Biochemical Systematics and Ecology 70 (2017) 304–310

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

Morphometric and phytochemical profile of seeds of metallicolous and nonmetallicolous *Echium vulgare* populations

Sławomir Dresler [a, *], Wiesław Bednarek [b], Barbara Hawrylak-Nowak [c], Małgorzata Wójcik [a]

[a] Department of Plant Physiology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland
[b] Department of Agricultural and Environmental Chemistry, University of Life Sciences, Akademicka 15, 20-950 Lublin, Poland
[c] Department of Plant Physiology, University of Life Science in Lublin, Akademicka 15, 20-950 Lublin, Poland

A R T I C L E I N F O

Article history:
Received 5 October 2016
Received in revised form 13 December 2016
Accepted 18 December 2016

Keywords:
Boraginaceae
Heavy metal
Allantoin
Rosmarinic acid
Seed oil

A B S T R A C T

The morphometric and phytochemical parameters of seeds of three populations of *Echium vulgare* L. were compared. Two metallicolous populations (designated as MZ and MC) originated from waste deposits left over Zn–Pb ore smelting and processing and the reference population originated from an uncontaminated area (NM - nonmetallicolous population). The length, width, weight, and surface area of collected seeds were measured; additionally, the germination ability and vigour index for each population were determined. Moreover, heavy metal, seed oil, and secondary metabolite concentrations were measured. The results obtained indicated differentiation in the seed size and germination ability between the populations. The smaller size of seeds from the metallicolous populations was accompanied by lower germination ability; however, the seeds from the MC population achieved the highest vigour index. Furthermore, the MC population was characterized by the lowest concentration of allantoin and rosmarinic acid; additionally, the seeds of the MC population contained higher content of seed oils and increased contribution of saturated fatty acids and monounsaturated fatty acids by ca. 60 and 45%, respectively, and a decreased (by 16%) fraction of polyunsaturated fatty acids compared with the NM population. The strong differentiation of the MC population coincided with the high seed metal accumulation.

1. Introduction

Echium vulgare L. (Boraginaceae) is a biennial plant commonly known as viper's bugloss or blueweed. It occurs on calcareous and light dry soils; however, as a pseudometallophyte, *E. vulgare* can also inhabit metalliferous areas such as waste heaps created by processing of heavy metal (HM) ores (Klemow et al., 2002; Dresler et al., 2014). In traditional folk medicine,

* Corresponding author.
E-mail addresses: slawomir.dresler@poczta.umcs.lublin.pl (S. Dresler), wieslaw.bednarek@up.lublin.pl (W. Bednarek), bhawrylak@yahoo.com (B. Hawrylak-Nowak), mwojcik@poczta.umcs.lublin.pl (M. Wójcik).

http://dx.doi.org/10.1016/j.bse.2016.12.012
0305-1978/

E. vulgare has been recommended for a wide range of applications, e.g. as a remedy for fever, headache, inflammation, promotion of wound healing, or mood stimulation (Klemow et al., 2002; Moallem et al., 2007). Moreover, the plants were used for both prevention and as a remedy for serpent bites. However, hepatotoxic pyrrolizidine alkaloids were identified in *E. vulgare*, which limits its use for traditional medicinal purposes. Nevertheless, the species still has a potentially wide range of use. Nićiforović et al. (2010) pointed out that plant extracts of *E. vulgare* collected in different regions of Serbia had significant antioxidant activity. *E. vulgare* may also be a source of shikonin, rosmarinic acid, and allantoin (Kuruüzüm-Uz et al., 2004; Dresler et al., 2015a, 2017b). Additionally, seeds of *E. vulgare* were defined as a source of oil, which reaches almost 20% of total seed oil content (Özcan, 2008). The *E. vulgare* seed oil, unlike the common oils including soybean or rapeseed oils, is rich in unique fatty acids such as 18:3*n*-6 (γ-linolenic acid) or 18:4*n*-3 (stearidonic acid), which constitute 11 and 13% of total seed oil, respectively (Król and Kowalski, 2004; Czaplicki et al., 2009).

Previous studies indicated that populations of *E. vulgare* exposed to HMs increased the synthesis of some metal chelating agents, such as low molecular weight organic acids and phytochelatins (Dresler et al., 2014). Additionally, a field study of metallicolous and nonmetallicolous populations indicated a strong influence of adverse growth conditions prevailing on metalliferous sites on the composition of secondary metabolites. Based on chemometric analysis, significant differences in phytochemical fingerprint between plants from contaminated and uncontaminated sites were shown (Dresler et al., 2016). Moreover, a pot experiment provided evidence that plant growth on metalliferous substrate significantly increased accumulation of allantoin in the shoots and roots and shikonin in the roots (Dresler et al., 2017a).

In the present paper, we show further insight into the characteristics of *Echium vulgare* L. populations inhabiting highly HM-contaminated areas (Dresler et al., 2014, 2015b, 2016, 2017a). In particular, the aims of the study were to check whether metalliferous areas: i) influence the seed size and alter the composition of oil and secondary metabolites; ii) pose a challenge to plant reproduction by reduction of the germination ability and/or vigour index; iii) are connected with the ability to restrict metal accumulation in the seeds.

2. Experimental

2.1. Materials

Seeds of *E. vulgare* were collected in August 2014 from three plant populations: two from metallicolous populations spontaneously inhabiting waste deposits created by mining and smelting of Zn–Pb ores in Piekary Śląskie and Brzeziny Śląskie (MZ, MC populations, respectively), and one nonmetallicolous population inhabiting an uncontaminated site in Kazimierz Dolny (NM population). The seeds were collected from plants of similar appearance with height of 50–70 cm. The metal concentrations in the waste deposits were: 0.361 g kg^{-1}; 10.7 g kg^{-1}, 38.2 g kg^{-1}, 1.59 g kg^{-1}, 132.0 g kg^{-1} in Piekary Śląskie (MZ) and 0.385 g kg^{-1}, 17.5 g kg^{-1}, 93.1 g kg^{-1}, 0.063 g kg^{-1}, 156.0 g kg^{-1} in Brzeziny Śląskie (MC) for Cd, Pb, Zn, Cu, and Fe, respectively (Dresler et al., 2014; Wójcik et al., 2014). The concentrations of Cd, Pb, Zn, Cu, and Fe in the non-polluted soil from the Kazimierz Dolny site were: 0.0028, 0.067, 0.031, 0.138, and 4.10 g kg^{-1}, respectively. The detailed description of the areas of the population occurrence was provided previously (Dresler et al., 2014, 2016, 2017a).

2.2. Morphometric characteristics of seeds and vigour index

For morphometric measurements, 150 seeds from each population were photographed using an Olympus SZX16 stereomicroscope (Olympus, Tokyo, Japan). The seed length, width, and surface area were determined individually by Stream Motion 1.7 software and were given in millimetres/square millimetres. Additionally, 8 replications of 100 seeds from each population were weighted. For vigour index determination, three replications of 25 seeds from each population were placed in a row on moistened filter paper, rolled up, and transferred to a germination chamber at 30 °C. After four days, the seedlings were photographed and measured using ImageJ software (ImageJ 1.48v., National Institutes of Health, Bethesda, MD, USA). The vigour index was calculated using the following formula:

$$\text{Seedling vigour index} = \text{mean seedling length (mm)} \times \text{germination percent}$$

2.3. Analysis of fatty acids

The fatty acid content was determined in composite samples of seeds collected from 2 to 3 individuals according to the method described by Król and Kowalski (2004). Briefly, the percentage of crude fat was measured using Soxhlet's extraction-gravimetric method. Fatty acids were analysed as methyl esters using the gas chromatography technique after preliminary fat saponification and acid esterification according to the AOAC-969.33 and 963.22 procedures (AOAC, 2000 a; b). The measurement was performed with a Unicam 610 Series GC (Unicam Analytical, Cambridge, UK) gas chromatograph equipped with a flame-ionization detector and a 60 m × 0.25 mm column of 0.25 μm i.d. coated with a film of HP-23. The concentration of each fatty acid in the seed samples was determined based on previous analysis of standard mixtures and calculation of individual correction coefficients (Król and Kowalski, 2004).