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Moderate salinity reduced phenanthrene-induced stress in the halophyte plant model *Thellungiella salsuginea* compared to its glycophyte relative *Arabidopsis thaliana*: Cross talk and metabolite profiling

Moez Shiri [a, b], Mokded Rabhi [b, d], Chedly Abdelly [b], Alain Bouchereau [c], Abdelhak El Amrani [a, *]

[a] University of Rennes 1, CNRS/OSUR-UMR 6553, Ecosystemes-Biodiversity-Evolution, Campus de Beaulieu, Bâtiment 14A, 35042 Rennes Cedex, France
[b] Laboratory of Extremophile Plants, Centre of Biotechnology of Borj Cedria, P.O. Box 901, 2050 Hammam-Lif, Tunisia
[c] INRA Agrocampus Ouest, University de Rennes 1, UMR 1349 Institut of Genetic, Environnement and Protection of Plants, F-35653 Le Rheu Cedex, France
[d] University of Hafr Al Batin, College of Science and Arts in Nairiyah, 31981, Nairiyah, Saudi Arabia

HIGHLIGHTS

- Moderate salinity enhances halophyte tolerance to phenanthrene induced stress.
- Phenanthrene tolerance was related to adapted metabolism in halophyte plant.
- These findings will improve the “green” remediation of organic pollutants.

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ABSTRACT

It was shown that halophytes experience higher cross-tolerance to stresses than glycophytes, which was often associated with their more powerful antioxidant systems. Moreover, salinity was reported to enhance halophyte tolerance to several stresses. The aim of the present work was to investigate whether a moderate salinity enhances phenanthrene stress tolerance in the halophyte *Thellungiella salsuginea*. The model plant *Arabidopsis thaliana*, considered as its glycophyte relative, was used as reference. Our study was based on morpho-physiological, antioxidant, and metabolomic parameters. Results showed that *T. salsuginea* was more tolerant to phenanthrene stress as compared to *A. thaliana*. An improvement of phenanthrene-induced responses was recorded in the two plants in the presence of 25 mM NaCl, but the effect was significantly more obvious in the halophyte. This observation was particularly related to the higher antioxidant activities and the induction of more adapted metabolism in the halophyte. Gas Chromatography coupled with Mass Spectrometry (GC-MS) was used to quantify alcohols, ammonium, sugars, and organic acids. It showed the accumulation of several metabolites, many of them are known to be involved in signaling and abiotic stress tolerance. Moderate salinity and phenanthrene cross-tolerance involved in these two stresses was discussed.

1. Introduction

Halophytes (Jithesh et al., 2006; Flowers and Colmer, 2008) are defined as plants able to accomplish their life cycle in the presence of 200 mM NaCl or more (Flowers and Colmer, 2008). Their higher antioxidant capacity in comparison with that of glycophytes has been suggested to confer them a higher ability to tolerate extreme environmental conditions in natural ecosystems (Flowers and

* Corresponding author. University of Rennes 1, CNRS/OSUR-UMR 6553, Ecosystemes-Biodiversity-Evolution, Campus de Beaulieu, Bâtiment 14A, 35042 Rennes Cedex, France.
E-mail address: abdelhak.elamrani@univ-rennes1.fr (A. El Amrani).

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Abbreviations

E	Extinction coefficient
EDTA	Ethylenediaminetetraacetic acid
F_0	Minimal fluorescence of dark-adapted leaves
F_m	Maximal fluorescence of dark-adapted leaves
F_v/F_m	Maximal quantum efficiency of PSII photochemistry
F_v	Variable fluorescence ($F_v = F_m - F_0$)
FW	Fresh Weight
GC-MS	Gas Chromatography coupled with Mass Spectrometry
GPX	Guaiacol peroxidase
GR	Glutathione reductase
GSSG	Glutathione disulfide
HCA	Hierarchical Cluster Analysis
MDA	Malondialdehyde
MS medium	Murashige and Skoog medium
MSTFA	*N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NBT	Nitro Blue Tetrazolium
PAHs	Polycyclic Aromatic Hydrocarbons
PAR	Photosynthetically Active Radiations
PCA	Principal Component Analysis
PVPP	Polyvinylpolypyrrolidone
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
UPLC	Ultra Performance Liquid Chromatography
PC1	The first principal component
PC2	The second principal component
P5CS	pyrroline-5-carboxylate synthetase
PSII	Photosystem II

Colmer, 2008). However, most experiments performed to understand the plasticity of plant behavior to abiotic constraints often investigate responses to individual stresses. But, in their natural habitat, they face simultaneously a variety of combined stresses (Ben Hamed et al., 2013; Shiri et al., 2015). In this context, halophytes were shown to present higher cross-tolerance than glycophytes, which can be attributed to their higher antioxidant capacity (Ben Hamed et al., 2013; Shiri et al., 2015). Recent studies proved that plant response to combined stresses is specific and cannot be directly deduced from the sum of its responses to the individual effects of each stress (Pnueli et al., 2002; Rizhsky et al., 2004; Bansal et al., 2013). Indeed, the interaction between stresses can induce additive (the sum of the individual effects), antagonistic (lower than additive) (Bansal et al., 2013), or synergistic (higher than additive) effects. The fact that responses to a given stress ameliorate tolerance to another is referred as cross-tolerance (Genoud and Métraux, 1999).

Interestingly, salinity has been shown to increase tolerance to other stresses in halophytes (See the review of (Ben Hamed et al., 2013)). For instance, Talbi-Zribi et al. (2012) showed that in *Hordeum maritimum*, moderate salinity alleviated phosphorus deficiency effects. Additionally, it was also shown that salinity can enhance tolerance to drought (Slama et al., 2007) and to heavy metals (Ghnaya et al., 2005; Zaier et al., 2010) in *Sesuvium portulacastrum*. This halophyte was also shown to increase Cd extraction effectiveness under saline conditions (Ghnaya et al., 2007).

Besides, a multitude of environmental stresses similarly induce an excessive production of ROS, driving an oxidative stress and ultimately cell death (Sharma et al., 2012). Hence, plant tolerance to oxidative stress can be highly correlated with cross-tolerance to several stresses (Iseki et al., 2013). In addition, some signaling compounds such as calcium, salicylic acid, and ROS are considered as key regulators of cross-tolerance *via* cross-talk between signaling pathways.

The halophyte plant model *Thellungiella salsuginea* (Brassicaceae) showed a better response to oxidative stress in comparison with its relative *Arabidopsis thaliana* (glycophyte) through a higher level of antioxidant molecules and the induction of several transcripts of antioxidant enzyme encoding genes under non-stressing conditions (Amtmann, 2009). In a previous work (Shiri et al., 2014), we showed through physiological, anatomical, and antioxidant responses that *T. salsuginea* was more tolerant to phenanthrene (a model molecule of Polycyclic Aromatic Hydrocarbons (PAHs)) than *A. thaliana*. In this work, we used metabolomic approach and antioxidant responses to investigate whether a moderate salt dose confers significant tolerance to the halophyte in comparison with the glycophyte.

2. Materials and methods

2.1. Plant material and growth conditions

Germination of *A. thaliana* (Columbia-0 or Col-0) and *T. salsuginea* (Shandong wild-type) seeds was performed in square Petri dishes under axenic conditions, after surface sterilization for 10 min in bayrochlore/ethanol mixture (1/1, v/v), rinsing in absolute ethanol then drying overnight. To enhance germination rate and homogenate young seedling growth, *A. thaliana* and *T. salsuginea* seeds were stratified at 4 °C for 2 and 10 days, respectively. The used growth medium was composed of 2% sucrose, 0.8% (w/v) agar in 1/2 × Murashige and Skoog (MS) basal salt mix (M5519, Sigma-Aldrich) adjusted to pH 5.7. Seedlings were grown in a growth chamber under 22°C/17 °C day/night temperature regime, a 16-h photoperiod, and a light intensity of 85 μmol m^{-2} s^{-1} PAR (Photosynthetically Active Radiations). Plantlets were then treated for 3 weeks using a fresh MS/2 agar medium contaminated or not with 25 μM phenathrene (dissolved in ethanol) and added or not with 25 mM NaCl. After three weeks of treatment, plants were harvested and weighed for growth measurements.

2.2. Chlorophyll fluorescence measurements

Just before harvest, chlorophyll fluorescence measurement was performed by a portable modulated fluorimeter (Mini-PAM, Heinz Walz, Germany). For dark test, plants were dark-adapted for 30 min then the minimal (F_0) and the maximal (F_m) fluorescence values were measured at <0.05 and 10,000 μmol m^{-2} s^{-1} for 1.8 μs, respectively. The maximal quantum efficiency of PSII photochemistry (F_v/F_m with $F_v = F_m - F_0$) was estimated using F_m and F_0 measurement couples.

2.3. Determination of MDA concentration

Malondialdehyde (MDA) concentration was measured following the thiobarbituric acid colorimetric method as described by Hodges et al. (Hodges et al., 1999).

2.4. Enzymatic assays

Harvested leaf samples were lyophilized for enzymatic assays.