Conference Paper

Genetic Diversity in Local *Taraxacum officinale* L. Populations from Habitats Varied in Toxic Load

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Abstract

The level of lipid peroxidation (a stress indicator) and the quantitative traits of leaf tissue (the number and size of mesophyll cells and chloroplasts) were determined in *Taraxacum officinale* L. plants from five habitats near the city of Nizhniy Tagil that differ in terms of the heavy metal content in the soil. It is shown that in polluted habitats, the content of heavy metals (Cu^{2+} and Zn^{2+}) in leaves is significantly higher compared to plants from background areas, with leaf thickness, mesophyll cell volume and chloroplast number per cell increasing. Both Inter Simple Sequence Repeat (ISSR) analysis and cluster analysis of dandelion genetic diversity based on eight primers have revealed that four groups of plants are closely related genetically. Of the observed differences between five local populations, 78% are caused by intrapopulation variability and 22% by interpopulation variability. It is supposed that *Taraxacum officinale*'s tolerance to heavy metal contamination in the studied localities is not genetically fixed adaptation, but acclimation within genetically selected ranges of tolerance.

Keywords: heavy metals, local populations, ISSR markers, leaf traits

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1. Introduction

The growth of industry and mining has led to an increase in the toxic load on ecosystems. In conditions of chronic pollution, an excessive amount of heavy metals (hereafter, HM) is found in natural media and biota components. Plants are forced to adapt to these new conditions. The mechanisms of adaptation can be either genetic or phenotypic, so plants can have adaptations or acclimations that allow individuals, populations and communities to exist in technogenic environments for a long time.

Nizhny Tagil is a large industrial center. About 50 industrial enterprises (mining, metallurgical, chemical and machine-building), 6 large quarries and 3 mines are concentrated in the city, which leads to a high level of air, water and soil pollution. Nevertheless, a lot of ruderal plants grow there. For several years, the accumulation of heavy metals in soils and different plant organs has been detected in widespread plant species in various localities of Nizhny Tagil [1–3]. Also, some physiological and biochemical mechanisms of plant adaptation to heavy metals have been studied [4, 5]. However, it is not clear whether the adaptations acquired by plants are genetically determined.

The data on the genetic diversity of dandelion populations and other plant species in sites with different levels of pollution are contradictory. In B. Keane et al.'s [6] study, it is mentioned that although statistically significant correlation between the level of pollution and genetic diversity of plants was not found, the data indicated that pollution-induced selection could contribute to lower genetic diversity in polluted sites. D. G. Ackova et al. [7] concluded that long-term HM impact caused genotoxic stress in several common plant species, including the dandelion. In contrast, reduction of genetic diversity in *Silene paradoxa* L. populations from contaminated areas was not shown [8, 9]. A. Slomka et al. [10] revealed an increased level of polymorphism and genetic diversity in the *Viola tricolor* L. population in habitats contaminated by HM.

Our goal was to compare phenotypic traits and genetic diversity in 5 local dandelion populations from habitats with different levels of toxic load.

2. Methods

The study was conducted in five habitats in the city of Nizhny Tagil and its surroundings: (i) Pokrovskoye village (57°58′33.1″ N 60°14′40.4″ E); (ii) ship's cape (57°53′02.6″ N 60°00′28.0″ E); (iii) copper mine dumps (57°58′20.6″ N 60°00′16.0″ E); (iv) Alapaevskaya railway branch (57°58′13.4″ N 60°06′02.0″ E); and (v) the main quarry dumps of the Vysokogorsky metallurgical enterprise (57°54′12.6″ N 59°54′27.5″ E). HM concentration in the soils of the regional background (i) and technogenically disturbed territories (ii–v) differed significantly, especially in zinc and copper [11]. According to the amount of HM in the soil, the index of toxic load (S_i) was calculated for each habitat as (1):

$$S_i = (1/n)\Sigma S_i/S_f \quad (1)$$

(i) was chosen as the background, (ii–iv) as the buffer and (v) as impact habitats [5, 11]. The morphological form *Taraxacum officinale* f. *dahlstedti* was chosen for the study because of its larger leaf surface compared to other morphs. Soil and plant samples in each habitat were collected randomly from the whole habitat area.

2.1. HM assay in plants

Leaf and root samples were taken separately from 10 plants in each habitat. 0.7–1.0 g of dry plant material was dissolved in ethanol and burned (150°C for 4–6 hours, 500°C for 10–15 hours). The metals were extracted by 5% HNO_3 over 40 h. Zn^{2+} and Cu^{2+} concentrations in acid extracts were determined by an AAnalyst^{TM} 300 (PerkinElmer®, USA) atomic absorption spectrophotometer. The value of C_n was calculated taking into account the concentration of the corresponding element in a blank sample.

2.2. Lipid peroxidation (LPO)

The intensity of LPO was evaluated by assaying thiobarbituric acid reactive substances (TBARS) – malondialdehyde (MDA) as described in [12] based on values of optical density at 532 nm and 600 nm and expressed in μmol TBARS/mg.

2.3. Quantitative traits of leaf mesophyll

A typical leaf was detached from each of the 10 flowering plants in every habitat: 5 samples from each leaf were fixed in 3.5% glutaraldehyde in a phosphate buffer (pH 7.2). Samples were processed according to the modified method of A. T. Mokronosov and R. A. Borzenkova [13]; the number and volume of cells and chloroplasts were determined using the SIAMS MesoPlant^{TM} system of image analysis (SIAMS, Russia). The number of repeated measurements were: 20 replicates for counting cells in macerated tissue using Goryaev haemocytometer, 30 replicates for estimating the number of chloroplasts in a cell and 30 replicates for measuring cell size and volume.

2.4. DNA isolation

20 young bright green leaves from non-flowering plants were collected for molecular genetic study. The isolation of nucleic acids (NA) was conducted in accordance with S. Porebski's method [14]. NA amount and purity was measured by a microplate

spectrophotometer Infinite® 200 PRO NanoQuant^{TM} (Tecan Group Ltd., Switzerland). Qualitative analysis of NA was conducted after horizontal electrophoresis in a 0.8% agarose 1× TBE gel containing ethidium bromide.

2.5. ISSR–PCR

Eight UBC primers were selected for ISSR–PCR. The PCR reaction mixture (20 µl) contained a 1x buffer (2.5 mM Mg^{2+}), a 1 mM dNTP, a 0.5 mM primer, 2 units of HS Taq polymerase and 25–100 ng NA. PCR was programmed as preliminary denaturation at 95°C for 5 min, 40 cycles of 40 s denaturation at 95°C, 50 s annealing at 45, 47, 48 or 51.5°C and a 40 s extension at 72°C, followed by a 10 min final elongation at 72°C. PCR products were separated by electrophoresis in a 1.2% agarose gel containing ethidium bromide buffered with 1× TBE and then visualized by a Gel Doc^{TM} XR Documentation System (Bio-Rad Laboratories, Inc., USA). The reliability of PCR results was confirmed in three series of reactions for each primer with random sampling.

2.6. Data analysis

The electrophoregrams were processed by the Image J program, while the binary matrix of data was composed manually. PAST 3.15 [15], GeneAlex 6.5 [16] and the software STATISTICA^{TM} 6.0 (Statsoft Inc., USA) were used to process the data.

3. Results

Depending on the level of toxic load (S_i), dandelion plants accumulated a lot of toxic HM, including copper, zinc, cobalt and chromium, both in the underground and aboveground organs. The strongest accumulation was detected for Cu^{2+} and Zn^{2+} (Table 1). The amount of these ions in the underground parts increased about 4-fold both for copper and zinc on the impact site compared to the background. In shoots, Cu^{2+} increased 15-fold, although there was only 4 times as much Zn^{2+}. Both elements are essential for plants, but excessive concentrations (more than 1–5 µg/g) cause stress. One of the stress indicators is the level of membrane lipid peroxidation (LPO) caused by reactive oxygen species formed under stress [17–19]. Though the level of LPO in *T. officinale* plants did not change drastically in different habitats (Figure 1(a)), living in polluted localities for decades caused changes in the leaf traits of dandelion plants.

Table 1: Copper and zinc ion concentration in the above- and underground organs of *T. officinale* (M ± SE).

Locations	Cu^{2+}, µg/g		Zn^{2+}, µg/g	
	Underground	Aboveground	Underground	Aboveground
1 (S_i = 1.00)	20.04 ± 1.86	3.53 ± 0.03	11.72 ± 0.39	8.30 ± 0.28
2 (S_i = 3.33)	22.36 ± 0.95	3.13 ± 0.21	21.55 ± 3.32	12.91 ± 0.99
3 (S_i = 6.19)	29.54 ± 0.64	7.26 ± 2.15	27.58 ± 0.40	9.73 ± 0.67
4 (S_i = 8.36)	22.00 ± 2.06	6.76 ± 0.69	28.25 ± 0.83	22.50 ± 4.24
5 (S_i = 22.78)	95.02 ± 6.25	46.34 ± 1.43	40.75 ± 4.10	29.43 ± 5.68

Source: Author's own work.

It was shown that leaf thickness gradually increased in plants in the impact area by 31%; mesophyll thickness increased by 26% and the thickness of the upper and lower epidermis by 58% (Figure 1(b)). Comparative analysis of leaf mesophyll characteristics have shown that in plants from contaminated habitats the volume of sponge cells significantly ($P < 0.0001$) increased compared to plants from the background area (1.5-fold), while the volume of palisade cells did not change (Figure 1(c)). The number of cells per unit leaf area and the number of chloroplasts per cell were significantly ($P < 0.001$) higher in plants growing in impact sites (Figure 1(d)). Thus, the observed changes in leaf mesophyll structure in plants under prolonged exposure to HM could be considered a protective reaction to pollution, a kind of regulation which allows plants to effectively assimilate CO_2 for growth, development and HM tolerance: this needs metabolic and energy resources.

The genetic characteristics of the local populations were identified by ISSR markers. The analyses of the binary matrix have revealed that the maximal number of bands in the primer UBC 807 was 23, the maximal number of bands for the sample was 19 (UBC 835) and the average number of bands for the sample was 9. These indicators are quite high for the ISSR method, so fewer primers are required to get 100 bands, which is generally considered to be sufficient for evaluating genetic diversity. The maximal length of the band was 2500 bp, while the minimum was 30 bp for all samples. The maximal number of monomorphic bands per primer was estimated as one. Monomorphic bands were found in 5 of 8 primers: UBC 807, UBC 810, UBC 811, UBC 814 and UBC 834. In general, 154 loci were found. The percentage of polymorphic loci (Table 2) in differed localities varied from 74.5 to 83.89. The total percentage of polymorphism was 96.75%. The maximum, minimum and mean values of the number of bands for each primer were calculated (Figure 1(f)).

Principal Coordinates Analysis allows us to visualize the distance between samples according to the distance matrix (Figure 1(g)). According to the PCA, local dandelion

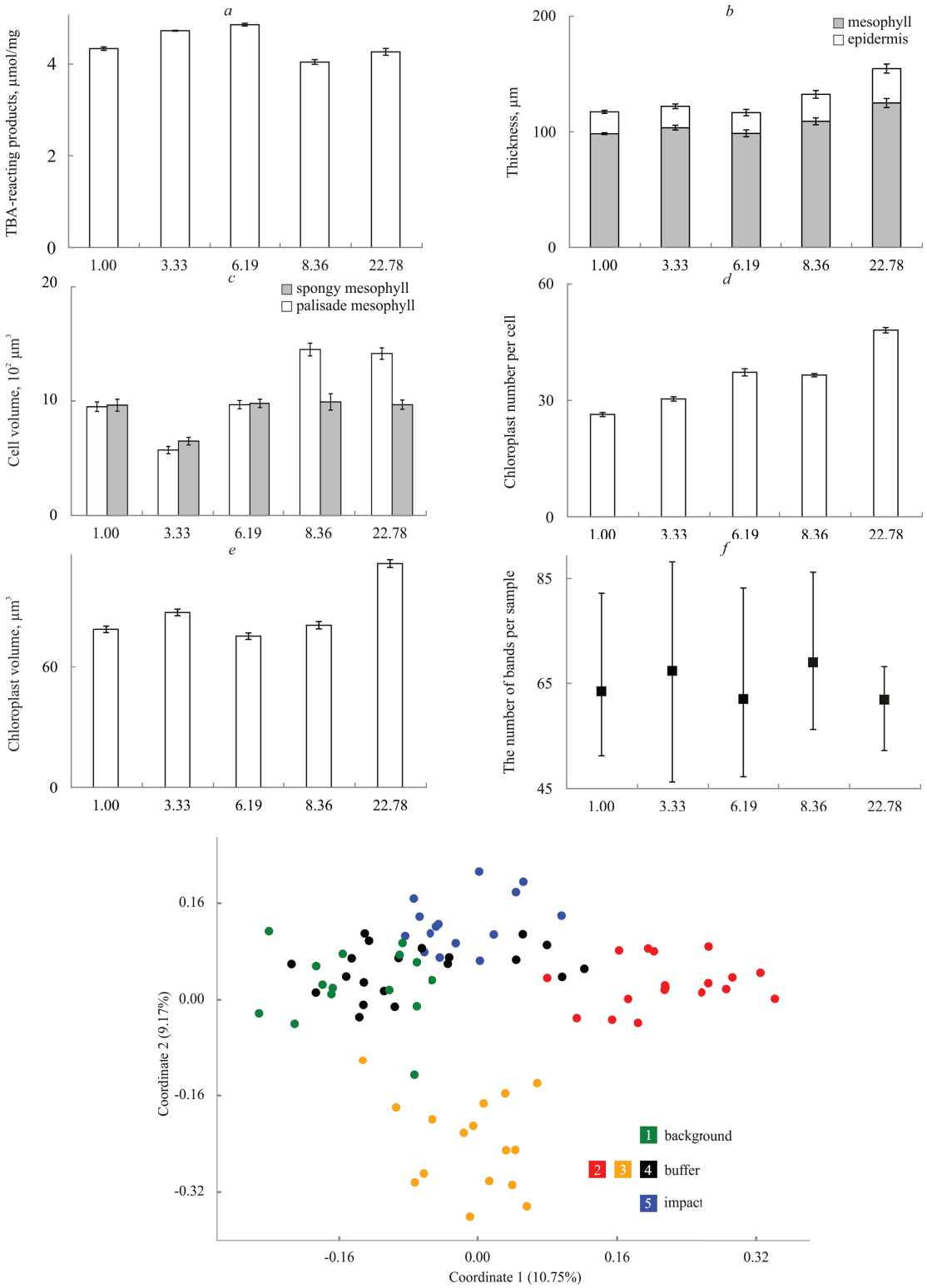

Figure 1: Morpho-physiological state of *T. officinale* plants from local populations with different levels of toxic load (*X*-axis): a – lipid peroxidation intensity; b – leaf mesophyll and epidermis thickness; c – mesophyll cell volume; d – chloroplast number per cell; e – chloroplast volume; f – number of ISSR loci per sample; g – dotted chart constructed from multidimensional scaling using the Jacquard metric. Mean values and standard errors are shown at a, b, c, d and e, the maximum, minimum and mean values – at f. Source: Author's own work.

Table 2: Genetic variability indicators of *T. officinale* local populations.

Indicator	Local Population				
	1	2	3	4	5
The calculated toxic load S_i, rel. units	1.0	3.33	6.19	8.36	22.78
Percentage of polymorphic loci	74.5	83.89	81.21	81.88	77.18
Number of effective alleles	1.412 ± 0.03	1.45±0.029	1.407 ± 0.027	1.447 ± 0.029	1.381 ± 0.029
Expected heterozygosity	0.244 ± 0.015	0.268 ± 0.015	0.249 ± 0.015	0.265 ± 0.015	0.229 ± 0.015
Shannon's information index	0.37 ± 0.021	0.408 ± 0.029	0.382 ± 0.02	0.4 ± 0.021	0.353 ± 0.021

Source: Author's own work.

population (iii) was the most isolated. Local population (i) was close to (iii), which allows us to suppose crossbreeding between these local populations. The other four local populations formed a common cloud, but local population (ii) was relatively distant from the others in the cloud. It is quite interesting that the local populations of the background site (i) and the most polluted area (v) were rather close to each other. These habitats are located within the city, but at a sufficiently large distance from each other. Local population (iv) has the largest variability among the populations. In general, all local populations were rather close to each other, which demonstrates an absence of barriers for crossbreeding between them.

Cluster analysis has shown that all populations were divided into four clusters. The most distant, according to this analysis, was local population (v), with the highest level of total toxic load, and part of (iv). A separate cluster was formed by local population (ii). The fourth cluster was formed by another part of local population (iv). This subdivision of population (iv) indicates its heterogeneity and can be explained by dissemination along the railway.

GeneAlex 6.5 software [16] was used to provide numerical indicators of genetic diversity level (Table 2). The percentage of polymorphic loci for background habitat (i) was 74.5%, while for impact (v) it was 77.18%. The highest percentage of polymorphic loci was observed in buffer habitats: (ii) – 83.89, (iii) – 81.21% and (iv) – 81.88%.

The N_e value indicates the effective number of alleles and was calculated as shown in (2):

$$N_e = 1/(p^2 + q^2). \quad (2)$$

The lowest value of this index was shown for impact population (v) – 1.381 ± 0.029. Generally, the N_e value varied from 1.381 to 1.45. The average expected heterozygosity, or H_e, was calculated as in (3):

$$H_e = 2 * p * q. \quad (3)$$

The minimal value of this indicator was found in impact habitat (v) – 0.229 ± 0.015. The variation of the H_e index ranged from 0.229 to 0.268.

The Shannon index is calculated as in (4):

$$I = -1 * (p * Ln(p) + q * Ln(q)), \quad (4)$$

and reflects the complexity of the community structure, based on the quantitative representation of species [20]. The Shannon index varied from 0.408 to 0.353, with the lower value being present in the impact zone.

The Nei standard genetic distance shows the genetic difference or similarity between populations and was calculated as in (5):

$$D_s = -lnI, \quad (5)$$

where I is the normalized genetic identity for the random sampling of loci [20]. The Nei standard genetic distance varied from 0.090 to 0.140 and was 0.100 between the background and impact habitats. The Nei genetic similarity varied from 0.914 to 0.870 and was 0.905 between the background and impact habitats.

Also, the intrapopulation and interpopulation variability was calculated as 78% and 22%, respectively: this indicates the main variability within the local populations. Φ_{ST} was 0.219 ($P \geq 0.001$).

The impact population has revealed minimal values for the number of effective alleles, average heterozygosity and the Shannon index. The values of these indexes for the background, buffer and impact populations overlapped: this does not support the original hypothesis about less genetic diversity in local populations of *T. officinale* in highly polluted sites.

4. Conclusion

The genetic similarity of local dandelion populations from varied polluted habitats was accompanied by some variety in plant morphology and physiology. Significant differences in HM concentration in roots and shoots (Table 1) indicated a high degree of protection against these pollutants in the aboveground parts of the plant. Apparently,

there is a barrier that allows shoots to avoid high concentrations of HM: this is a way of protecting the reproductive and assimilating organs. Therefore, if the reproductive system is not subjected to a high concentration of HM, then natural selection is not directed and there is no genetic isolation of the local populations.

It can also be assumed that contamination could disrupt the amphimixis, which would lead to a high frequency of apomixis and, as a result, to a decrease in the level of genetic diversity.

Enterprises have been polluting the buffer and impact habitats in Nizhny Tagil for no less than a century: this is probably insufficient time for the populations to pass through microevolution and acquire specific genetic traits and adaptations. It is likely that *T. officinale* plant tolerance to HM contamination in the studied localities does not represent genetically fixed adaptations, but acclimation reactions within genetically selected tolerance ranges. So, the revealed leaf mesophyll changes in local *T. officinale* populations from habitats varied in terms of toxic load cannot be related to genetic diversity as studied through ISSR markers.

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