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RESEARCH ARTICLE

Edaphic factors as genetic selection agents and adaptation drivers of native plant species in harsh environments of the Brazilian savanna

Leide R. M. Andrade · Fabiana G. Aquino · Guillaume Echevarria · Jamile S. Oliveira · Cícero D. Pereira · Juaci V. Malaquias · Karina S. Souza · Emmanuelle Montargès-Pelletier · Fábio G. Faleiro · Fábio B. Reis Junior · Zenilton G. Miranda · Edson E. Sano · Lourdes Isabel Velho do Amaral

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Abstract
Background The highly diversified flora in the Brazilian Cerrado (savanna) region is attributed to several factors, including the high concentrations of metals in soils, especially Al in widespread Ferralsols and Ni in soils derived from ultramafic rocks. We hypothesized that adaptation mechanisms are responsible for the genetic diversity of the following native plant species that are found in the abovementioned environments: *Euploca salicoides* (ES), *Justicia lanstyakii* (JL), and *Oxalis hirsutissima* (OH).
Objectives We aimed to analyse the main edaphic factors that differentiate ultramafic from Al-rich environments, and act as drivers of the evolution of physiological mechanisms underlying plant adaptation to these harsh environments.
Methods We analysed the chemical attributes of four ultramafic soils (SAP5, SAP7, SAP9, LAT) and an Al-rich soil (CAM), and the elemental composition and DNA of the three species growing in both environments. ES was used as a model species to analyse changes in the levels of non-structural carbohydrates (NSCs) and Ni localization in plant leaves.
Results The soil types presented significant differences in available nutrients and heavy metals. The DNA sampled from the same species from ultramafic sites was genetically closer, but different from that in the Al-rich sites. In ultramafic soils, ES accessions had high levels of NSCs and Ni accumulated in trichomes.
Conclusions The genetic diversity observed in plants growing in both areas is probably related to plant adaptation to the contrasting edaphic conditions of these environments. The raffinose production and Ni allocation to trichomes are mechanisms employed by ES to overcome metal toxification in ultramafic environments.

Keywords Cerrado biome · Ni-hyperaccumulators · Plant adaptation and conservation · Ultramafic soils

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In memoriam of Lourdes Isabel Velho do Amaral.

L. R. M. Andrade (✉) · F. G. Aquino · J. S. Oliveira · C. D. Pereira · J. V. Malaquias · F. G. Faleiro · F. B. Reis Junior · Z. G. Miranda · E. E. Sano
Embrapa Cerrados, Planaltina, FD, Brazil
e-mail: leide.andrade@embrapa.br

G. Echevarria
Laboratoire Sols et Environment, Université de Lorraine–INRAE, 54000 Nancy, France

G. Echevarria
Centre for Mined Land Rehabilitation–Sustainable Minerals Institute, University of Queensland, 4072 St. Lucia, QLD, Australia

K. S. Souza · L. I. V. do Amaral
Departament of Botany, IB, University of Brasilia, Brasília, FD, Brazil

E. Montargès-Pelletier
Université de Lorraine–CNRS, LIEC, 54000 Nancy, France

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Introduction

The Brazilian tropical savanna (Cerrado biome) occupies more than 203 million hectares in the central part of the country, corresponding to more than 23% of the national territory. The climate is classified as Aw in the Koppen's classification system, i.e., tropical savanna with two well-defined seasons: a six-month dry season (May to October) and a six-month rainy season (November to April) (Silva et al. 2008). It is one of the most biodiverse tropical savannas in the world, with 11,046 species of phanerogams, of which 40% are endemic (Mendonça et al. 2008; Myers et al. 2000). The high plant diversity and degree of endemism found in this biome result from its significant habitat heterogeneity. A factor that determines this diversified flora is the soil geochemistry, especially due to the high concentrations of metallic elements, such as Al in acidic Ferralsols (Goodland and Pollard 1973) and Ni in soils derived from ultramafic rocks (Filgueiras 2002).

Naturally low-nutrient and Al-rich soils derived from clastic, acidic, and lateritic rocks occupy more than 80% of the Brazilian central region (Martins et al. 2010). Ferralsols are the most common soil type in this biome (>46% of the surface area). They are products of long-term development on geologically stable surfaces and are among the world's oldest and most weathered soils (Burak et al. 2010). The Cerrado's Ferralsols are deep, well-structured, well-drained, and highly acidic (usually pH < 5), with a lack of primary minerals, silica leaching, and complete loss of exchangeable base cations (Ca^{2+}, Mg^{2+}, and K^{+}) in the soil profile. They also present residual enrichment of Fe and Al oxides (Gomes et al. 2004). The long-term weathering process has resulted in deficiencies in P and micronutrients (Co, Cu, Mn, Mo, and Zn), low base saturation, and Al^{3+} accounting for most of the cation exchange capacity (CEC). Aluminium is quite toxic to several species, with strong effects on root development, plant growth, and absorption of water and essential elements, sometimes leading to plant death. The Cerrado's vegetation is composed of a mosaic of grasses, shrubs, and trees in different proportions, depending on the availability of water and soil nutrients (Ratter et al. 1997). Currently, 45% of this biome presents some type of land occupation, especially cultivated pastures, and annual crops (Alencar et al. 2020).

Ultramafic rocks occupy only 0.2% of the Cerrado biome. They occur in small fragments and geochemical islands that vary in size and distance from each other. Approximately 70% of the Cerrado's ultramafic soils (percentage of area) correspond to the mafic-ultramafic complex of Canabrava, Niquelândia and Barro Alto, located in the central part of the Goiás State (Martins et al. 2010). This complex has high economic value due to the mineral exploitation of metals, mainly Ni. The municipality of Barro Alto has one of the world's largest reserves of Ni. Its ore deposits are estimated to total 120 million tons, with 1.25 million tons of Ni (Anglo American 2011). Similar to other ultramafic soils in the world, the Cerrado's soils derived from serpentinized ultramafic rocks contain more than 70% of mafic minerals rich in Fe, Mg, Cr, Ni, Co, Mn, Zn, and Cu. On the other hand, they are poor in Si, Al, Ca, P, and K and have a low Ca:Mg ratio. Sometimes, they are stony, with a particular chemical and mineralogical constitution. The B horizon is variable in both the extent and degree of development (Echevarria 2018; Vidal-Torrado et al. 2006). They are considered harsh environments and can act as strong agents of ecotypic selection on plants, indicating that they are important areas for biological conservation (Kruckeberg 2002; Martins et al. 2010), as well as for the ecological restoration in the case of mining areas, when applied as a strategy for reducing biodiversity loss (Gann and Lamb 2006).

Ni bioavailability is usually high in ultramafic soils. Once plants absorb Ni, it acts as a cofactor for urease, an enzyme that hydrolyses urea into NH_3 and CO_2, in many plant species. Nickel is also involved in several other physiological processes that may include nutrient transport to seeds and movement of Fe into cells. Thus, Ni is an essential micronutrient for plant metabolism (Brown et al. 1987; Seregin and Kozhevnikova 2006), although it can be toxic if absorbed in high concentrations. Ni toxicity causes water and nutrient imbalances, reduces plant growth, and induces reactive oxygen species (ROS) production, which affects numerous physiological and biochemical processes (Shahzad et al. 2018).

Metal hyperaccumulator plants are rare (less than 0.5% of known angiosperm species) and are

generally found in metalliferous environments (naturally occurring or anthropogenically contaminated soils), suggesting that they evolved in situ in these soils (Cappa and Pilon-Smits 2014). Ni is the metal most commonly accumulated in plants. Among approximately 700 plant species reported to accumulate metals, 500 accumulate Ni (Reeves et al. 2021; Aquino et al. 2011) identified more than 200 native species in the ultramafic complex of Barro Alto. Approximately 10% of these species were considered as Ni hyperaccumulators, as they contained > 1000 mg/kg (0.1%) Ni in dry matter (Andrade et al. 2015). These species have high biotechnological potential since they can be used in the processes of Ni phytoextraction or Ni phytomining (agromining) (van der Ent et al. 2015). However, Ni mining activities in the ultramafic region cause significant loss of the local biodiversity. Al hyperaccumulators (Al > 0.1% in dry matter) are reported to occur in several wild species, present in more than 45 botanical families (Jansen et al. 2002). Most of them are woody and evergreen species, native to acidic soils in tropical and subtropical regions (Jansen et al. 2002). Al-tolerant species, hyperaccumulators or not, are important botanical materials for studies of gene and biomolecule prospection related to tolerance to soil acidity and to the metal itself (Oliveira et al. 2019).

Ni-rich ultramafic and Al-rich soils in the Cerrado region restrict the growth of plants not adapted to these edaphic conditions. To overcome Ni and Al toxicities and nutrient deficiencies, plants growing on both ultramafic and non-ultramafic Cerrado soils have evolved specific mechanisms to tolerate metal toxicity or deficiency. It is noteworthy that several hundreds of Ni and Al hyperaccumulator species evolved in such environments (Andrade et al. 2011; Jansen et al. 2002; Reeves et al. 2007). These are the cases for *Justicia lanstyachii* (Acanthaceae), *Euploca salicoides* (Boraginaceae) and *Oxalis hirsutissima* (Oxalidaceae), the native herbaceous species that occur in the ultramafic zones of the Goiás State as well as in the acidic, low-fertility soils through all of the of Central Plateau of Brazil. They accumulate large amounts of Ni (>0.1% in dry matter) when grown in soils with high Ni bioavailability. In acidic, low-Ni content soils, the Ni concentration in their tissues is consistent with the low availability of Ni in this type of environment. These species are therefore facultative metallophytes, as they can grow in both types of environments (Bothe and Słomkab 2017; Pollard et al. 2014). However, in an exploratory botanical survey on non-ultramafic areas, we found that *E. salicoides* is also an Al hyperaccumulator (>0.1% Al in dry matter).

The study aimed to understand how the edaphic factors contribute to the genetic selection and adaptation of native species in metal-rich environments in the Cerrado biome. The specific objectives of this work were: (i) to analyse the main edaphic factors that differentiate ultramafic from typical Cerrado environments; (ii) to determine the genetic diversity of three facultative metallophyte species, namely, *J. lanstyachii*, *E. salicoides* and *O. hirsutissima*, that occur in both ultramafic and Al-rich Cerrado soils; and (iii) to determine the edaphic factors that may contribute to the evolution of mechanisms underlying adaptation to harsh environments in *E. salicoides*, a **plant model** that hyperaccumulates metals.

Materials and methods

Site description

The selected study areas are located in the municipality of Barro Alto, Goiás State (four sites: SAP5, SAP7, SAP9, and LAT) and in the Federal District (FD) (two sites: CAM and LV) (Fig. 1). The soil and plant sampling locations and the characteristics of the sampling sites are shown in Table 1.

The first study area is located in the Barro Alto mafic-ultramafic complex. According to topography and rock properties (dunite, harzburgite, and others), different soils have evolved in the area from deep laterites (Geric Ferralsols, LAT) to shallower saprolitic soils (Magnesic Ferric Cambisol, SAP) (Fig. 1). The saprolitic sites (SAP5, SAP7, and SAP9) are found in the hills whereas the lateritic site (LAT), with a deeper soil profile, is located in lower and flat portions of the landscape. The natural vegetation is adapted to high levels of heavy metals and to the prolonged water deficits. It is composed mainly of herbs with sparsely distributed shrubs and rare trees. According to the Cerrado's vegetation classification system proposed by Ribeiro and Walter (2008), there is a mosaic of phytophysiognomies composed of *Campo Sujo* (shrub Cerrado, dominant grassland with sparse shrubs and rare trees), *Campo Rupestre*

Table 1 Location and characteristics of the soil and plant sampling sites

Landscape	Location and characteristics of the sites
Ultramafic environment	
	SAP5 Vegetation: *Cerrado Rupestre* Topography: strongly to gently sloping with outcrops Soil: rocky and shallow (< 0.50 m depth) (Cambisols) Bedrock: ultramafic rocks Latitude: 15° 06′ 07.2″ S Longitude: 49º 00′ 39.3″ W Elevation: 903 m
	SAP7 Vegetation: *Cerrado Rupestre* Topography strongly sloping; frequent outcrops Soil: rocky and shallow (< 0.50 m depth) (Cambisols) Bedrock: ultramafic rocks Latitude: 15° 06′ 20.6″ S Longitude: 49° 01′ 02.0″ W Elevation: 836 m
	SAP9 Vegetation: *Cerrado Rupestre* with patches of *Campo Sujo* Topography: gently sloping, frequent outcrops Soil: shallow (< 0.50 m depth) (Cambisols) Bedrock: ultramafic rocks Latitude: 15° 06′ 10.57″ S Longitude: 49° 01′ 54.8″ W Elevation: 822 m
	LAT Vegetation: *Campo Sujo* Topography: gently sloping with few outcrops Soil: deep B horizon (> 1 m depth) (Ferralsols) Bedrock: ultramafic rocks with laterite Latitude: 15° 06′ 29″ S; Longitude: 49° 01′ 14.7″ W Elevation: 860 m
Typical Cerrado environment	
	CAM Vegetation: *Cerrado sensu stricto,* anthropogenic area Topography: gently sloping with few outcrops Soil: shallow (< 0.50 m depth) (Cambisols) Bedrock: laterite Latitude: 15° 48′ 17.8″ S Longitude: 47° 47′ 34.6″ W Elevation: 1028 m
	LV Vegetation: *Cerrado sensu stricto,* mostly anthropogenic area Topography: flat Soil: deep B horizon (> 1 m depth) (Ferralsols) Bedrock: ferruginous detrital-lateritic deposits Latitude: 15° 45′ 59.8″ S Longitude: 47° 51′ 48.1″ W Elevation: 1032 m.

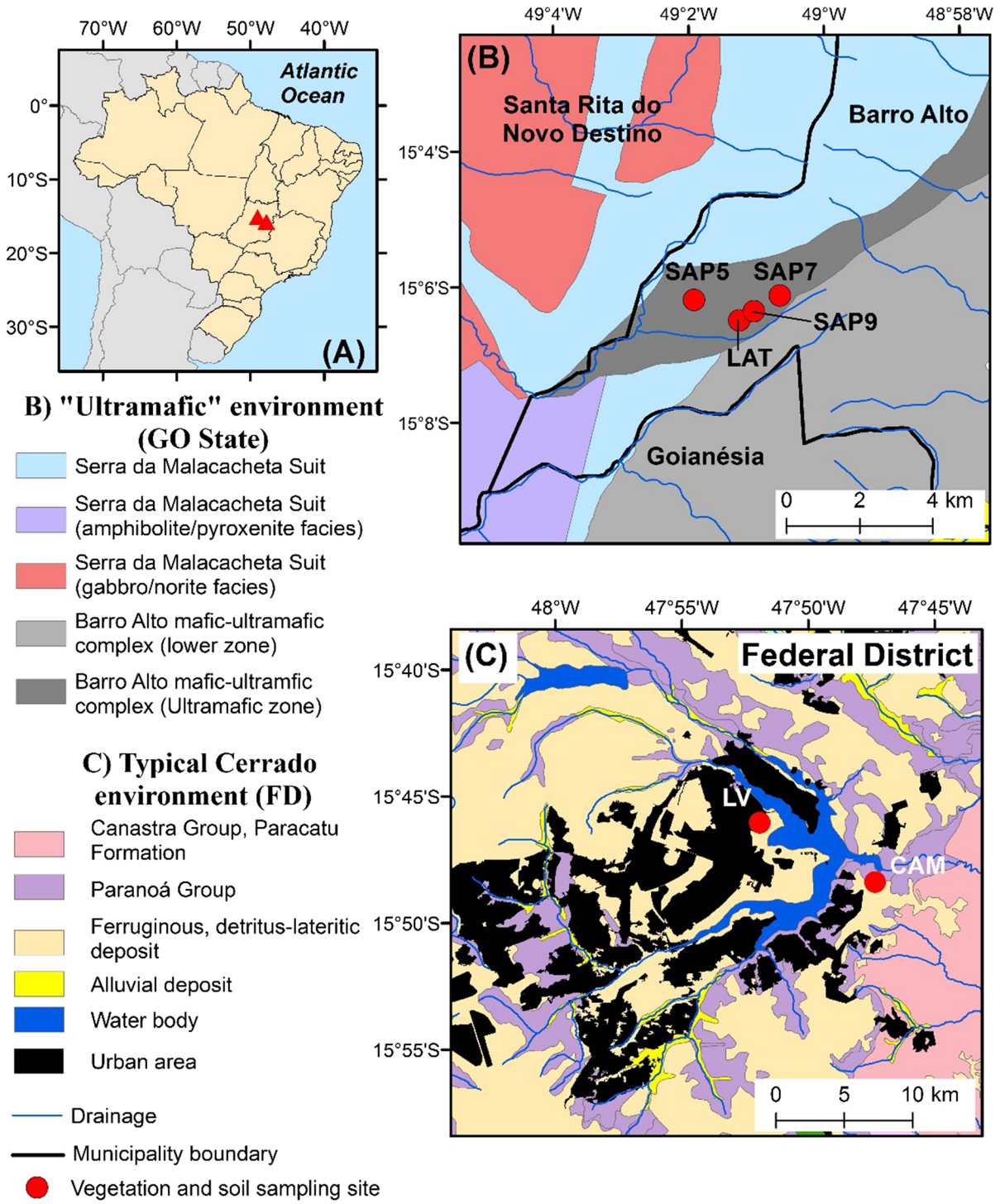


Fig. 1 Location of the study areas in the Goiás State and in the Federal District of Brazil (**A**). The locations of the vegetation and soil sampling sites in the municipality of Barro Alto, Goiás State and in the Federal District are shown in (**B**) and (**C**), respectively. The background map corresponds to the 1:500,000-scale geological map proposed by the Brazilian Geological Survey (CPRM). (adapted from Moreira et al. 2008)

(rupestrian grassland, dominant grassland with outcrops), and *Cerrado Rupestre* (rupestrian Cerrado, dominant shrublands with outcrops).

The second study area is located in the central part of the Federal District of Brazil and corresponds to the Tertiary/Quaternary, ferruginous, detritic and lateritic deposits covering most of the area (Moreira et al. 2008) (Fig. 1). The CAM site presents spaced outcrops and a shallow soil horizon (< 0.50 m; Cambisols) and is located close to the Lake Paranoá Lake reservoir. The LV site is located in a flat area with a deep diagnostic B horizon (> 1.0 m; Ferralsols), located approximately 15 km apart from the CAM site, on the campus of the University of Brasília. We selected these two sites because of distinct geological and edaphic formations from the Barro Alto ultramafic environment (Freitas et al. 1978; Moreira et al. 2008). Cerrado vegetation adapted to acidic, Al-rich, and low-fertility soils used to cover both areas; however, they are now intensively occupied by urban environments.

Soil sampling

Soil sampling was carried out at the ultramafic (SAP5, SAP7, SAP9, and LAT) and typical Cerrado (CAM) areas described in Table 1. Three composite soil samples, consisting of 10 subsamples each, were collected from each site at a 0–20 cm soil depth, using a stainless-steel "Edelmann"-type auger with 20 cm-long blades and a 7.6-cm diameter (Sondaterra Equipamentos Agronômicos Ltda. n.d.). Site LV (Table 1) was selected for having a geological and edaphic formation distinct from that of the ultramafic environment of Barro Alto, as confirmed by records of previous studies (Freitas et al. 1978, Moreira et al. 2008). Therefore, for the purpose of this study, a new chemical and physical characterization of that site were unnecessary.

The soil samples were air-dried and ground using a 2-mm sieve. The soil chemical characterization was carried out in the Soil Laboratory of Embrapa Cerrados following the procedures described by Embrapa (1999) for organic matter (OM); pH in water (1:2.5); exchangeable Ca^{2+}, Mg^{2+}, and Al^{3+} extracted by 1 mol L^{-1} KCl; and P and K extracted with Mehlich-1 solution (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4). P was determined by UV–Vis spectrophotometry, K by flame photometry, Ca^{2+} and Mg^{2+} by atomic absorption spectrophotometry, OM by Walkley—Black method, and Al^{3+} by titration with 0.025 mol L^{-1} NaOH solution as the titrant and bromothymol blue as a turning point indicator. The potential acidity (H + Al) was detected with 0.5 mol L^{-1} calcium acetate at pH 7. Co, Cu, Fe, Mn, Ni, and Zn were extracted with a DTPA solution (0.005 mol L^{-1} DTPA + 0.01 mol L^{-1} $CaCl_2$ + 0.1 mol L^{-1} triethanolamine, pH 7.3) (Baker and Amacher 1982). We determined the concentration of metals in the DTPA extracts was determined by inductively coupled plasma optical emission spectroscopy (ICP–OES, Thermo Fisher Scientific 7000).

Plant species

The three native plant species selected in this study occur in well-drained terrain, covered by savanna physiognomies. They are herb or subshrub species with dry fruits. *Euploca salicoides* (Cham.) J.I.M.Melo & Semir (Boraginaceae) reaches heights of 20–90 cm, with yellow flowers; *Justicia lanstyachii* Rizzini (Acanthaceae) reaches heights of 15–90 cm, with red flowers; and *Oxalis hirsutissima* Mart. ex Zucc. (Oxalidaceae) reaches heights of 15–60 cm, with yellow flowers (Flora do Brasil 2020) (Table 2).

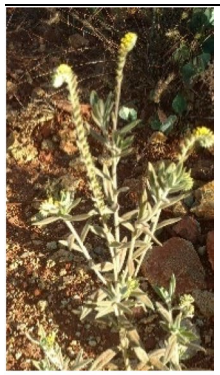
At least one individual of these species collected in the ultramafic area was shown to hyperaccumulate Ni in the aboveground tissues (Andrade et al. 2015). The *E. salicoides* specimens sampled in Al-rich soils (hereafter, named typical Cerrado soils) also hyperaccumulate Al in their tissues (> 1,000 mg kg^{-1} Al in dry matter). Therefore, we chose the *E. salicoides* metallophyte species as a model for studying the morphological, biochemical, and metal compartmentation mechanisms underlying plant adaptation to the metal-rich environments.

Characterization of plant genetic diversity and mechanisms of plant adaptation to the environment

Sample processing for DNA extraction

At the ultramafic (SAP5, SAP7, and SAP9) and typical Cerrado (CAM) sites, two or three individuals of *E. salicoides* (ES), *O. hirsutissima* (OH), and *J. lanstyakii* (JL) were collected to form a composite sample of each plant species, totalling 14 botanical accessions. The plants were in good condition and were growing at least two metres apart. We stored the collected materials in plastic bags and then in polystyrene boxes with ice. We sent the

Table 2 Plant species accessions and corresponding identification codes

Plant species image	Plant species (botanical family)	Environment	Soil site*	Botanical accessions	DNA accession
	*Euploca salicoides* (ES) (Boraginaceae)	Ultramafic	SAP5	ES-P5	1
			SAP7	ES-P7	2
			SAP9	ES-P9	3
			SAP5	ES-SAP5**	-
			LAT	ES-LAT**	-
		Typical Cerrado	CAM	ES-CAM1	12
				ES-CAM2	14
			LV	ES-LV**	-
	*Justicia lanstyakii* (JL) (Acanthaceae)	Ultramafic	SAP5	JL-P5	7
			SAP7	JL-P7	8
			SAP9	JL-P9	9
		Typical Cerrado	CAM	JL-CAM	10
	*Oxalis hirsutissima* (OH) (Oxalidaceae)	Ultramafic	SAP5	OH-P5	4
			SAP7	OH-P7	5
			SAP9	OH-P9	6
		Typical Cerrado	CAM	OH-CAM1	11
				OH-CAM2	13

*SAP5, SAP7, and SAP9 are soils associated with saprolites, while LAT is related to lateritic formation, all derived from serpentinic ultramafic rocks. CAM and LV are soils derived from ferruginous detrital-lateritic deposits. ** ES-SAP5, ES-LAT, and ES-LV accessions were used only for morphological features and non-structural carbohydrate (NSC) determinations. A voucher specimen of each species sampled in the field was deposited at the Herbarium of Embrapa Genetic Resources and Biotechnology (Cenargen) in Brasília, Federal District

boxes to the Laboratory of Plant Genetics and Molecular Biology of Embrapa Cerrados on the same day of plant sampling. They were processed and aliquoted for DNA analysis.

We extracted genomic DNA samples from each accession using the modified cetyltrimethylammonium bromide (CTAB) method (Faleiro et al. 2003) and by washing in sorbitol buffer (0.35 M). We estimated the amount of DNA by spectrophotometry at 260 nm (A260). The purity and quality of the samples were evaluated by the A260/A280 ratio (Sambroock et al. 1989). The DNA samples from each accession were diluted to 5 ng μL^{-1}. The amplification reactions to obtain *inter-simple sequence repeat* (ISSR) markers were carried out with 4.9 µL of Milli-Q water, 1.3 µL of buffer, 0.39 µL of 50 mM $MgCl_2$, 0.26 µL of deoxyribonucleotides (dATP, dTTP, dGTP and dCTP) at 10 µM, 1.95 µL of a 2 µM primer (Operon

Technologies Inc., California, USA), 0.2 µL of Taq DNA polymerase enzyme (1 unit), and 3 µL of DNA (15 ng) (total volume of 13 µL). Initially, 18 ISSR primers were tested (Table 3). From these tests, we selected eight primers and used them to obtain ISSR markers that generated a more significant number of polymorphic bands and presented better amplification quality.

Sample preparation for chemical analysis of shoot tissues

We sent all remaining tissue material (leaves and stems together) from each sample, after removing the aliquot for DNA analysis, to the Laboratory of Plant Chemical Analysis of Embrapa Cerrados for element characterization. Other plant specimens of each species were collected at the same sites to increase the number of plant replicates for chemical analysis. Each replicate was composed of at least three individuals. The total number of replicates was dependent on the availability of individuals of each species present in the area during the field campaign. In the laboratory, the plant materials were slightly immersed in tap water and then in the deionized water to remove soil particles. They were air-dried at room temperature. The materials were then placed in paper bags and oven-dried at a temperature of 40 °C until a constant weight. Next, the plant materials were finely ground in a knife mill and mineralized by moist digestion with a mixture of perchloric acid and hydrogen peroxide in a 2:1 proportion (v/v) to determine the contents of Ca, Mg, P, K, S, Co, Cu, Fe, Mn, Ni, and Zn by inductively coupled plasma–optical emission spectrometry (ICP–OES). We digested the plant tissues by the micro-Kjeldahl method for N analysis and analysed by UV–Vis spectrophotometry or flow injection analysis (FIA, Lachat Quikchem 6000 system) coupled with UV/VIS spectrophotometry.

Table 3 Primers tested and used to obtain the *inter-simple sequence repeat* (ISSR) markers for 14 accession sequences (5 '→ 3') and the number of polymorphic bands (PBs)

Primer ISSR	Sequence (5'→3')	PBs
1-TriAAG3'RC	AAGAAGAAGAAGAAG	-
2-TriACA3'RC	ACAACAACAACAACA	-
3-RriCAA3'RC	CAACAACAACAACAA	-
*4-TriAAC3'RC	AACAACAACAACAAC	15
*5-TriAGC3'RC	AGCAGCAGCAGCAGC	13
*6-TriAGG3'RC	AGGAGGAGGAGGAGG	16
7-TriCAG3'RC	CAGCAGCAGCAGCAG	-
8-DiGA5'C	CGAGAGAGAGAGAGA	-
*9-DiCA3'YG	CACACACACACACAC	7
*10-DiCA5'CR	CACACACACACACAC	8
11-DiGT3'YG	GTGTGTGTGTGTGTG	-
12-DiCA3'G	CACACACACACACAC	-
*13-DiGA3'C	GAGAGAGAGAGAGAG	21
*14-DiGA5'CY	AGAGAGAGAGAGAGA	12
15-DiGT5'CY	GTGTGTGTGTGTGTGT	-
*16-DiGA3'YC	GAGAGAGAGAGAGAG	16
17-DiGA3'T	GAGAGAGAGAGAGAG	-
18-DiCA3'RG	CACACACACACACAC	-
Total		108

* Primers selected and used to generate ISSR markers in the 14 accessions tested

Morphological features and non-structural carbohydrate(NSC)determination in E. salicoides plants

Accessions of the *E. salicoides* model species, a species that can both hyperaccumulate Ni and Al in their tissues, were collected at two ultramafic sites, previously characterized as contrasting in terms of DTPA bioavailable levels of Ni: LAT (~100 mg Ni dm^{-3}; ES-LAT accession) and SAP5 (~650 mg Ni dm^{-3}; ES-SAP accession), for measurements of some morphological features and for non-structural carbohydrate (NSC) determination in the Laboratory of Plant Physiology of University of Brasília. An ES control accession originating from plants growing at the LV site (<1 mg Ni dm^{-3}; ES-LV accession), located at the campus of University of Brasilia, in FD, was included in the analysis.

At the peak of the dry season (June to August), we selected three plants growing 2–3 m apart, cut them near the ground, and collected them for determination of the number of inflorescences/plant, number of branches/stems, stem length, and internode length. We measured stem length from the cut base of the plant to the shoot tip. The length of the internodes corresponded to the distance between the insertions of two leaves on the main stem. The measurements were obtained using a pachymeter. After completing the measurements, we sent the plant materials for destructive analysis.

Leaf samples of ES-SAP, ES-LAT, and ES-LV accessions were lyophilized and ground (10 mg) for total soluble sugar (TSS) content determination. We extracted soluble sugars four times with 80% ethanol (500 µL) at 80 °C for 40 min. After centrifugation (10,000 g, 10 min), the supernatants were combined and depigmented by the modified Shannon method (Shannon 1968). In a separating funnel, we added the ethanolic fraction (2.0 mL), absolute ethanol (0.5 mL), chloroform (3.0 mL), and water (5.5 mL) in sequence. Separation occurred after a period of approximately 12 h. We measured TSSs according to the phenol-sulfuric method (Dubois et al. 1956). For comparison purposes, a standard glucose curve (SIGMA) was used at the concentrations of 0, 5, 10, 20, 40, and 80 µg mL^{-1}. We also collected and preserved supernatants containing TSSs for further analysis. The precipitate was used to remove the other components.

For TSSs analysis, the alcoholic fractions were dried, resuspended in water (1 mL), passed through an anionic and cationic exchange column (Dowex), and analysed by a High–Performance Anion–Exchange Chromatography coupled with Integrated Pulsed Amperometric Detection (HPAEC/IPAD) in a CarboPac PA-10 column (Dionex Corporation, Sunnyvale, Ca, USA) using an elution gradient with 200 mM NaOH in water for 30 min. We compared the detector responses with the patterns of glucose, fructose, sucrose, and raffinose at 0.625, 1.25, 2.5, 5.0, 10.0, and 20.0 µM. The standard curve for each sugar was used to calculate the carbohydrate content in the leaves.

Localization of Ni in cellular compartments of E. salicoides leaves

We randomly selected and collected three *E. solicoides* (ES-P5) plants at the SAP5 site for use in microscopic analysis for the localization, distribution, and relative concentration of Ni in the leaf cells. We cut the third pair of leaves that preceded the youngest leaflet into small segments with a stainless-steel blade. The cuttings were made so that leaf blade tissue and secondary ribs were in the same segment and placed in a small test tube (10 mL) containing 5 mL of 70% ethanol. They were maintained in this environment until processing for analysis. In the laboratory, we transferred approximately 10 segments of each sample collected in the field, containing the upper and lower epidermis, to Petri dishes with 100% ethanol. Then, we cut the segments into thin pieces (1 mm × 3 mm length), fixed them with adhesive tape, placed them on carbon cassettes ("stubs") and dried them at room temperature. In these samples, we observed the localization of Ni accumulation in tissues by using a scanning electron microscope with energy dispersive X-ray analysis (SEM/EDXS) (Hitachi-S4800 SEM) with accelerating power ranging from 5 to 15 kV.

Statistical analysis

Since the assumption of normality was not met, we analysed the data of metal content from soils and in plant tissues based on a *non-parametric* statistical approach. Comparisons between treatment *medians* were made with the Kruskal–Wallis or the Mann–Whitney U test at a significance level of $p \leq 0.05$ (Doria-Filho 1999). We considered the principal component analysis (PCA) to verify the site groups that eventually formed based on the soil chemical characteristics and plant tissue accumulation patterns. The value used for communality was > 0.70 (Hair-Jr et al. 2009).

In the DNA analysis, the ISSR markers were converted into a binary matrix from which the genetic dissimilarity between the different genotypes was estimated based on the complement of the Nei and Li similarity coefficient (Nei and Li 1979) using the Genes program (Cruz 2013). The genetic similarity (GS) was given by $Gsij = 2Nij/(Ni + Nj)$, where: Nij is the number of bands present in both genotypes i and j and Ni and Nj are the numbers of bands present in genotypes i and j, respectively. We obtained GS by subtracting the unit's GS value by 1 (1 - GS). The genetic dissimilarity matrix was used to conduct cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) approach (Sneath and Sokal 1973) as the clustering criterion and the graphic dispersion based on multidimensional scales using the main coordinate method.

The effects of treatments (soil type) on the production of total sugars by *E. salicoides* plants were analysed using a one-way analysis of variance (ANOVA),

followed by Tukey's test when the significance level was < 0.05.

Statistical analyses were performed using SAS (SAS Institute Inc. 2008), Statistica (StatSoft Inc. 2007), and R software, version 3.6.0 (R Core Team 2019).

Results

Soil fertility

The ultramafic soils in the Barro Alto region (SAP5, SAP7, SAP9, and LAT) presented pH in water > 6 (6.3–6.8), medium to high levels of cation exchange capacity (CEC) at pH 7 (T) (9.4–13.3 $cmol_c\ dm^{-3}$), high base saturation (V) (57–73%), and high Mg (5.7–7.1 $cmol_c\ dm^{-3}$), Co (1.7–5.1 mg dm^{-3}), Cu (3.0–5.9 mg dm^{-3}), Mn (11–111 mg dm^{-3}), Ni (108–1025 mg dm^{-3}), and Zn (1.1–5.5 mg dm^{-3}) bioavailabilities (Table 4). The average Mg contribution to the T values (Mg/T, %) observed in ultramafic soils was 55% (minimum of 42.5% and maximum of 63.1%) in contrast to less than 4% found at the typical Cerrado soil (CAM), in the FD. The CAM soil also showed high exchangeable Al^{3+}(0.6 $cmol_c\ dm^{-3}$), contributing to more than 40% of its CEC, low pH (5.6), V (17%), and Mg:Ca (< 0.5 mg dm^{-3}) values. It was also deficient in all micronutrients, except Fe, which availability (398 mg dm^{-3}) was significantly higher than the one observed in ultramafic soils (average 31 mg dm^{-3}). The soils of both environments presented very low P (< 1.0 mg dm^{-3}), medium availability of K (< 0.15 $cmol_c$ mg dm^{-3}) and Ca (< 1.0 $cmol_c$ mg dm^{-3}), except the SAP5 soil in which K and Ca were high (> 1.5 $cmol_c$ Ca dm^{-3}), and medium to high levels of OM (2.4–5.5%).

The Fig. 2b shows the PCA of the plant-potential bioavailable metals (DTPA). The first principal component explained 69.1% of the variance, with the contents of Co, Ni, and Zn as the correlation factors (communalities $r^2 \geq 0.70$), where SAP7 and SAP5 were the soils that presented the highest levels of those metals (Table 4; Fig. 2b). In contrast, Al^{3+} and Fe were strongly related to the CAM soil. The second principal component explained 19.9% of the variance, indicating higher bioavailability of Mn and Cu in the SAP9 soil.

The PCA describing chemical attributes of the soils showed a clear separation between the ultramafic soils and the typical Cerrado (CAM) (Fig. 2a). The first principal component explained 52.5% of the variance and was associated with pH, T, SB, Mg, and Mg/Ca. The CAM soil presented negative values of the first principal component while the ultramafic soils presented positive values. The CAM soil presented a significant influence of the Al^{3+} attribute. The second principal component explained 35.4% of the variance and was associated with K, P, H + Al, Ca, and OM (communalities for $r^2 \geq 0.7$). Regarding the ultramafic sites, two subgroups were formed: SAP5 soils located in the first quadrant, with higher levels of K, P, H + Al, Ca, and OM, and the other three soils (LAT, SAP7, and SAP9) in the second quadrant, having significantly higher pH, Mg, Mg:Ca, and SB.

Elemental characterization of botanical accessions

The nutrient concentrations in plant shoots varied widely among the *E. salicoides* (ES), *J. lanstyakii* (JL), and *O. hirsutissima* (OH) and between populations of the same species growing at the different sites. The superposition of the correlation circles from the centroid values relative to the ultramafic Cambisols (SAP5, SAP7, and SAP9) indicated that the differences in nutrient concentrations were insufficient to discriminate the three species (Fig. 3). However, the circle of correlations relative to the CAM soil was highlighted. The average concentrations of chemical elements in the plant tissues of the three selected species growing in ultramafic (SAP_T soil) and *typical* Cerrado (CAM soil) environments are shown in Table 5. The three species accumulated similar amounts of P and S growing in both soil types. Plants growing in the ultramafic environment had higher levels of Ni and Co in their tissues.

ES absorbed similar amounts of Al and nutrients in both soils, but less Fe when grown in CAM soil. JL and OH absorbed more Ca, Cu and Al when grown in CAM soil. The Mg:Ca and Ca:Ni ratios were higher in the plant tissues growing in the ultramafic environment.

Table 4 Chemical attributes of soils from the ultramafic zone of Barro Alto and from the typical Cerrado

A.Soil environment		Ultramafic				Typical Cerrado
Soil attribute	Unit	SAP5	SAP7	SAP9	LAT	CAM
pH	H_2O	6.29 d	6.52 c	6.80 a	6.68 b	5.60 e
sd		0.067	0.05	0.08	0.04	0.11
OM	%	5.50 a	4.27 a	2.77 b	2.43 bc	2.05 c
sd		0.17	0.1	0.37	0.59	0.08
H+Al	$cmol_c\ dm^{-3}$	5.65 a	4.74 a	3.52 b	2.49 c	3.86 b
sd		0.56	0.13	0.91	0.22	0.1
Al^{3+}		0.02 bc	0.02 b	0.01 bc	0.00 c	0.60 a
sd		0.01	0.01	0.01	0.01	0.14
Ca^{2+}		1.70 a	0.63 c	0.77 b	0.90 b	0.51 c
sd		0.27	0.08	0.06	0.19	0.14
Mg^{2+}		5.65 ab	7.13 a	6.99 a	5.93 a	0.17 b
sd		0.98	0.59	1.68	0.86	0.08
K^+		0.25 a	0.13 b	0.09 c	0.07 c	0.13 b
sd		0.02	0.01	0.02	0.02	0.02
P	$mg\ dm^{-3}$	0.64 a	0.44 bc	0.48 ab	0.34 c	0.52 ab
sd		0.02	0.02	0.06	0.1	0.15
Co	$mg\ dm^{-3}$	2.21 b	5.08 a	4.78 a	1.70 b	0.01 c
sd		0.42	0.27	0.96	0.51	0.00
Cu		3.61 b	2.97 b	5.86 a	3.03 b	0.15 c
sd		0.65	0.72	1.19	0.28	0.03
Fe		16.27 e	27.58 d	47.44 b	34.05 c	398.78 a
sd		7.8	1.75	3.52	1.57	11.55
Mn		11.12 c	43.03 b	111.44 a	33.46 b	1.79 d
sd		7.8	17.6	25.13	10.57	0.26
Ni		461.03 b	1025.44 a	394.63 b	107.96 c	< 0.01 d
sd		69.86	27.81	131.7	13.64	-
Zn		3.48 b	5.48 a	2.81 c	1.06 d	0.81 e
sd		0.57	0.09	0.6	0.14	0.03
T	$cmol_c\ dm^{-3}$	13.25 a	12.63 a	11.36 ab	9.39 bc	4.66 c
sd		1.62	0.69	2.52	1.14	0.27
SB		7.60 a	7.88 a	7.84 a	6.90 ab	0.81 b
sd		1.09	0.56	1.75	0.99	0.22
CEC		7.61 a	7.90 a	7.85 a	6.90 ab	1.41 b
sd		1.09	0.56	1.75	0.99	0.08
V	%	57.26 d	60.99 c	69.14 b	73.32 a	16.76 e
sd		1.55	1.2	3.81	2.26	3.8
m		0.20 bc	0.33 ab	0.13 bc	0.08 c	43.24 a
sd		0.15	0.15	0.07	0.09	12.42
Mg:T	%	42.47 c	56.39 b	61.43 a	63.07 a	3.61 d
sd		2.18	1.7	3.18	2.85	1.25
Mg:Ca		3.35 d	11.38 a	9.07 b	6.70 c	0.33 e
sd		0.59	2.18	1.53	1.07	0.05

The numbers correspond to the average values obtained from three composite samples per site, collected at a 0–20 cm soil depth. One composite sample consisted of 10 subsamples; sd = standard deviation; OM = organic matter; H + Al = titratable acidity; Al^{3+}, Ca^{2+}, and Mg^{2+} extractable by 1 mol L^{-1} KCl; K and P extractable with Mehlich-1 solution; Co, Cu, Fe, Ni, Mn, and Zn extractable with DTPA solution; SB = sum of bases; CEC = cation exchange capacity at soil pH; T = CEC at pH 7; V = base saturation; m = Al^{3+} saturation in CEC; means followed by the same letters within the same line are not significantly different according to the Kruskal–Wallis test ($p \geq 0.05$)

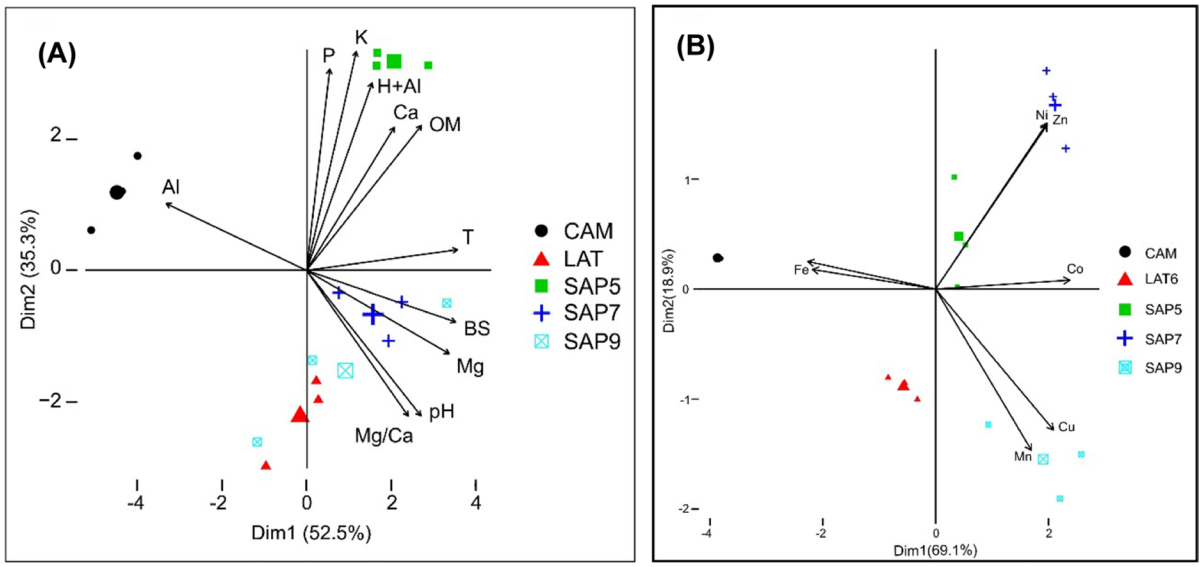


Fig. 2 Principal component analysis based on the soil chemical attributes (**A**) and bioavailable Al and micronutrients (**B**). The arrows represent the ability of each attribute to separate the sites where the botanical accessions were collected. The values in parentheses on each axis refer to the percentage of variance explained

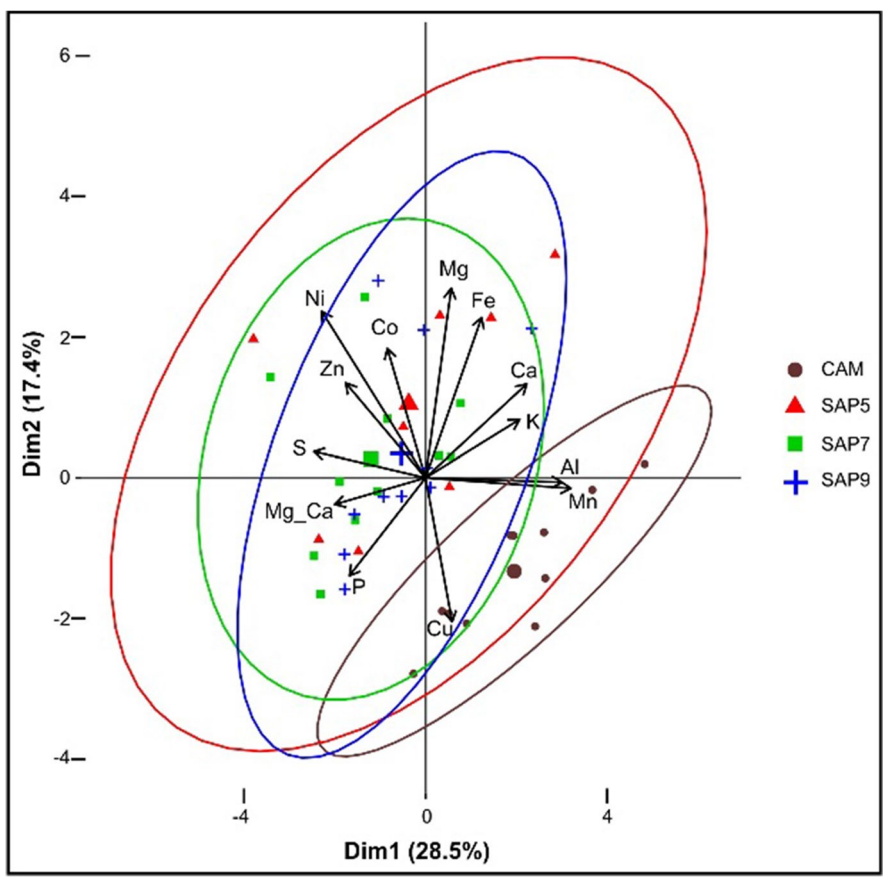


Fig. 3 Principal component analysis representing the correlation of the sites according to the chemical elements in the plant tissues of the accessions collected in ultramafic (SAP5, SAP7, and SAP9) and typical Cerrado (CAM) environments. The arrows represent the ability of each attribute to separate the sites where the botanical accessions were collected. The values in parentheses on each axis refer to the percentage of variance explained

Table 5 Chemical element concentrations in aboveground tissues of native plants in the ultramafic (SAP_T) and typical Cerrado (CAM) environments

Site	N	Ca	Mg	K	P	S	Mg:Ca	Al	Co	Cu	Fe	Mn	Ni	Zn	Ca:Ni
	g kg^{-1}							mg kg^{-1}							
	Euploca salicoides (ES)														
***SAP_T**	13.8	21.7 A	8.5 A	9.3 A	0.9 A	2.4 A	0.44 A	245 A	9.3	12.8 A	2244 A	79 A	1154	43 A	24.73
sd (8)	1.6	9.0	1.6	2.8	0.1	0.4	0.07	106	3.4	3.8	1604	24	112	12	13.88
CAM	16.6	16.0 A	4.5 A	10.8 A	0.8 A	1.6 A	0.29 B	340 A	0.12	9.3 A	279 B	126 A	28	24 A	> 1000
sd (3)	**	9.6	2.6	6.2	0.33	0.8	0.05	275	***	5.6	253	79	***	23	> 1000
	Justicia lanstyakii (JL)														
SAP_T	17.4	5.5 B	10.6 A	9.9 B	1.2 A	2.3 A	2.36 A	188 B	6.0 A	0.1 B	1290 A	104 B	1874 A	43 A	2.88
sd (12)	1.5	0.2	3.4	1.9	0.2	0.7	0.44	31	0.9	0.1	147	14	615	3	0.91
CAM	19.1	23.1 A	10.1 A	17.4 A	1.0 A	1.4 A	0.47 B	525 A	0.2 B	7.4 A	521 A	365 A	4 B	16 B	> 1000
sd (4)	**	11.5	4.2	4.8	0.1	0.3	0.19	205	0.1	4.9	296	46	4	16	> 1000
	Oxalis hirsutissima (OH)														
SAP_T	20.2	1.0 B	5.7 A	9.5 A	1.5 A	3.1 A	6.17 A	115 B	6.5 A	7.9 B	966 A	61 A	699 A	31 A	1.46
sd (9)	3.31	0.2	1.2	3.3	0.3	0.9	0.44	96	3.6	0.9	1106	30	34	3	0.27
CAM	22.1	5.6 A	2.4 A	10.5 A	1.5 A	2.2 A	0.45 B	387 A	0.4 B	16.9 A	357 A	139 A	25 B	27 A	> 1000
sd (4)	8.6	1.9	1.0	6.2	0.5	0.9	0.27	136	0.1	4.4	100	105	30	5	> 1000

*SAP_T = average values of elements absorbed by plants from soils SAP5, SAP7 and SAP9; sd (n) = standard deviation (number of samples, each one composed of at least three plants). ** = the collected material was insufficient to perform more than one analysis of N; *** = Ni or Co was detected in only one sample; > 1000 = very low Ni concentration values generate very high Ca:Ni ratio values. Means followed by the same letters within the same column and species are not significantly different according to the non-parametric Mann–Whitney U test at the 5% probability level

Plant diversity

The analysis of the 14 accessions studied using eight primers generated 108 ISSR markers, with an average of 13.5 markers per primer. The high percentage of polymorphic markers and the high average number of markers per primer demonstrated the high genetic variability among the accessions of the three species. Genetic dissimilarities ranged from 0.17 to 1.00 (Table 6).

The greatest distances were observed for the accessions of *E. salicoides* collected in the typical Cerrado soil (ES-CAM1 and ES-CAM2) and the other accessions of *E. salicoides* collected in the ultramafic area (ES-P5, ES-P7 and ES-P9), and with the other species (1.0). However, *O. hirsutissima* accessions OH-P7 and OH-P9 (0.17), *E. salicoides* accessions ES-P5 and ES-P7, and ES-P7 and ES-P9 (0.18), and *J. lanstyakii* accessions JL-P7 and JL-P9 (0.19) showed small genetic distances. Cluster and graphical dispersion analyses showed the divergence between accessions (Fig. 4).

In addition to the divergence between the accessions, the cluster analysis also showed the formation of five similarity groups, considering a genetic distance of 0.6 as the threshold: Group 1 formed by the ES-P5, ES-P7, ES-P9, and ES-CAM1 accessions (numbers in the graph: 1, 2, 3, and 12, respectively); Group 2 formed by the OH-P5, OH-P7, and OH-P9 accessions (numbers in the graph: 4, 5 and 6, respectively); Group 3 formed by JL-P5, JL-P7, JL-P9, and JL-CAM accessions (numbers in the graph: 7, 8, 9, and 10, respectively); Group 4 formed by the OH-CAM1 and OH-CAM2 accessions (numbers in the graph: 11 and 13, respectively); and Group 5 formed by only the ES-CAM2 accession (number in the graph = 14).

Plant morphology and carbohydrate production

Euploca salicoides collected at the SAP5 and LAT sites presented thinner branches, with well-spaced internodes and well-developed inflorescences, showing larger plant sizes. On the other hand, plants growing in the non-ultramafic site LV had reduced internode distances, with accentuated hairiness on the leaves, stems, and buds in the leaf axils (Fig. 5).

Table 6 Genetic dissimilarity matrix between 14 accessions calculated based on the complement of the Nei and Li similarity coefficient, using 108 ISSR markers

Accessions	ES-P7	ES-P9	OH-P5	OH-P7	OH-P9	JL-P5	JL-P7	JL-P9	JL- CAM	OH- CAM1	ES-CAM1	OH- CAM2	ES-CAM2
ES-P5	0.18	0.33	0.80	0.83	0.87	0.95	0.85	0.81	0.88	1.00	0.57	0.92	1.00
ES-P7		0.18	0.81	0.84	0.88	0.96	0.88	0.85	0.89	0.93	0.60	0.85	1.00
ES-P9			0.74	0.77	0.80	0.88	0.88	0.86	0.95	0.94	0.56	0.87	1.00
OH-P5				0.40	0.38	0.70	0.66	0.68	0.92	1.00	1.00	1.00	1.00
OH-P7					0.17	0.83	0.73	0.79	0.88	0.95	1.00	1.00	0.94
OH-P9						0.82	0.74	0.78	0.96	0.95	1.00	1.00	0.94
JL-P5							0.34	0.40	0.58	0.89	1.00	0.94	1.00
JL-P7								0.19	0.55	0.93	1.00	1.00	0.92
JL-P9									0.48	0.91	1.00	0.95	0.89
JL-CAM										0.85	1.00	1.00	0.79
OH-CAM1											1.00	0.50	1.00
ES-CAM1												0.87	1.00
OH-CAM2													1.00

There were no significant differences in the numbers of branches, leaves, and inflorescences per plant obtained from specimens collected from different sites and environments (Fig. 6A). However, plants growing in the ultramafic environment (LAT and SAP5) showed differences in growth, with longer branches (Fig. 6B) and internodes (6C) than those from the typical Cerrado (LV).

To understand whether the basal metabolism of plants is altered in the presence of greater availability of metals (Ni and Al) in the soil, analyses of the content of total soluble sugars content (TSSs) and types of soluble sugars were carried out in plants growing in ultramafic soils (SAP5 and LAT) and typical Cerrado soil (LV). The specimens collected at SAP5 showed higher levels of TSSs than those collected at the LAT and LV sites (Fig. 7A). Additionally, the pentose carbohydrate raffinose was detected only in plants growing in SAP soil (Fig. 7B).

Ni localization in the leaf tissues of *Euploca salicoides*

In our study, using SEM coupled to an EDXS probe, we searched for Ni in various cell compartments where other metals were reported to accumulate in leaves (e.g., epidermal cells and guard cells). We observed that the surface architecture on both sides of *E. salicoides* leaves was characterized by a dense pubescence formed by non-glandular trichomes. The trichome surface was microsculptured with numerous prominent protrusions. Ni localization was observed only for the protrusions of trichomes of *E. salicoides* leaves collected at the ultramafic site (Fig. 8).

Discussion

Edaphic factors that differentiate ultramafic soils from typical Cerrado soils

Some metal availability in soils is regulated by redox reactions influenced by soil pH and by the metal's concentration in the parent materials. The syndrome of ultramafic soils, i.e., 'serpentine syndrome', is related to the combined physical, chemical, and biological factors associated with serpentine soils (Kruckeberg 2002). It manifests as

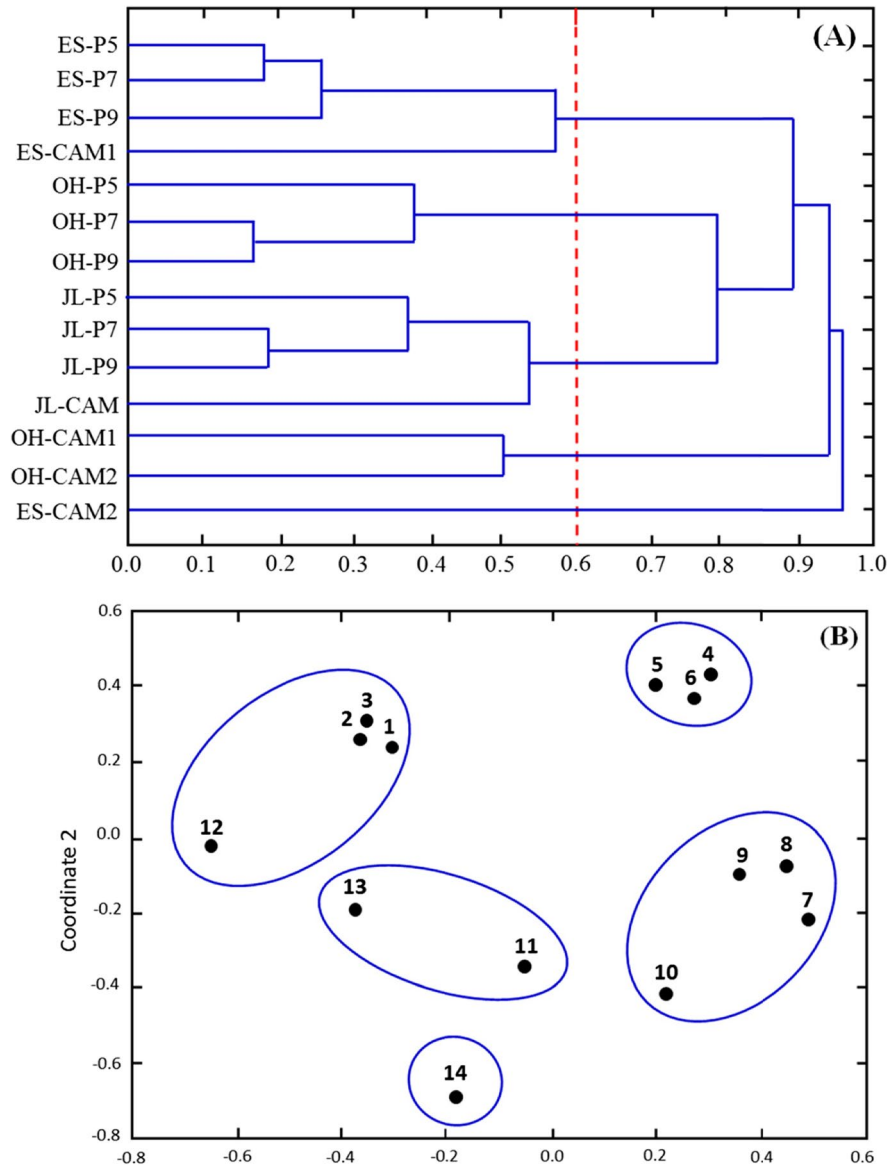


Fig. 4 Cluster analysis (**A**) and graphical dispersion (**B**) of 14 accessions based on the genetic dissimilarity matrix calculated from 108 ISSR markers. The value of the coffonetic correlation coefficient (r) was 0.88. The accession numbers in the scatter plot correspond to the accession numbers shown in Table 2

competition between Mg, with high availability in the environment, and Ca for exchange sites on the root surfaces, resulting in a Mg:Ca ratio > 1 and Ca deficiency in plants. In our study, exchangeable Mg was much more abundant than Ca in the ultramafic soils resulting in a high Mg:Ca ratio (ranging from 3 to 11), which can be unfavorable for the development of non-adapted plants. The predominance of Mg over Ca, due to the presence of Mg-rich weathering minerals, in addition to the presence of bioavailable heavy metals (Ni, Co, Cr, Cu, Mn, Zn, Cr, and others) at high levels, and the low levels of Al are the most significant soil features derived from ultramafic rocks in the world (Echevarria 2018). Soils developed from ultramafic rocks (peridotite and pyroxenite) in the Central Plateau of Brazil (ultramafic complexes of Niquelândia and Barro Alto) are reported to be particularly rich in Ni and Cr (Garnier et al. 2006; Lima 2010). On the other hand, typical Cerrado soils are well documented as having, except Al and Fe, low levels of these heavy metals, most of which are essential for plant development (Gomes et al. 2004; Marques et al. 2004).

Fig. 5 Field photos of *E. salicoides* plants growing at an ultramafic site (SAP5) (**A**) and at a typical *Cerrado* site (LV) (**B**). In (**C**) and (**D**), the corresponding details of a branch with long internodes and a hairy branch with short internodes are shown

Sensitive plants growing in acidic soils, with high Al saturation at cation exchange sites, have reduced root systems. As a consequence, they experience reduced water absorption and a variety of nutrient deficiency symptoms, such as induced Ca deficiency.

Plant-soil relationships

Calcium and Mg are essential elements for plant growth. Calcium's roles in root development and the alleviation of growth inhibition by salts and metal stresses are well known (Aziz et al. 2014; Kinraide 1998). Plants can grow satisfactorily when Ca is in low concentration in soil solution since other divalent ions (as Mg) are also maintained at low concentrations (Mengel et al. 2001). In typical Cerrado soils, the availability of Ca is usually higher than that of Mg, although both are at very low levels (Ca + Mg < 1 cmolc kg of soil; Mg/Ca < 0.5). Haridasan (1982) found a Mg:Ca relation of approximately 0.5 in tissues of Cerrado Al-hyperaccumulators species. In our studies, ultramafic soils showed a Mg:Ca ratio > 3. *E. salicoides* growing in both environments absorbed higher amounts of Ca than Mg, resulting in a Mg:Ca ratio < 1 in plant tissues. It seems this species shows selectivity, i.e., more efficiency in absorbing Ca in the presence of high concentrations of Mg in soil, as pointed out by Kazakou et al. (2008). Contrary to what is frequently observed in several species growing in ultramafic areas worldwide (Reeves et al. 2022), in *J. lansthyakii* and *O. hirsutissima* the concentration of these nutrients in the tissues reflected the trend of absorption of the element with greater availability in the environment: SAP: Mg:Ca ratio > 1, and CAM: Mg:Ca ratio < 1. Despite these differences, the levels of Ca and Mg in plant tissues of the three species are compatible with those observed in native species of both environments studied (Haridasan 1982; Reeves et al. 2007).

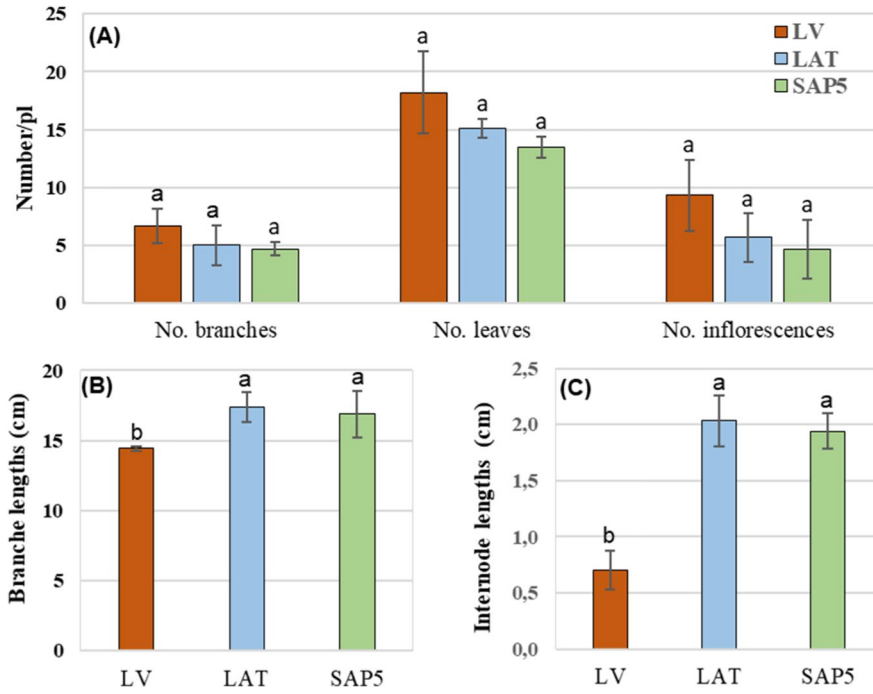


Fig. 6 Numbers of branches, leaves and inflorescences per plant (**A**), average length of branches (**B**), and average length of internodes (**C**) of *E. salicoides* plants growing at the LV, LAT and SAP5 sampling sites. n = three plants per site. Bars represent the standard deviation of the mean. Means followed by the same letters are not significantly different ($p > 0.05$)

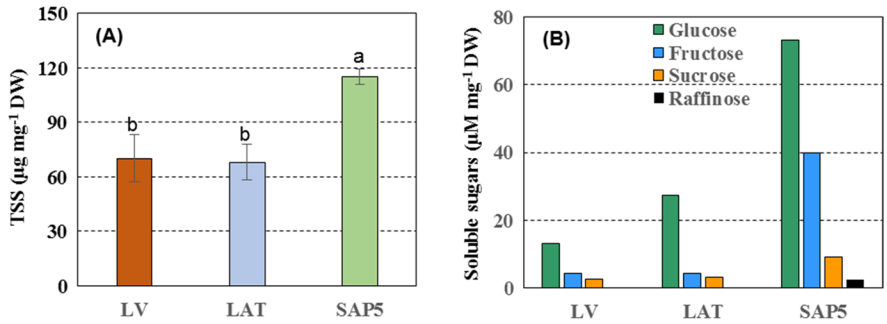


Fig. 7 Carbohydrate contents in *E. salicoides* plants growing at the LV, LAT, and SAP5 sampling sites considering the total soluble sugars (TSSs) (**A**), and content of soluble sugars, such as monosaccharides (glucose and fructose) and oligosaccharides (sucrose and raffinose) (**B**). Bars represent the standard deviations of the mean values. Means followed by the same letters are not significantly different ($p > 0.05$)

The nutrient concentrations in plants usually tend to increase with external availability. In typical Cerrado soils, the three plants species accumulated, on average, less than 30 mg kg^{-1} Ni in their shoot tissues, suggesting that the critical level of Ni for these species is closer to that of typical plants, which is approximately 10 mg kg^{-1}, as stated by Chang et al. (1992). Despite being an Al-rich environment, the concentrations of this metal in the plant tissues are also considered normal in Cerrado native species (approximately 400 mg kg^{-1} in dry matter) (Haridasan 1982).

The DTPA-extractable Ni in the ultramafic soils from Barro Alto can exceed 1000 mg kg^{-1}, a value among the highest ever recorded for ultramafic soils globally (Lopez et al. 2019). Surprisingly, according to our laboratory measurements, there seems to be no difference in Ni uptake for any of the three plant species (ranging from 700 to 1900 mg kg^{-1} N dry wight) growing in the three ultramafic sites, although they

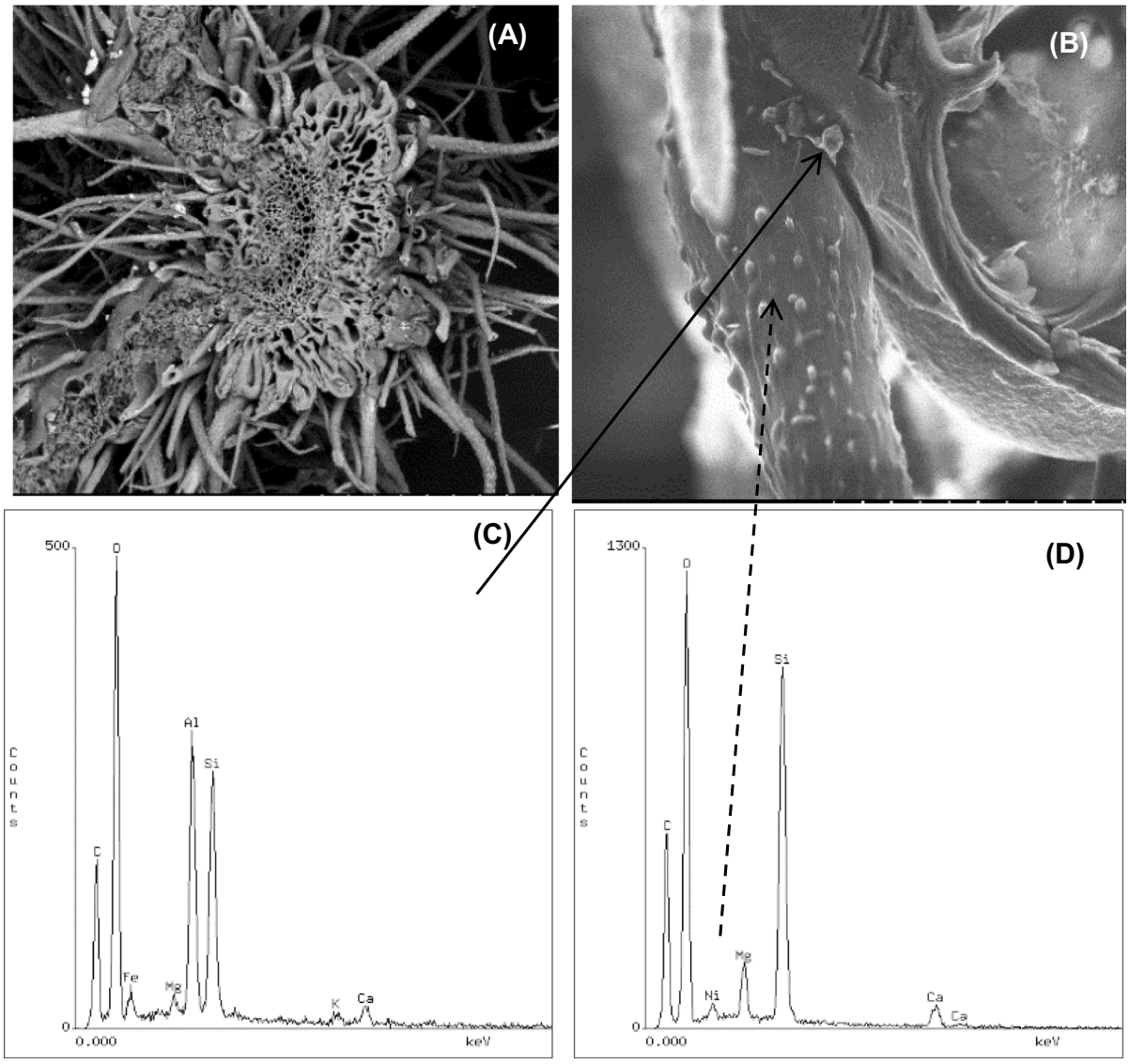


Fig. 8 Scanning electron microscopy (SEM) images of a central rib (**A**) and trichomes (**B**) on leaf blades of E. *salicoides* grown in soil with high Ni bioavailability (SAP5) and X-ray emission spectrum intensities of elements in "dust" trapped between trichomes (**C**) and in trichomes (**D**). Ni peak is shown only for protrusions of trichomes (kV = 10)

display apparent differences in Ni availability. Perhaps the level of available Ni is sufficiently high in the three soils, i.e., not limiting Ni uptake. The level of Ni accumulation by the three species is considered excessive for most species (phytotoxic concentration ranging from 10 to 250 mg kg^{-1}) (Kabata-Pendias and Mukherjee 2007). Although, we did not observe exceptional levels of Ni hyperaccumulation when comparing our results with those from other worldwide serpentine flora (Kierczak et al. 2021).

Genetic diversity of plants from ultramafic and typical Cerrado environments

Genetic studies of the plant populations from three Barro Alto ultramafic areas showed close DNA relatedness, indicating genetic similarity among ultramafic populations. On the other hand, they were different from the plant population sampled in the typical Cerrado environment. Pereira et al. (2004) also evaluated the genetic

structure of Cerrado native trees growing in serpentine and nonserpentine soils. Due to a possible occurrence of gene flow between the populations, they detected only a tendency for divergence between them. They suggested that the two plant populations followed different evolutionary paths after their colonization of ultramafic environments. In this study, the distance between sampling sites for plant accessions was approximately 250 km, leading to a low probability of gene flow between the native groups in the Barro Alto ultramafic zone and the non-ultramafic region in Brasília. Although there is genetic diversity between the populations, it is not possible to determine whether the groups diverged to the point of being considered different species or whether they should be considered separate ecotypes that developed specific adaptations to different habitats with very distinct edaphic characteristics.

Physiological and biochemical mechanisms of plant adaptation to high metals in soils

Plants adapt to natural environments through biochemical and physiological mechanisms. The ultramafic and typical Cerrado environments present contrasting edaphic characteristics that are hostile to the development of non-adapted plants. Thus, it is worth questioning what types of internal adjustments the three native species trigger to survive in environments with excess and scarcity of essential nutrients (P, Ca, Mg, Zn, Mn, Cu), presence and absence of high acidity, and phytotoxic elements (Ni, Al).

Increased soluble sugar production

Metal-tolerant plants are able to reduce the absorption of toxic elements or have some means of internal detoxification after uptake. Plants subjected to various biotic and/or abiotic stresses exhibit, as a common characteristic, the accumulation of organic metabolites (soluble carbohydrates, amino acids, phenolic compounds, and others) in shoot or root tissues Drzewiecka et al. 2017; Khan et al. 2000, O'Brien et al. 2014). Carbohydrates comprise a variety of different compounds that are involved in the primary and secondary functional processes of the plant. Some of these functions, including growth, are under complex regulatory control in response to environmental signals (Hartmann and Trumbore 2016). The production of raffinose, a polysaccharide with an important transport function in the phloem of some species, is associated with an adaptive response to environmental stresses, such as water stress and metal toxicity (Costa and Spitz 1997; Hartmann and Trumbore 2016). In the present study, Ni availability was 4.3 times higher in the SAP5 soil (461 mg Ni kg^{-1}) than in LAT soil (108 mg Ni kg^{-1}) (Table 4), while Ferralsols of Central Plateu of Brazil (as LV soil) are reported to have very low content of Ni (Burak et al. 2010). In addition to presenting higher Ni availability, the SAP5 soil is shallow (< 0.50 cm), creating an environment where plants are more susceptible to water stress during the dry season than those grown in LAT and LV soils (both with a soil depth > 1 m). In the natural environment, adult plants of *E. salicoides* native to ultramafic soils presented similar plant architecture (numbers of branches, leaves, and inflorescences) to the plants found in typical Cerrado soils. However, the plants on ultramafic soils (ES-SAP5 and ES-LAT) grew more (longer branches and internodes) than on the typical Cerrado soils (ES-LV). The greater production of aerial biomass, therefore, suggests that higher Ni contents in SAP5 and LAT soils did not affect negatively the photosynthesis in these plants. Moreover, plants grown in SAP5 soil produced more glucose and fructose (reducing sugars) and sucrose and raffinose (non-reducing sugars) than plants grown in LAT and LV soils. The increased production of sucrose and raffinose led to decreased levels of reducing sugars potentially harmful to cells and suggests a response to metal detoxification mechanisms, that in this case might be Ni.

Ni compartmentation in cells

Heavy metal ions, after being absorbed by the roots, generally accumulate in different parts of the plant, where they interfere with the activities of several enzymes essential for normal metabolism (Jha and Dubey 2004). Studies on the distribution of Ni in hyperaccumulating plants have shown that leaves are the primary site of the metal accumulation. The

accumulation occurs mostly in epidermal cells far from guard cells (Paul et al. 2020; Psaras and Manetas 2001; Robinson et al. 2003; van der Ent et al. 2019). In our study, Ni allocation was observed in trichomes of *E. salicoides* leaves collected at ultramafic sites (Fig. 8), where it cannot affect essential processes of plant metabolism. McNear et al. (2005) also observed Ni accumulation in the non-glandular trichome structure of *Alyssum murale.* These findings reveal the functional role of trichomes in metal storage and detoxification. Therefore, the localization of Ni in trichomes is a mechanism developed by *E. salicoides* plants to deal with the excessive absorption of Ni from the soil and its translocation to the aboveground tissues.

Conclusions and future research

Results of this research indicate that the phenomenon of metal hyperaccumulation in the studied species may not be directly linked to the ability to survive in ultramafic or typical Cerrado environments, but rather requires the development of adaptation mechanisms. The genetic differences found in the accessions collected at different sites and in different environments indicate that the plant species are adapted to local edaphic conditions. Nevertheless, this adaptation is the product of a series of internal physiological adjustments, as suggested by Goolsby and Mason (2016) and Pollard et al. (2014). These adjustments might culminate in changes in the genetic structures of the populations that developed in both harsh environments.

The adaptation of plants to environments rich in metals, such as ultramafic soils, increases their importance for cultivation in Ni-mining degraded soils. The hyperaccumulator species have the potential to be used in phytoremediation processes for areas heavily contaminated with metals or in phytoremediation or phytomining activities. Our results reinforce the need for mining companies to consider the use of Ni-tolerant plants in the biodiversity conservation plans of ultramafic massifs of Barro Alto, which have been explored. This practice can ensure the maintenance of the variability of the gene pool of the native populations adapted to the metalliferous environment, as pointed out by McKay et al. (2005).

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Declarations

Not applicable.

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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