such as the amino acid L-proline (Steyn et al., 2002; Szabados and Savouré, 2009).

Progress in plant genetic transformation and the availability of potentially useful genes from different sources has encouraged the use of transgenic approaches to minimize yield loss in many crops (Hirayama and Shinozaki, 2010). It is well known that salt

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tolerance is a complex trait involving the function of many genes (Munns and Tester, 2008), however the transgenic approaches pursue the development of salinity tolerant cultivars based on specific traits controlled by only one gene, e.g. a transcription factor or an important ion channel (Munns, 2011). Most of these strategies are highly criticized due to poor evaluation methodology on carrying out such studies under field conditions (Ashraf et al., 2008). In addition, although constitutive expression of a specific transgene can increase tolerance to stress conditions, this usually leads to a decrease in yield (Heil and Baldwin, 2002). It would be highly desirable that defence genes were expressed only under stress conditions.

Plants are able to develop numerous defence strategies to endure stress. Amongst them higher plants are capable of demonstrating some stress 'memory', or stress imprinting (Conrath, 2011; Pastor et al., 2013). Stress imprinting is usually defined as genetic or biochemical modifications induced by a first stress exposure that leads to enhanced resistance to a later stress. Preliminary stress exposure is indeed known to enhance the stress tolerance of the plant through induction of adaptation responses (Conrath, 2011; Pastor et al., 2013), while tolerance can be linked to an array of morphological, physiological and biochemical responses that decrease stress damage or facilitate repair in damaged systems (Potters et al., 2007). By using this "memory" or imprinting mechanism it is possible to induce responses to a range of biotic and abiotic stresses. This is known as priming and is observed in plants and also in animals (Pham et al., 2007; Beckers and Conrath, 2007; Jung et al., 2009). Typically, primed plants display faster and/or stronger activation of defence mechanism, which are induced in response to abiotic stresses or following attack by microbial pathogens (Beckers and Conrath, 2007; Pham et al., 2007; Conrath, 2011 Pastor et al., 2013). Consequently, priming could provide low-cost protection in relatively high-stress environmental conditions (Van-Hulten et al., 2006).

Several molecules have been tested as priming agents which can increase plant resistance to different and unrelated stresses (Maffei et al., 2012; Filippou et al., 2013). Among them, menadione sodium bisulphite (MSB), a water-soluble vitamin K_3 o menadione derivative, is capable of inducing priming in Arabidopsis by foliar spray 24 h prior to inoculation with the virulent strain of *Pseudomonas syringae* pv. tomato DC3000 or against downy mildew in pre-treated pearl millet seeds (Borges et al., 2009; Pushpalatha et al., 2007), respectively. Additionally, our research group has patented practical applications of MSB in agriculture (Borges-Pérez and Fernández-Falcón, 1995, 1996), including one for inducing tolerance to salt stress (Borges et al., 2010) and several other MSB-based formulations have been commercially developed as plant defence elicitors. Here we present MSB as a molecule which protects plants against detrimental salt stress from the seed stage onward, through priming of existing defence mechanisms, avoiding undesirable resource allocation, energy costs and manipulation of the genome (Heil, 2002; Van-Hulten et al., 2006). The aim of this work was to understand how the priming agent MSB potentiates the Arabidopsis response to salinity stress (50 mM NaCl).

2. Materials and methods

2.1. In vitro conditions and MSB concentration

Arabidopsis thaliana ecotype Columbia-0 was used in the experiments. The MSB treatments were performed on seeds by soaking in the MSB solution during seed stratification for 2 days at 4 °C at the desired concentration (2, 20 and 40 mM of MSB). Control seeds were stratified with distilled water under identical conditions and both experimental treatments were sterilized according to Martinez-Zapater and Salinas (1998). MSB effects on germination ratio were studied in vitro on MS medium supplemented with 0.7% agar in Petri dishes. Plates were placed in a growth chamber at 22 °C, 16 h light (100–110 μmol m^{-2} s^{-1}) and 60–70% relative humidity. Germination rates were assessed after 1 week of growth, while fresh weights of the above-ground plants were measured after 2 weeks. Each MSB treatment was applied to 50 seeds per plate and repeated in four independent experiments. Statistical analyses were performed by one-way ANOVA and the significance of differences between experimental treatments was calculated using a Tamhane post hoc test (IBM SPSS 20).

2.1.1. Hydroponic conditions

Salinity studies were performed under hydroponic conditions. The containers (Araponics®, Liège, Belgium) were 1.9 L black plastic boxes accommodating 18 seed-holders with one plants each (Supplementary Fig. 1). A mixture of two granulometry sizes of river sand was used as a physical substrate. Seeds, each sown in seed-holders, were germinated in nursery trays filled with moistened river sand for 1 week in a growth chamber at 22 °C, 16 h light (100–110 μmol m^{-2} s^{-1}) and 100% relative humidity. Seed-holders with seedlings were transferred to the hydroponic system in the same growth conditions but 60–70% relative humidity. Following the previously optimized conditions, seedlings remained without aeration during the first week, and then the nutrient solution was renewed every 7 days and gentle aeration applied. The solution used was: KNO_3 1.25 mM, KH_2PO_4 0.5 mM, $MgSO_4 \times 7H_2O$ 0.75 mM, $Ca(NO_3)_2 \times 4H_2O$ 0.75 mM, H_3BO_3 50 μM, $MnSO_4 \times H_2O$ 10 μM, $ZnSO_4 \times 7H_2O$ 2 μM, $CuSO_4 \times 5H_2O$ 1.5 μM, $(NH4)_6Mo_7O_{24} \times 4H_2O$ 0.075 μM, Sequestrene® 44.8 μM (Syngenta, USA). Salinity treatments were applied 2 weeks after transferring seed-holders, by adding 50 mM NaCl to the nutrient solution.

Supplementary Fig. 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.envexpbot.2014.07.017.

2.2. Growth, chlorophylls, anthocyanin and proline determinations

All these quantifications were restricted to the aerial part of the plants. After fresh weight (FW) measurement, harvested plants were dried in a hot air oven at 70 °C for 72 h and dry weight (DW) determined. The relative growth rate (RGR) was calculated by using the following formula: $RGR = [\text{Ln}(DW_{D_2}) - \text{Ln}(DW_{D_1})]/D_2 - D_1$, where D_1 and D_2 are the initial and the final time-points of the analysis expressed in days.

Chlorophylls were analyzed in fully expanded leaves (50 mg FW), which were immediately grounded with liquid nitrogen and extracted with ice-cold acetone/water 85% (v/v) and 100% acetone. The extract was then centrifuged at 15,000 × *g* for 5 min and kept at −20 °C until analysis. Chlorophyll pigments were quantified according to Porra (2002) and anthocyanin following Mita et al. (1997). Approximately 100 mg fresh tissue was collected in Eppendorf tubes, flash-frozen in liquid N_2 and ground into powder. One ml of 1% (v/v) hydrochloric acid in methanol was added to each sample and the tubes were vigorously vortexed. After 1 day of incubation at 4 °C, the mixture was centrifuged at 15,000 × *g* for 15 min and absorbance of the supernatant was measured at 530 nm and 657 nm. Proline content was determined as Bates et al. (1973) with minor modifications as follows. Briefly, proline was extracted from approximately 100 mg fresh tissue, which was immediately ground in liquid nitrogen and extracted with 4 ml of 3% sulphosalicylic acid. Two μl of extract was reacted with 2 ml of acidic ninhydrin for 60 min at 100 °C, stopping the reaction in an ice-bath. The reaction mixture was extracted with 4 ml of toluene, and vortexed.