ABS

Data Article

Noccaea praecox leaf metabolomes from unpolluted and metal-polluted sites

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Abstract

Noccaea praecox is a hyperaccumulating plant, i.e. the Cd, Zn, and Pb concentrations in leaves exceed 100-fold the non-toxic levels. In this study, we report the untargeted leaf metabolome of *N. praecox* from an unpolluted site (Lokovec) and a metal-polluted site (Žerjav) in Slovenia. Using UPLC-MS/MS and metabolomic analysis, we identified 7,253 metabolites: 6,659 were non-annotated, and 594 were annotated. Among the identified metabolite classes, carbohydrates, terpenoids, flavonoids, and organic acids were the most dominant. Principal Component Analysis revealed distinct metabolomic profiles between the two *N. praecox* ecotypes, suggesting environmental-related effects in *N. praecox* physiology. This dataset is a valuable resource for studies on plant adaptation, metal accumulation, and metabolite biosynthesis under metal-stress conditions.

Keywords

Untargeted metabolome; metal hyperaccumulator; metabolomics; metal pollution

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Metabolomi listov *Noccaea praecox* z neonesnaženega in s kovinami onesnaženega območja

Izvleček

Rani mošnjak (*Noccaea praecox*) je hiperakumulacijska rastlinska vrsta, t.j. v svojih listih lahko kopiči zelo visoke koncentracije Cd, Zn in Pb. V tej študiji poročamo o netarčnem metabolomu v listih rastlin vrste *N. praecox* iz neonesnaženega (Lokovec) in s kovinami onesnaženega območja (Žerjav) v Sloveniji. Z uporabo UPLC-MS/MS in analize metaboloma smo identificirali 7253 metabolitov: 6659 je bilo neanotiranih, 594 pa anotiranih. Med identificiranimi razredi metabolitov so bili najpogostejši: ogljikovi hidrati, terpenoidi, flavonoidi in organske kisline. Analiza glavnih komponent (PCA) je pokazala razlike v metabolomskem profilu med obema analiziranima ekotipoma vrste *N. praecox*, kar kaže na značilen okoljski vpliv na fiziologijo te vrste. Pridobljen nabor podatkov je dragocen vir za prihodnje študije o prilagajanju rastlin, privzemu kovin in biosintezi metabolitov kot odziv na povečane koncentracije kovin v tleh.

Ključne besede

Untargeted metabolome; metal hyperaccumulator; metabolomics; metal pollution

Objectives

Capturing metabolite composition in leaves of field-collected *N. praecox* from two contrasting habitats, the metal-polluted and the unpolluted one. This dataset (comprising 594 annotated and 7,253 non-annotated metabolites) will support resolving effects of environmental perturbations on the ecology and physiology of *N. praecox*.

Data description

The dataset represents the untargeted leaf metabolome of *N. praecox* from the unpolluted site in Lokovec (Slovenia) and *N. praecox* from the polluted site in Žerjav (Slovenia). The latter site is characterised by high concentrations of lead (Pb), cadmium (Cd), and zinc (Zn) in the soil (Likar et al. 2009). Feature annotations are presented in a spreadsheet (.xlsx file), which describes metabolites detected in each sample, including their m/z ratios, chemical formulas, ion adducts and molecule identity.

The identified metabolites are representative of various metabolite families of both primary and secondary metabolites, including carbohydrates, terpenoids, flavonoids, alkaloids, organic acids, amino acids, and fatty acyls (Fig. 1).

Principal Component Analysis (PCA) ordination shows distinction in the set of metabolites between *N. praecox* ecotypes from the metal-polluted and the unpolluted site (Fig. 2).

Value of the Data

- The provided dataset of all metabolites from different ecotypes of *N. praecox* is valuable for researchers interested in studying differences between ecotypes of *N. praecox*.
- The identified metabolites are representative of various metabolite classes, including carbohydrates, terpenoids, flavonoids, alkaloids, organic acids, amino acids, and fatty acyls.
- The metabolite profiles depict differences between the two ecotypes and thus can be of interest to researchers studying metal accumulation in plants with a special focus on hyperaccumulators.

Experimental Design, Materials and Methods

Sampling

Three naturally occurring *N. praecox* plants were collected at Lokovec (46°2′39.2706″N, 13°46′8.9934″E) and Žerjav (46°28′26.1258″N, 14°51′56.0118″E). Lokovec is an unpolluted grassland, whereas Žerjav is a metal-polluted grassland site due to centuries-long mining and smelting activity (Likar et al., 2009). A healthy rosette leaf was collected from each plant, flash-frozen in liquid nitrogen for at least 15 minutes,

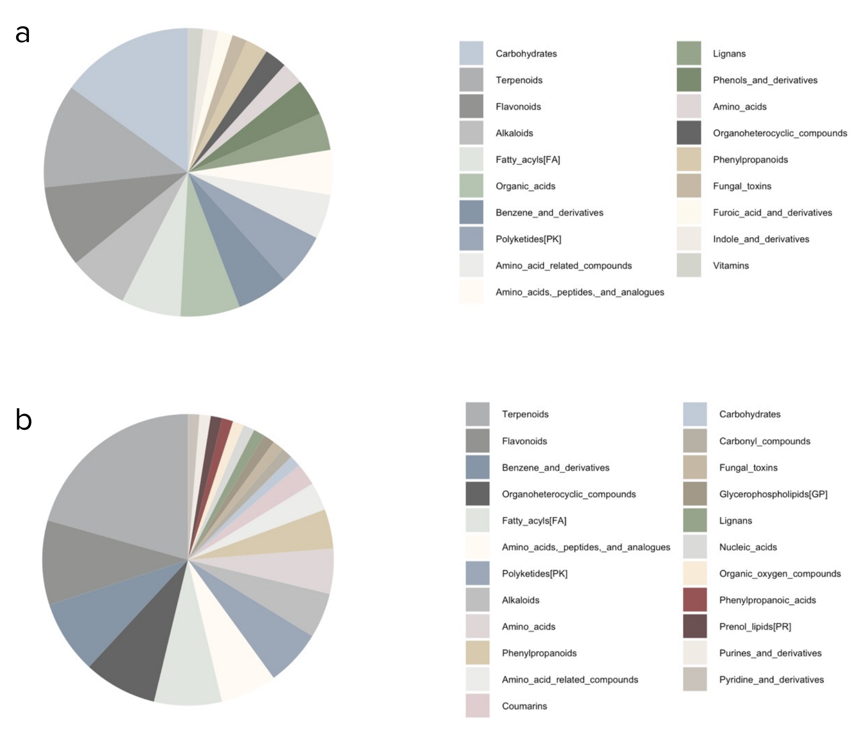

Figure 1. Piecharts of metabolite classes in leaves of field-collected *Noccaea praecox* from MS/MS measurement in both a) negative and b) positive ion mode. Only groups with more than one unique metabolite are represented.

Slika 1. Diagrami razredov metabolitov vrste *Noccaea praecox* iz meritev MS/MS v a) negativnem in b) pozitivnem ionskem načinu. Predstavljene so samo skupine z več kot enim edinstvenim metabolitom.

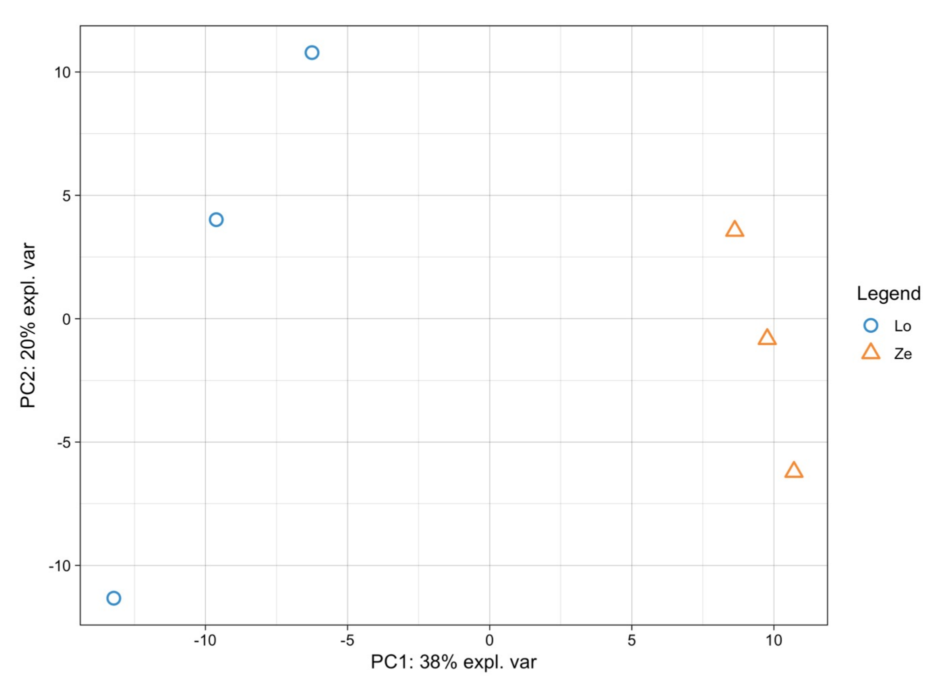

Figure 2. PCA ordination for metabolites of leaves of field-collected *Noccaea praecox* from the metal-polluted site Žerjav (Ze) and the unpolluted site Lokovec (Lo).

Slika 2. PCA ordinacija za metabolite listov rastlin *Noccaea praecox* z onesnaženega (Ze) in neonesnaženega (Lo) rastišča.

and then crushed into smaller fragments. The samples were freeze-dried for three days at −96 °C and 0.0012 mbar in a Coolsafe (Scanvac) LaboGene system (Allerød, Denmark) and stored at −80 °C before being sent to BGI Tech Solutions (Poland) for untargeted metabolomic analysis.

Metabolomics

For the analysis, 50 mg of each sample was extracted with 800 µL of a pre-cooled extraction solution (methanol: water = 7:3, v/v) and 20 µL of an internal standard mixture (d3-Leucine, 13C9-Phenylalanine, d5-Tryptophan, and 13C3-Progesterone). The samples were homogenized by grinding and ultrasonication. After incubation at −20 °C for one hour, the extracts were centrifuged at 14,000 rpm for 15 minutes at 4 °C.

Metabolite separation and identification were carried out using a Waters UPLC I-Class Plus system (Waters, Milford, MA, USA) coupled with a Q Exactive high-resolution tandem mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation was performed on a Hypersil GOLD aQ Dim column (1.9 µm, 2.1 × 100 mm; Thermo Fisher Scientific) at a flow rate of 0.3 mL/min and a column temperature of 40 °C. The mobile phase comprised solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). The initial mobile phase composition was 5% solvent B, which was held for 2 minutes, then ramped to 95% solvent B over 20 minutes (2–22 min) and maintained for 5 minutes (22–27 min). The injection volume was 5 µL. Mass spectrometry was performed with a scan range of 125–1,500 m/z for positive ions and 100–1,500 m/z for negative ions, using a resolution of 70,000 for MS acquisitions and 30,000 for MS/MS. Fragmentation energies of 20, 40, and 60 eV were applied. The sheath gas flow rate and auxiliary gas flow rate were set at 40 and 10, respectively. Spray voltages for positive and negative ion modes were 3.80 kV and 3.20 kV, respectively. The ion capillary and auxiliary gas heater temperatures were 320 °C and 350 °C, respectively.

Data preprocessing was performed using the metaX library (Wen et al., 2017) in R. Metabolite classification and functional annotation was conducted using the KEGG and HMDB databases, yielding KEGG IDs, HMDB IDs, categories, and associated KEGG pathways.

Specifications Table

Subject	Omics: Metabolomics; Environmental biology
Specific subject area	Metabolic differences between ecotypes of N. praecox from polluted and unpolluted sites.
Type of data	Excel file
How the data were acquired	Metabolite separation and identification were carried out using a Waters UPLC I-Class Plus system (Waters, Milford, MA, USA) coupled with a Q Exactive high-resolution tandem mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).
Data format	Excel tables
Description of data collection	Leaves of three mature plants were collected per site; 50 mg sample from each plant was extracted with 800 µL of pre-cooled extraction solution (methanol: H2O = 7:3, v/v); chromatographic separation on Hypersil GOLD aQ Dim column on 5-95% water-ACN gradient. The scan range was 125–1,500 m/z for positive ions and 100–1,500 m/z for negative ions, with a resolution of 70,000 for MS acquisitions. For MSMS acquisition, 30,000 was used. Data preprocessing was performed using metaX in R. Classification and functional annotation analysis via KEGG and HMDB databases were performed to obtain KEGG ID, HMDB ID, category, and KEGG Pathway.
Data source location	Plant material was collected by Valentina Bočaj at Lokovec (46°2′39.2706″N, 13°46′8.9934″E) and Žerjav (46°28′26.1258″N, 14°51′56.0118″E).
Data accessibility	Metabolome data tables were deposited at Zenodo (10.5281/zenodo.10991915).
Related research article	Bočaj, V.; Pongrac, P.; Fischer, S.; Likar, M. Species-Specific and Pollution-Induced Changes in Gene Expression and Metabolome of Closely Related Noccaea Species Under Natural Conditions. Plants 2024, 13, 3149. https://doi.org/10.3390/plants13223149

Author Contributions

Conceptualization, M.L. and V.B.; formal analysis, P.P. and V.B.; investigation, V.B., writing—original draft preparation, V.B. and M.L.; writing—review and editing, P.P. and M.L.; visualization, M.L. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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