Plant Physiology and Biochemistry 115 (2017) 380–389

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Effect of short-term Zn/Pb or long-term multi-metal stress on physiological and morphological parameters of metallicolous and nonmetallicolous *Echium vulgare* L. populations

Sławomir Dresler [a, *], Magdalena Wójciak-Kosior [b], Ireneusz Sowa [b], Grzegorz Stanisławski [a], Izabela Bany [a], Małgorzata Wójcik [a]

[a] *Department of Plant Physiology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland*
[b] *Department of Analytical Chemistry, Medical University of Lublin, Chodźki 4a, 20-093, Lublin, Poland*

ARTICLE INFO

Article history:
Received 13 February 2017
Received in revised form
11 April 2017
Accepted 12 April 2017
Available online 13 April 2017

Keywords:
Allantoin
Antioxidant capacity
Chlorogenic acid
Echium vulgare L.
Metal tolerance
Organic acid
Rosmarinic acid

ABSTRACT

The aim of the study was to determine the response of metallicolous and nonmetallicolous *Echium vulgare* L. populations to chronic multi-metal (Zn, Pb, Cd) and acute Zn (200, 400 μM) and Pb (30, 60 μM) stress. Three populations of *E. vulgare*, one from uncontaminated and two from metal-contaminated areas, were studied. Two types of experiments were performed – a short-term hydroponic experiment with acute Zn or Pb stress and a long-term manipulative soil experiment with the use of soils from the sites of origin of the three populations. Growth parameters, such as shoot and root fresh weight and leaf area, as well as organic acid accumulation were determined. Moreover, the concentration of selected secondary metabolites and antioxidant capacity in the three populations exposed to Pb or Zn excess were measured. Both metallicolous populations generally achieved higher biomass compared with the nonmetallicolous population cultivated under metal stress in hydroponics or on metalliferous substrates. Plants exposed to Pb or Zn excess or contaminated soil substrate exhibited higher malate and citrate concentrations compared with the reference (no metal stress) plants. It was observed that Zn or Pb stress increased accumulation of allantoin, chlorogenic and rosmarinic acids, total phenolics, and flavonoids. Moreover, it was shown that Pb sequestration in the roots or Zn translocation to the shoots may play a role in enhanced metal tolerance of metallicolous populations under acute Pb/Zn stress.

1. Introduction

In the last few decades, increasing interest in stress adaptive processes of plants inhabiting heavy metal (HM) polluted areas has been observed (Wójcik et al., 2017). It is now evident that abiotic stress factors, including HM contamination, in the long term induce microevolutionary changes which may lead to development of distinguished ecotypes (Wierzbicka and Rostański, 2002). Many populations from metalliferous areas differ from populations originating from uncontaminated sites in respect of tolerance to deficiency of water and essential elements and/or HM stress adaptation.

Comparison of plant populations inhabiting metalliferous sites with populations from uncontaminated areas is a common approach to reveal adaptive tolerance developed through microevolutionary processes or constitutive tolerance present in most populations/phenotypes of the species (Wójcik et al., 2015). Regardless of the tolerance source, i.e. adaptive/constitutive, both types of tolerance strive to maintain cell homeostasis and normal plant function in the presence of HM excess in the environment. Generally, plants have developed several HM detoxification mechanisms, which, except for hyperaccumulators, are mostly based on the exclusion phenomenon relying on restriction of metal uptake and/or translocation to shoots. Such a strategy involves several processes including reduction of HM phytoavailability e.g. by secretion of root exudates. Exclusion of HM from the protoplast is achieved by sequestration thereof in plant parts with low biological activity, such as the cell wall or trichomes, or HM translocation into old leaves followed by shedding thereof (Verkleij and Schat, 1990). On the other hand, if HMs enter the cell, several intracellular tolerance mechanisms mostly based on chelation and sequestration processes, are involved to cope with HM excess.

---

* Corresponding author.
E-mail address: slawomir.dresler@poczta.umcs.lublin.pl (S. Dresler).

http://dx.doi.org/10.1016/j.plaphy.2017.04.016

Several types of ligands participate in HM binding, e.g. phytochelatins, low molecular weight organic acids, or amino acids (Wójcik et al., 2017 and references therein). Moreover, some reports provide evidence that secondary metabolites (SMs), including phenolic compounds, also serve as metal chelators (Michalak, 2006). Kováčik and Klejdus (2008) pointed out that, since SMs participate in cell wall lignification, they could contribute to a decrease in the HM entry into the plant cell. Furthermore, phenolic compounds also play a crucial role in antioxidant activity protecting against lipid peroxidation, which is one of the toxicity action of HM excess in plant tissue (Kováčik et al., 2010; Nasim and Dhir, 2010; Sytar et al., 2013).

The HM tolerance mechanisms depend highly on the duration and level of HM exposure (Sanitá di Toppi and Gabbrielli, 1999). It has been suggested that, for example, acute Cd stress induced polygenetic integrated response called ‘fan-shaped’ response while ‘real’ metal tolerance is only revealed under chronic metal exposition (Sanitá di Toppi and Gabbrielli, 1999). In this context, a question arises of the role of adaptive/constitutive tolerance processes in response to acute and chronic HM stress. The most common approach to reveal plant response to acute HM stress is hydroponic cultivation of plants in the presence of excessive concentrations of metals (Sanitá di Toppi and Gabbrielli, 1999). However, since hydroponic acute HM stress experiments are very often incompatible with the natural environmental conditions, long-term soil experiments could partially solve this problem and ensure better recognition of plant HM tolerance mechanisms.

Echium vulgare L. has been extensively studied recently in terms of its physiological response to severe environmental conditions (Dresler et al., 2014a, 2016, 2017a, 2017b). Comparative studies have proved phytochemical, physiological, and genetic differentiation (Dresler et al., 2015b) between metallicolous and nonmetallicolous *E. vulgare* populations.

It was found that metallicolous populations of *E. vulgare* exhibited higher tolerance to Cd compared with plants derived from uncontaminated areas, which allowed them to produce more biomass (Dresler et al., 2014a). Organic acids and phytochelatins were suggested to be involved in Cd detoxification in metallicolous populations. However, analysis of plants from manipulative soil experiments as well as from the sites of their origin has shown substantial plasticity of *E. vulgare* to change the concentration of some secondary metabolites depending on growth conditions (Dresler et al., 2017a). For instance, a great increase (even 20-fold) in the allantoin and shikonin concentrations was noted in plants exposed to stressful growth conditions. Given the considerable changes in the secondary metabolite profile, including the concentrations of allantoin, shikonin, p-hydroxybenzoic acid, chlorogenic acid, or rutin, in plants growing under environmental stress in metalliferous areas (Dresler et al., 2017a), there is a need for better understanding of the HM influence on phytochemical composition. Since the results obtained previously indicated high *E. vulgare* changeability in SM accumulation in response to environmental stress (Dresler et al., 2017a), a few questions remain unclear: firstly, the role of HM, including the type of the metal or/and its concentration in the nutrient medium in changing the SM profile and, secondly, the role of SMs in adaptive/constitutive tolerance mechanisms against HM stress in *E. vulgare*. Additionally, as we found several differences in response to HM stress between metallicolous and nonmetallicolous populations (Dresler et al., 2014a), another research goal was to reveal the role of low molecular weight organic acids and HM root-to-shoot translocation in tolerance under chronic/acute metal stress.

In this study, we investigated the response of nonmetallicolous and metallicolous populations of *E. vulgare* to acute Zn and Pb stress. As a continuation of the study on the response of *E. vulgare* populations to long-term multi-metal stress (Dresler et al., 2017a), this study provides further evidence of increased tolerance of metallicolous plants to chronic HM stress.

In particular, the aims of the study were: (1) estimation of the level of tolerance to chronic multimetal and acute Zn/Pb stress of metallicolous and nonmetallicolous *E. vulgare* populations based on their growth parameters; (2) evaluation of HM root-to-shoot translocation as a strategy of metal tolerance; (3) assessment of the role of malate and citrate in increased tolerance to Zn and Pb under acute and chronic metal stress; finally, (4) estimation of the significance of secondary metabolites in plants exposed to Pb and Zn excess.

2. Material and methods

2.1. Plant material and experimental design

Two *Echium vulgare* L. (Boraginaceae) populations spontaneously inhabiting waste deposits created by processing of Zn-Pb ores were studied (metallicolous, MP, MB populations). The MP population originated from a relatively young (30-year-old) slag waste deposit left over a former Pb and Zn smelter localised in Piekary Śląskie, while the MB population originated from an old (90-year old) waste heap formed of by-products of gravitational enrichment of a Zn-Pb ore localised in Brzeziny Śląskie. Additionally, one population from an uncontaminated site localised in Kazimierz Dolny (nonmetallicolous, NM population) was tested as reference plants. The detailed description of the study areas including localisation, soil properties, and origin is presented in the Suppl. Table 1. Seeds of each population collected from natural sites in August 2014 were used in the hydroponic Zn/Pb treatment and long-term soil experiment. Both types of growth experiments were conducted under controlled conditions in the growth chamber at 17/25 °C (n/d) 16-h photoperiod at photosynthetic active radiation of 150 μmol m^{-2} s^{-1} and 75% relative air humidity. Since the concentrations of secondary metabolites as well as the antioxidant capacity and heavy metal accumulation in the plants from a reciprocal soil experiment were presented in a previous paper (Dresler et al., 2017a), only morphometric parameters and organic acids concentration in the plants exposed to chronic multi-metal stress are shown here.

2.1.1. Zn/Pb treatment hydroponic experiment

The seeds germinated on moist filter paper and 3-day old seedlings were transferred into pots (one plant per pot) filled with 0.5 L 1/2 concentrated Hoagland nutrient medium (pH 5.6) (Hoagland and Arnon, 1950). To avoid precipitation of insoluble $Pb_3(PO_4)_2$, the nutrient solution was deprived of phosphate ions. After 3 weeks, the nutrient solution was supplemented with 0-control, 30, or 60 μM Pb (as $Pb(NO_3)_2$) or 200, or 400 μM Zn (as $ZnSO_4 \cdot 7\ H_2O$). Eight to twelve pots (plants) were used in each treatment. After addition of appropriate metal concentrations, the plants were cultivated for 14 days. During the experiment, the nutrient medium was continuously aerated and exchanged every 7 days, while its losses were supplemented daily with distilled H_2O. Harvested plants were divided into aboveground (shoot) and belowground (root) parts and weighed. The leaves were photographed and the total leaf area was determined using image analysis software (ImageJ 1.46r, National Institutes of Health, Bethesda, Maryland USA). Prior to further analysis of organic acids, secondary metabolites, and total antioxidant capacity, the shoot and root samples were frozen in liquid nitrogen and stored at −80 °C.