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Anatomical, karyotypic, and nuclear DNA content studies in four morphotypes of wild lettuce

Estudos anatômicos, cariotípicos e de conteúdo de DNA nuclear em quatro morfotipos de alface silvestre

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ABSTRACT

Wild lettuce (*Lactuca* aff. *canadensis* L.), belonging to the Asteraceae family, occurs subspontaneously in Brazil and may originate from Africa, Asia, Europe and North America. Popularly known as Canada lettuce, it is an unconventional leafy vegetable. Studies on this species are scarce in Brazil, and its scientific name is debated among experts. It has high morphological variability and controversial botanical classification. This study characterized the stomata, organized the karyotype, and determined the nuclear DNA content of four morphotypes of wild lettuce to facilitate correct classification. The genetic material used was acquired from the non-conventional vegetable germplasm in UFLA. The leaves of wild lettuce morphotypes are hypoamphiestomatic with a greater number of stomata in the abaxial epidermis. There were similarities between the morphotypes (green and purple) and between the smooth purple types (narrow leaf and broad leaf) for the number and size of stomata. No variation was found in the number of chromosomes (2n = 18) or DNA content among the four morphotypes. The separation of the morphotypes of wild lettuce did not match the morphological classification or the karyological data. The four morphotypes evaluated were placed under the same species, and the results obtained when compared to other studies led us to infer that the four morphotypes of wild *Lactuca* belonged to the species *L. indica* and not *L. canadensis* as previously assumed. Further investigation may provide insights into the evolutionary history of this species.

Index terms: Asteraceae; botany; Brazil; taxonomy; unconventional vegetable.

RESUMO

A alface silvestre (*Lactuca* aff. *canadensis* L.), pertencente à família Asteraceae, ocorre de forma subespontânea no Brasil e pode ser originária da África, Ásia, Europa e América do Norte. Popularmente conhecida como alface canadense, é um vegetal folhoso não convencional. Os estudos sobre esta espécie são escassos no Brasil e seu nome científico é debatido entre especialistas. Possui alta variabilidade morfológica e classificação botânica controversa. Este estudo caracterizou os estômatos, organizou o cariótipo e determinou o conteúdo de DNA nuclear de quatro morfotipos de alface silvestre para facilitar a classificação correta. O material genético utilizado foi adquirido do germoplasma vegetal não convencional da UFLA. As folhas dos morfotipos de alface silvestre são hipoanfiestomáticas com maior número de estômatos na epiderme abaxial. Houve semelhanças entre os morfotipos (verde e roxo) e entre os tipos roxos lisos (folha estreita e folha larga) quanto ao número e tamanho dos estômatos. Nenhuma variação foi encontrada no número de cromossomos (2n = 18) ou no conteúdo de DNA entre os quatro morfotipos. A separação dos morfotipos de alface silvestre não correspondeu à classificação morfológica nem aos dados cariológicos. Os quatro morfotipos avaliados foram colocados sob a mesma espécie, e os resultados obtidos quando comparados com outros estudos nos levaram a inferir que os quatro morfotipos de *Lactuca* selvagem pertenciam à espécie *L. indica* e não a *L. canadensis* como se supunha anteriormente. Investigações adicionais podem fornecer conhecimento sobre a história evolutiva desta espécie.

Termos para indexação: Asteraceae; botânica; Brasil; taxonomia; vegetal não convencional.

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Introduction

Wild species of *Lactuca* occur subspontaneously (adapt to new environments) in several Brazilian biomes and are unconventional leafy vegetables (Monge et al., 2016). *Lactuca* L. is the most widely distributed genus in the Cichorieae tribe. More than 154 species are known, and they originated in Africa, Asia, Europe, and North America (Kilian, Gemeinholzer, & Lack, 2009). These species belonging to the Asteraceae family have been identified as *Cichorium intybus* L. (Pedrosa et al., 2012), *Lactuca canadensis* L. (Santos, Silva, & Fonseca, 2016; Kinupp et al., 2021; Pallaoro et al., 2020; Batista, et al., 2021), or *Lactuca indica* L. (Monge et al., 2016; Ranieri, 2017; Ossani et al., 2020). This divergence in scientific nomenclature occurs due to the great morphological variability found within species belonging to the genus *Lactuca* (Hassan et al., 2021), which raises doubts about

the correct nomenclature of the species. Thus, the species needs to be correctly identified for its application in plant breeding.

Different morphological types found in the south and southeast regions of Brazil were collected and identified by Monge et al. (2016) as *L. canadensis* and *L. indica* based on morphological descriptors. The oldest collection and first report of *L. canandensis* in South America was made in 1999, and the first report of L. indica was made in 2003; that study reported the species for the first time not only in Brazil but also on the American continent. However, accurate information on how these species arrived in South America is lacking (Monge et al., 2016).

Monge et al. (2016) also found that both species are morphologically similar, although they reported some morphological differences; for example, *L. indica* has a whitish latex, alternating leaves in a whole spiral, flowers with a yellow base, dyed purple abaxially, yellow anthers with purplish longitudinal lines and a glabrous, and purplish apex, whereas, *L. Canadensis* has a light yellow or light orange latex, alternate whorled leaves, pinnate or lobed, yellow flowers on both surfaces, yellow anthers, and glabrous and yellow apex.

In the last few decades, studies on the anatomy of vascular plants and their application in taxonomy have changed considerably (Bahadur et al., 2022). Anatomical features of the leaf epidermis, such as stomata, trichomes, and other characters, are useful tools, as the features reflect variations within the species, genus, or family (Ahmad et al., 2010). Similarly, phenotypic characterization and anatomical comparisons are extensively used to determine the genetic diversity of plant populations and facilitate accurate identification of species and understanding of their phylogenetic relationships (Moradi, Khaleghi, & Khadivi, 2023; Lemos et al., 2020).

The taxonomy of the Asteraceae family is extremely complex due to the presence of phenotypic plasticity. Intraspecific and interspecific variations, mainly in leaf morphology, occur in natural populations, hindering correct taxonomic identification (Martin & Mort, 2023; Moradi, Khaleghi, & Khadivi, 2023). This issue greatly hinders the genetic improvement of the species. Although studies have investigated the leaf anatomy of some species in this family (Rivera et al., 2019), studies with the above-mentioned morphotypes are scarce.

Most members of Asteraceae are amphistomatic, with stomata distributed on the adaxial and abaxial surfaces. Generally, species from the tribe Lactucae have tetracytic and anisocytic stomata with a papillose surface, while the stomatal pore is very large and varies in shape (Bahadur et al., 2023; Janaćković, Susanna, & Marin, 2019). *Lactuca indica* has tetracytic stomata, which are anisocytic in the abaxial surface of the leaf (Bahadur et al., 2023). Studies on the anatomy of the leaf epidermis of *L. canadensis* are scarce. Lebeda et al. (2012) found that trichomes are always present on the underside of the leaves in most of the samples of *L. canadensis*. Anatomical studies on the trichomes, stomata, and leaves (including their waxes) of the genus *Lactuca* have been neglected (Lebeda et al., 2019a).

Cytogenetic studies have found that *C. intybus* has 18 chromosomes (diploid) (Hauser, Jørgensen, & Toneatto, 2012), *L. canadensis* has 34 chromosomes (diploid) and nuclear DNA content of 17.96 pg, and *L. indica* has 18 chromosomes (diploid) (Chung et al., 2020; Wei et al., 2017) and DNA content of 11.87 – 14.12 pg (Doležalová et al., 2002). This genus contains species from Europe and the Himalayas (with eight chromosomes; haploid), from the Middle East, Africa, and India (with nine chromosomes; haploid), and from North America (distributed from Canada to Florida) (with 17 chromosomes; haploid) (Doležalová et al., 2002; Lebeda et al., 2012; El-Esawi & Sammour, 2014; Lebeda et al., 2009; Abdel Bar et al., 2023). Karyotypic analysis and determination of the DNA content have been performed to elucidate phylogenetic relationships in the genus *Lactuca* (Doležalová et al., 2002; Doležel & Bartoš, 2005; El-Esawi & Sammour, 2014; Koopman, 2000; Lebeda et al., 2009). Some studies compared species or assessed variations between individuals of the same species. Information on the nuclear DNA content and number of chromosomes of each species is required for breeding programs, especially for species with high genetic variability and whose evolutionary mechanisms are not fully understood (Silva et al., 2017).

Given the great morphological variability observed among individuals and few records on phenological, micromorphological, anatomical, and cytogenetic characteristics, in this study, we characterized the anatomy of the leaf epidermis, constructed the karyotype, and determined the nuclear DNA content of different morphotypes of wild lettuce for the accurate scientific nomenclature of the species.

Material and Methods

Study area

The experiment was performed in the Olericulture sector of the Federal University of Lavras (UFLA), in the city of Lavras, MG (21°14'18"S, 45°00'07"W, and 913 m altitude). This region has a humid temperate type of climate, with hot summers and dry winters, classified as type Cwb in the Köppen classification (Alvares et al., 2013).

The seeds were obtained from cultivated plants (in 2017) from a collection of plant germplasm located in the same place where the experiment was conducted. Seeds were collected from four morphotypes of wild *Lactuca* (Figure 1), which were designated according to their morphology as green pointy (VR), purple with entire and narrow leaf (RFIE), purple pointy (RR), and purple with entire leaf and wide (RFIL). Exsiccates were deposited in the PAMG Herbarium (Herbarium of the Agricultural Company of Minas Gerais – EPAMIG, Belo Horizonte, Minas Gerais, Brazil) with codes 58657, 58658, 58659, and 58656, respectively.

Figure 1: Plants of the wild lettuce (*Lactuca*): green peaked - VR (A), purple with entire and narrow leaves - RFIE (B), purple peaked - RR (C), and purple with entire and broad leaves -RFIL (D).

Sowing for the production of seedlings was conducted in polystyrene trays containing a commercial substrate of the Tropstrato® type. The trays were placed on suspended benches in the greenhouse and irrigated via suspended and inverted micro sprinklers. In the seedling development phase, three foliar fertilizations were conducted, with the commercial fertilizer Biofert® [188.96 g L^{-1}] (NPK 6-4-4; 0.5% Mg; 1% S; 0.02% B; 0.3% Cl; 0.02% Co; 0.1% Cu; 0.05% Mn). The plants were kept in a greenhouse from February 2017 to January 2018 and monitored until the final phase of botanical development; all parts of the plants, including flowers and seeds, were monitored during this period. At the end of the cycle, seeds of the four morphological types were collected and stored in a cold room.

Transplanting was performed 35 days after sowing; specifically, the plants were transplanted into 5-L polyethylene pots containing 4.5 L of soil and 0.5 L of organic compost. After transplanting, the pots were also placed in a greenhouse, with only one plant per pot. At this stage, each plant had three to four well-developed leaves.

The plants were fertilized following the recommendation of Silva et al. (2018) based on soil analysis. Subsequently, nitrogen fertilization was conducted with ammonium sulfate (35.2 g L^{-1}), applying 100 mL of the solution per pot every 21 days. Drip irrigation was performed with five lines distributed and 30 cm spacing between drippers per plant.

Anatomical studies

Plant tissue samples were collected from the midsection of 10 fully developed leaves 56 days after sowing and fixed in Karnovsky solution (2.5% glutaraldehyde, 2.0% paraformaldehyde in 0.05 M sodium cacodylate buffer, 0.001 M $CaCl_2$, pH 7.2) and stored at 4 ºC for 24 h. Then, they were washed in 0.05 M cacodylate buffer three times for 10 min, followed by dehydration in an acetone gradient (25%, 50%, 75%, 90%, and 100%). The samples were kept at each concentration of acetone for 10 min and treated three times with 100% acetone for 10 min.

In Balzers CPD 030 critical point dryer, acetone was replaced by CO_2. The samples were metalized with gold using a sputtering device (Balzers SCD 050) and then observed using a Zeiss LEO EVO 40 scanning electron microscope.

The number of stomata in the adaxial and abaxial epidermis, stomatal polar diameter (DPE), stomatal equatorial diameter (DEE), and types of trichomes were analyzed. Images were captured at 500x magnification to count the number of stomata and determine stomatal density (number of stomata per mm^2). Additionally, images were captured at 3,000x magnification to measure DPE and DEE, while those taken at 500x and 1,000x were used to determine the types of trichomes. The data on DPE and DEE were used to determine stomatal functionality (FE) by calculating the DPE/DEE ratio (Castro, Pereira, & Paiva, 2009).

The data were evaluated by analysis of variance, and the mean values were compared using the Tukey test, with a probability of 5%, using the R software (R Core Team, 2020).

Cytogenetic analysis

For the cytological study, root meristems obtained from recently germinated seeds were placed in a Petri dish with a

paper substrate in a BOD incubator at 25 °C. These seeds (<1 cm in size) were collected from the fourth day onward, as they germinated. For mitotic analysis, the material was pre-treated with 0.002 M 8-hydroxyquinoline (8HQ) for 5 h at 15 °C, then fixed in 3:1 Carnoy's solution (absolute ethanol: glacial acetic acid, v/v). The slides were prepared following the method described by Guerra and Souza (2002), hydrolyzed in 5 N HCl at room temperature for 20 min, prepared using the squash technique in 60% acetic acid, and stained with 10% Giemsa (Schwarzacher, Ambros, & Schweizer, 1980) for 5 min.

The images were obtained using an AxioCam Microcamera ICc 1 coupled to an Axio Lab A1 bright-field microscope (Carl Zeiss Microscopy GmbH, Göttingen, Germany), and the Adobe Photoshop CS6 program was used to assemble the karyotypes.

Chromosome counts were performed on 20 meristematic cells per morphotype of wild *Lactuca*, and the karyotype was constructed using the KaryoType software (old NucType) vol. 2.0 (Altınordu et al., 2016). To construct the karyotype formula, the chromosomes were classified based on the categories established by Guerra (1986). The degree of karyotype asymmetry was determined according to the classification proposed by Stebbins (1971). Centromeric asymmetry was calculated according to Peruzzi and Eroğlu (2013), and the intrachromosomal (A1) and interchromosomal (A2) asymmetry indices were calculated according to Zarco (1986). The total length of the haploid chromosome group was calculated according to Peruzzi et al. (2009).

The classification of interphase meristematic nuclei was performed based on the analysis of 100 nuclei per morphotype of the wild lettuce *Lactuca*, observed in random fields of 10 slides, according to the nomenclature proposed by Guerra (1990). To obtain the interphase nuclear volume (INV), two nuclear diameter measurements were made to calculate the mean diameter and final radius of the nuclei, which were inserted in Equation 1, according to Das and Mallick (1993). Calculation of INV based on the core radius, expressed in micrometers (μm^3) Equation 1:

$$INV = \frac{4}{3\pi r^3} \quad (1)$$

Quantification of nuclear DNA content

To quantify nuclear DNA content, three samples of each *Lactuca* morphotype were analyzed, with approximately 20 mg of young leaf tissue. The same amount of young *Pisum sativum* L. leaf tissue served as an internal reference standard (2C = 9.09 pg).

The samples were fragmented in a Petri dish with 1 mL of Marie and Brown (1993) buffer to obtain the nuclear suspension. The interphase nuclear solution obtained after filtration was mixed with 25 µL of propidium iodide (1 mg L^{-1}) and 2.5 µL of RNase (50 µg mL^{-1}) for reading on a flow cytometer (FACS Calibur, Becton Dickinson and Company, San Jose, CA, USA) with the Cell Quest software (Becton, Dickinson). This data was analyzed using WinMDI (v.2.8). Genome size was estimated according to Doležel and Bartoš (2005).

The data were compared by analysis of variance, and the mean values were compared by conducting the Tukey test at 5% probability, considering a completely randomized design, using the R software (R Core Team, 2020).

Results and Discussion

Wild species of *Lactuca* in Brazil were investigated, and the anatomical parameters of their leaf epidermis, the DNA content, and the karyotypic profile were determined. These parameters can be used to establish phylogenetic relationships in systematics (Butt et al., 2020) and to assess the adaptation of species to the environment. Although many studies have investigated the anatomy of the leaf epidermis in *L. indica*, such studies for *L. canadensis* are rare.

Wild variants of *Lactuca* occur spontaneously in several Brazilian biomes and present wide morphological variability, making identification at the species level difficult. Correctly identifying species is necessary for genetic improvement. Monge et al. (2016) proposed a taxonomic key for separating these wild morphotypes based on morphological characters. They reported that *L. indica* generally has entire leaves, and *L. canadensis* generally has pinnate lobed leaves. In this study, two morphotypes had entire leaves (RFIE and RFIL), and two morphotypes had lobed leaves (RR and VR) (Figure 1). According to the taxonomic key of Monge et al. (2016), the VR and RR morphotypes belong to the species *L. canadensis*, and the RFIE and RFIL belong to *L. indica*. However, all morphotypes that occur in Brazilian biomes are speculated to belong to *L. canadensis* (Liberal et al., 2021).

The leaves of wild lettuce morphotypes showed differences in stomata density and biometric characters. The interaction between the morphotypes and the sides of the leaf epidermis did not affect the analyzed variables. The VR morphotype had the highest number of stomata (393; 19.06 mm^{-2}), while the RFIL morphotype had the lowest number of stomata (295; 16.08 mm^{-2}) (Table 1). The greatest difference in the number of stomata was found between the VR and RFIL morphotypes (approximately 100 stomata mm^{-2}).

Considering the DPE and DEE, a significant difference was found between the different morphotypes and the smallest stomatal sizes were observed in the purple whole-leaf types. The relationship between DPE and DEE indicates the shape of the stomata; the higher the DPE/DEE ratio, the more ellipsoid its shape and the greater its function. In contrast, the smaller this relationship, the smaller its format and function (Santos et al., 2022). The RR morphotype had the highest DPE/DEE ratio and the highest functionality (Table 1).

The morphotypes had 3.7 times more stomata in the abaxial epidermis compared to that in the adaxial epidermis. A small difference was found between the size of the stomata of the adaxial and abaxial epidermis. No statistical difference was found between the FE of the four morphotypes, which presented an average of 0.87 (Table 2). The difference in the number of stomata in the RR and RFIE morphotypes was not statistically significant.

The differences in the morphology of epidermal and stomatal cells are shown in Figures 2 and 3.

The leaves of the four morphotypes of wild lettuce *Lactuca* were amphistomatic, i.e., they had anomocytic-type stomata on both types of epidermis, with a greater number on the abaxial surface and with similar functions (Figures 2 and 3). Epicuticular wax and papillosis were found in all four morphotypes, with greater occurrence on the abaxial side.

The presence of amphistomatic leaves observed in the four morphotypes is a characteristic of most species of the Asteraceae family (Bahadur et al., 2022); therefore, it cannot be used to distinguish species. However, characteristics such as the length and width of the stomata are parameters that can be used to identify different species within the genus *Lactuca*.

The variables stomatal length, stomatal width, stomatal pore length, and stomatal pore width were used by Bahadur et al. (2022) to define the grouping of *L. indica* and *L. canadensis* within the genus *Lactuca*. Both species were grouped in different clusters and *Lactuca indica* presented stomatal length and width of 20.56 ± 0.47 and 15.16 ± 1.32, respectively, while the stomatal length and width in *L. canadensis* were 21.69 ±0.09 and 14.24 ±0.61, respectively. In this study, the VR and RR morphotypes presented stomatal length and width that were similar to those reported by Bahadur et al. (2023) for *L. canadensis*. The stomatal length and width in the RFIE and RFIL morphotypes were substantially lower than those observed by these authors.

Anomocytic and anisocytic stomata were found on the abaxial surface of *L. indica*, while tetracytic-type stomata were found in *L. canadensis* (Bahadur et al., 2023). The presence of papilloses on the surface of the leaf epidermis was also observed by these authors in *L. indica*, as reported in this study (Figure 2). No study has reported the presence of papilloses on the leaf epidermis of *L. canadensis*.

We found filiform multicellular tector trichomes, located close to the central vein or on the vein of the adaxial epidermis (Figure 4).

The presence of a particular type of trichome can frequently delimit species, genera, and even families. In this study, all evaluated genotypes had the same type of non-glandular trichome. Epidermal cells with a straight anticlinal cell wall were observed in *L. indica* as in this study, and wavy to sinuous patterns were found in *L. canadensis* (Bahadur et al., 2023). Overall, information available on the anatomy of the leaf epidermis of *L. canadensis* is considerably less than that available for *L. indica*.

The four morphotypes of wild lettuce *Lactuca* had a reticulated interphase nucleus, with an intensely stained chromatin reticulum and polarized chromatin, which was difficult to visualize. All four lettuce morphotypes had 18 chromosomes (diploid) (Figure 5).

Wild lettuce interphase nuclei were characterized for the first time, and information on the characteristics of interphase nuclei in other wild species of Asteraceae is limited. Cytological studies in this family are limited to the chromosome number and observations in meiosis (Jones et al., 2018; Lebeda et al., 2019b). The structure and organization of chromatin during interphase is generally constant within the same species (Guerra, 1985), and the four morphotypes presented the same structure.

Table 1: Stomatal density (number of stomata mm^{-2}) and measurements of stomatal polar diameter (DPE) and stomatal equatorial diameter (DEE) of the leaf epidermis of four morphotypes of the wild lettuce *Lactuca*.

Wild lettuce *Lactuca*	Number of stomata mm^{-2}	DPE (µm)	DEE (µm)	Functionality
Green peaked (VR)	393 (19.06) a	19.67 b	23.08 a	0.86 a
Purple with entire and narrow leaves (RFIE)	328 (17.06) ab	8.08 c	10.42 b	0.80 a
Purple peaked (RR)	369 (17.83) ab	21.88 a	23.13 a	0.95 a
Purple with entire and broad leaves (RFIL)	295 (16.08) b	8.83 c	10.02 b	0.88 a
CV (%)	14.23	7.87	9.39	18.74

Means followed by the same letter in the column do not differ from each other at 5% probability using the Tukey test.

Table 2: Stomatal density (number of stomata mm^{-2}) and measurements of DPE and DEE on the abaxial (AB) and adaxial (AD) sides of the leaf epidermis of wild lettuce *Lactuca*.

Face	Number of stomata mm^{-2}	DPE (µm)	DEE (µm)	Functionality
AB	545 (23.25) a	14.06 a	15.72 b	0.89 a
AD	147 (11.76) b	15.17 a	17.60 a	0.85 a
CV (%)	15.23	19.87	7.84	11.89

Means followed by the same letter in the column do not differ from each other at 5% probability using the F test.

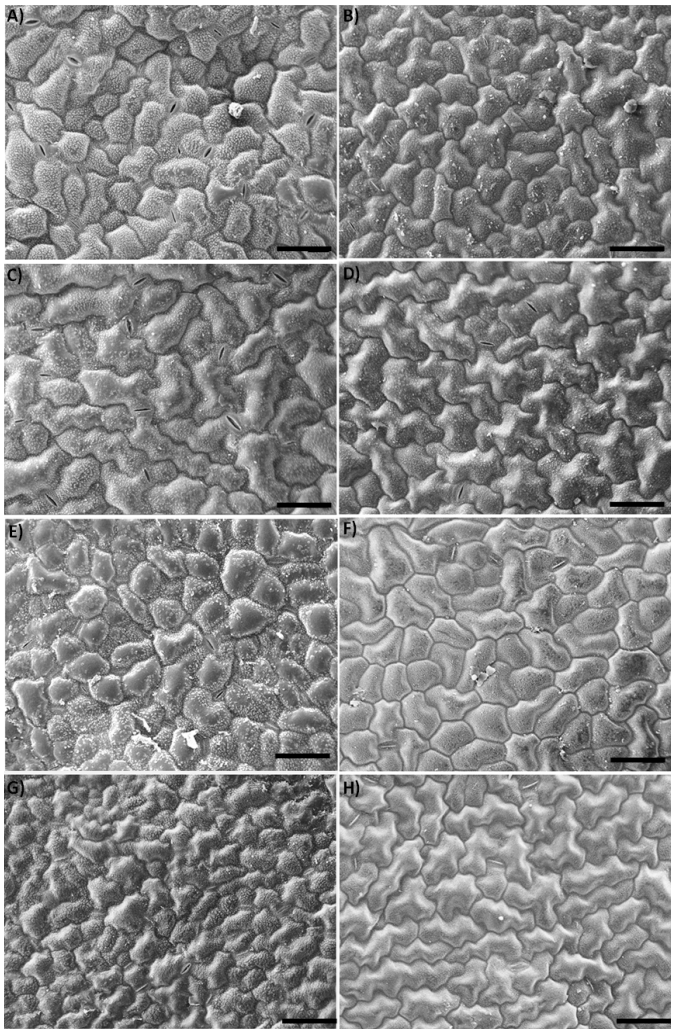

Figure 2: Scanning electron micrograph of stomata on the abaxial surface (A, C, E, and G) and adaxial surface (B, D, F, and H) of wild lettuce *Lactuca* morphotypes: Green peaked – VR (AB), Purple with entire and narrow leaves - RFIE (CD), Purple peaked - RR (EF), and Purple with entire and wide leaves - RFIL (GH); bar: 50µm.

The karyogram of the four morphotypes (Figure 6) showed few morphometric differences, although phenotypic variations were present (Table 3).

The relationships and genetic diversity within and between *Lactuca* species were evaluated by several researchers using karyological characteristics (Doležalová et al., 2002; Yang et al., 2009; El-Esawi & Sammour, 2014). The chromosome number (2n = 18) observed in this study was previously reported for several species in the family Asteraceae, including *L. indica* (Doležalová et al., 2002; El-Esawi & Sammour, 2014; Chung et al., 2020; Widarmi et al., 2020) and *C. intybus* (Kiers, van der Meijden, & Bachmann, 2000; Hauser, Jørgensen, & Toneatto, 2012), whose wild morphotypes are found in Brazil. The species *L. canadensis* was classified as an allotetraploid with 2n = 4x = 34 chromosomes (Tomb et al., 1978; Wei et al., 2017; Jones et al., 2018; Lebeda et al., 2019a).

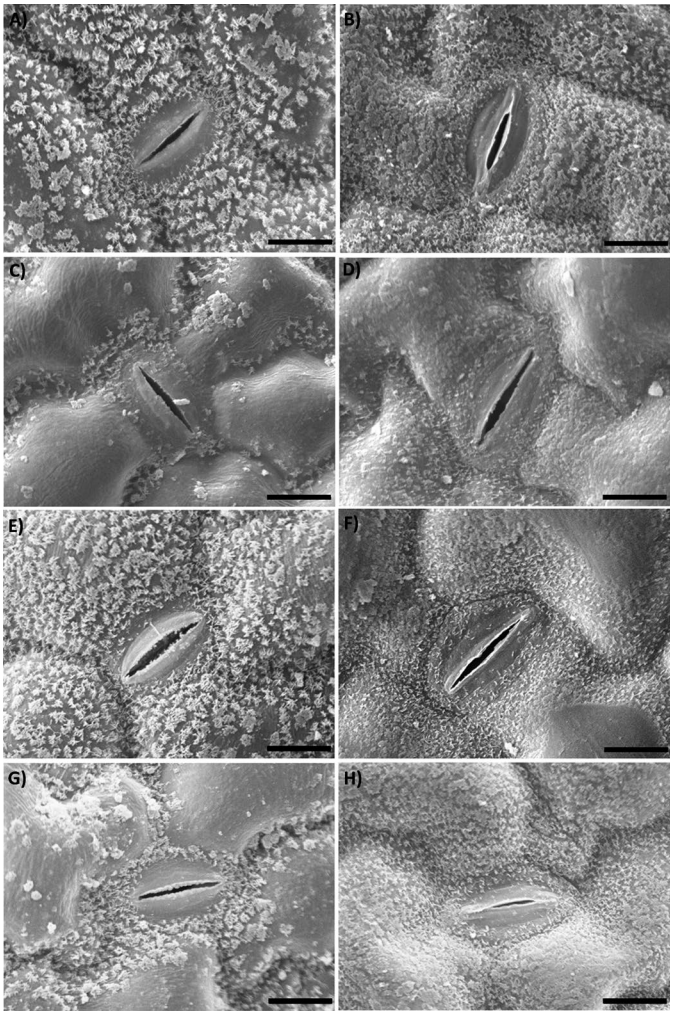

Figure 3: Scanning electron micrograph of stomata on the abaxial surface and the presence of pailoses (A, C, E, and G) and adaxial (B, D, F, and H) of wild lettuce *Lactuca* morphotypes: Green peaked -VR (AB), Purple with entire and narrow leaves - RFIE (CD), Purple peaked -RR (EF), Purple with entire and broad leaves - RFIL (GH); bar: 10 µm.

Reports suggest that wild *Lactuca* in Brazil was introduced from North America. However, according to Jones et al. (2018), the *L. canadensis* clade native to North America consists exclusively of allotetraploid species with 34 chromosomes (diploid). These authors also stated that this clade has predominantly brownish-orange latex. The morphotypes in this study were diploid individuals (2n = 18) and produced a pure white latex. Based on these parameters, we inferred that *L. canadensis* was not introduced from North America.

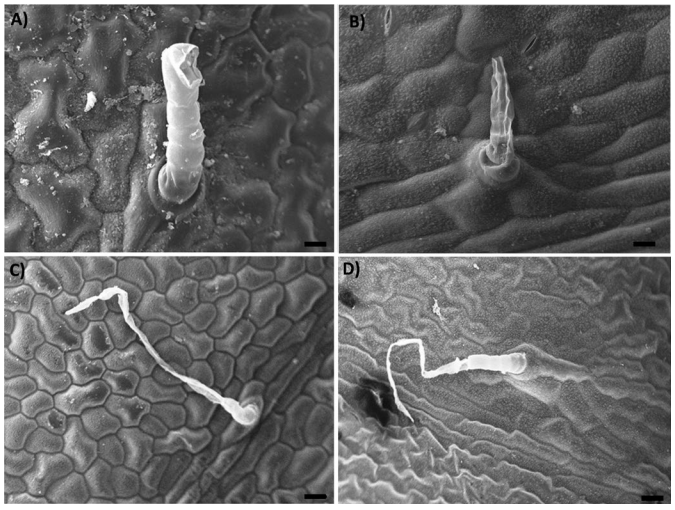

Figure 4: Scanning electron micrograph of trichomes on the adaxial surface of the leaf blade of *Lactuca* wild lettuce morphotypes: A) VR (10 µm), B) RFIE (10 µm), C) RR (20 µm), and D) RFIL (bar: 20 µm).

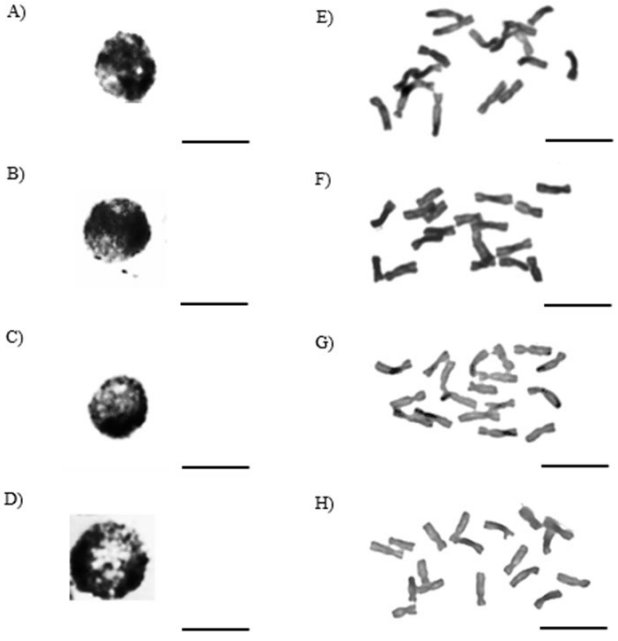

Figure 5: Interphase cell nuclei in the metaphase of the four morphotypes of wild lettuce *Lactuca*: VR (A); RFIE (B); RR (C); RFIL (D); Mitotic metaphases with 18 chromosomes (diploid) of the four Canadian lettuce morphotypes (E, F, G, and H); bars: 10 µm.

Karyotypic formulae presented metacentric (m), submetacentric (sm), and acrocentric (ac) chromosomes. The karyotypic formula of the peaked morphotype (12m+2sm+4ac) differed from that of the others, whose karyotypic formula was 10m+6sm+2ac (Table 3).

Figure 6: Karyograms of the four wild lettuce *Lactuca* morphotypes: VR (A); RFIE (B); RR (C); RFIL (D); bars: 10 µm.

In this study, no variation was observed in the karyotypic formula between the different *Lactuca* wild lettuce types, except for the RR type. The chromosomes were predominantly metacentric and submetacentric, with two acrocentric types (10m+6m+2ac). The RR morphotype presented the karyotypic formula with a greater number of acrocentric chromosomes (12m+2sm+4ac). Karyotypic formulae observed in *L. indica* were 6m+12sm (El-Esawi & Sammour, 2014) and 2m + 7sm (Yang, 2009). These authors did not find acrocentric chromosomes. No reports of karyotypic formulae were identified in *L. canadensis*.

Several researchers have investigated the relationships and genetic diversity within and between *Lactuca* species using karyological characteristics (Doležalová et al., 2002; Matoba et al., 2007; Lebeda et al., 2009; El-Esawi & Sammour, 2014). These authors found that along with a constancy in the number of chromosomes, the species of the genus exhibited uniformity in chromosomal morphology, and most chromosomes were metacentric or submetacentric, as found in this study.

All morphotypes studied had 18 chromosomes (diploid), and the differences in their karyotypes can be attributed to processes that do not affect the number of chromosomes, such as chromosomal rearrangements, pericentric inversion, and unequal translocation (El-Esawi & Sammour, 2014).

A detailed study of the karyotypic index provides a better understanding of the evolutionary direction, although, in some cases, more symmetrical karyotypes with low chromosome numbers may reveal early stages of evolution. Karyotypic index, has also been used between taxa to infer taxonomic aspects (Fernandes et al., 2020).

All morphotypes were classified as type 3A (10–50% of chromosomes having a longest/shortest chromosomal ratio < 2:1 and an arm ratio < 2:1), according to the scheme provided by Stebbins (1971).

Table 3: Measurements and proportions of the long (q) and short (p) arms of chromosome pairs, centromeric classification, centromeric asymmetry index (CA), intrachromosomal and interchromosomal asymmetry (A1 and A2), and karyotype formulae of the four morphotypes of *Lactuca*.

Pair	q (µm)	p (µm)	q + p (µm)	q/p	Classification centromeric	
Green peaked (VR)						
1	4.80	1.71	6.51	2.80	Sm	
2	4.43	1.51	5.94	2.93	Sm	
3	3.97	1.81	5.78	2.19	M	
4	3.99	1.59	5.58	2.51	Sm	
5	3.44	1.75	5.20	1.96	M	
6	3.99	1.02	5.01	3.92	B.C	
7	2.91	2.00	4.92	1.45	M	
8	3.17	1.34	4.51	2.36	M	
9	2.67	1.80	4.47	1.48	M	
Karyotype formula		10m+6sm+2ac		A1 = 0.54	A2 = 0.13	AC = 38.57
Purple with entire and narrow leaves (RFIE)						
1	3.86	1.46	5.32	2.65	Sm	
2	3.53	1.41	4.94	2.51	Sm	
3	3.16	1.34	4.51	2.36	M	
4	3.24	1.15	4.39	2.81	M	
5	2.70	1.64	4.34	1.65	M	
6	3.24	1.05	4.29	3.08	B.C	
7	2.18	1.66	3.84	1.32	M	
8	2.53	0.99	3.52	2.56	Sm	
9	2.55	0.96	3.51	2.66	M	
Karyotype formula		10m+6sm+2ac		A1 = 0.55	A2 = 0.14	AC = 39.37
Purple peaked (RR)						
1	3.87	1.58	5.46	2.45	M	
2	3.63	1.79	5.42	2.03	M	
3	3.65	1.51	5.16	2.42	M	
4	3.70	1.36	5.07	2.71	Sm	
5	3.36	1.54	4.90	2.18	M	
6	3.19	1.37	4.56	2.34	M	
7	3.63	0.86	4.49	4.24	B.C	
8	2.55	1.73	4.28	1.47	M	
9	3.24	0.90	4.14	3.59	B.C	
Karyotype formula		12m+2sm+4ac		A1 = 0.58	A2 = 0.10	AC = 42.03
Purple with entire and broad leaves (RFIL)						
1	3.76	1.29	5.05	2.91	Sm	
2	3.59	1.34	4.93	2.67	Sm	
3	3.59	1.34	4.93	2.68	Sm	
4	3.04	1.65	4.69	1.84	M	
5	3.13	1.49	4.61	2.10	M	
6	3.03	1.30	4.33	2.32	M	
7	2.70	1.59	4.29	1.69	M	
8	2.31	1.49	3.81	1.55	M	
9	2.45	0.67	3.11	3.67	B.C	
Karyotype formula		10m+6sm+2ac		A1 = 0.55	A2 = 0.14	AC = 38.83

Regarding the size of the chromosomes, the total length of the arms decreased, reaching a size reduction of 31.14–34.8% between the longest and shortest chromosome pairs. The total length of the arms showed negligible variation between the four morphotypes, with an average length of 29.70 ± 2.97 µm for the long arm and 12.75 ± 1.26 µm for the short arm.

Intrachromosomal and interchromosomal asymmetries (A1 and A2) showed slight variations. The differences in the position of the centromere, represented by centromeric asymmetry (CA) and chromosome size (q+p), are reflected in the values of A1 and A2, in which the morphotypes presented similar levels of chromosomal asymmetry, with A1 varying from 0.54 to 0.58 and A2 varying from 0.10 to 0.14 (Table 3).

Centromeric asymmetry was 39.7% on average (Table 3), and the total length of the haploid set was 42.45 ± 4.19 µm on average (Table 4), with greater chromosomal size for VR and RR leaves. Similar values of relative length, centromeric asymmetry, and total haploid set length indicated a small variation in genome size or chromosome biometric characteristics between the morphotypes. The 1C content of nuclear DNA varied from 5.44 to 6.08 pg among the four morphotypes (Table 4), with no significant difference between them.

Table 4: Nuclear DNA content, interphase nuclear volume (INV), and total haploid set length (TLHS) of the four wild lettuce *Lactuca* morphotypes.

Morphotypes	DNA=1C (pg)	INV (µm ^{3})	TLHS (µm)
Green peaked (VR)	6.08 a	346.63 ab	47.92
Purple with entire and narrow leaves (RFIE)	5.93 a	367. 72 ab	38.64
Purple peaked (RR)	5.44 a	309.90 b	43.47
Purple with entire and broad leaves (RFIL)	6.08 a	431.28 a	39.76
CV (%)	11.6%	20.7%	

Means followed by the same letter do not differ according to the F test and Tukey test at 5% probability.

Similar values of relative length, centromeric asymmetry, total length of the haploid set, symmetrical karyotype with metacentric predominance and submetacentric chromosomes of similar size (Paszko, 2006), and values of intrachromosomal and interchromosomal asymmetry (A1 and A2) indicated negligible variation in genome size or biometric characteristics of chromosomes of wild morphotype lettuce; these findings were validated in this study, considering that the nuclear 1C DNA content ranged from 5.44 to 6.08 pg among the four wild lettuce morphotypes, with no significant difference between them.

These values were similar to those reported by Bennett and Leitch (2011) (1C DNA =5.9 pg) and Doležalová et al. (2002) (2C = 11.87 – 14.12) in *L. indica*. The same authors found a value of 17.96 pg for *L. canadensis.* Some researchers have considered the nuclear DNA content to be a species-specific character (Doležel et al., 1998) and used the corresponding information to establish intraspecific and interspecific taxonomic relationships in the genus *Lactuca* (Lebeda et al., 2009).

Our results showed slight variation in the micromorphology analysis of the leaf surface and in the karyotype between the four wild lettuce morphotypes, although they showed considerable morphological differences. Intraspecific variability occurs widely within the genus *Lactuca* (Křístková et al., 2008; Lebeda et al., 2009; Lebeda et al., 2012).

Therefore, based on specific anatomical parameters, such as the presence of papilloses, a specific characteristic of *L. indica,* anomocytic stomata, and considering the cytogenetic studies that reported 18 chromosomes in *L. indica* (Chung et al., 2020) and allotertaploidy in *L. canadensis* (34 chromosomes) (Jones et al., 2018; Lebeda et al., 2019a), the four morphotypes were inferred to belong to the same species. Unlike the findings of other studies, they were found to be more strongly correlated with *L. indica* than *L. canadensis*. Both *L. indica* and *L. canadensis* occur subspontaneously in Brazil (Monge et al., 2016).

Conclusions

The four morphotypes evaluated belonged to the same species. The results indicated that the four morphotypes of wild *Lactuca* were a part of *L. indica* and not *L. canadensis* as previously assumed.

Author Contribution

Conceptual idea: Avelar, R. I. S.; Resende, L. V.; Methodology design: Avelar, R. I. S.; Gavilanes, M. L.; Avelar, R. I. S.; Souza, D. C.; Data collection: Avelar, R. I. S.; Castro, E. M.; Martins, A. D.; Mendes, M. H. A.; Ferraz, R. M. Data analysis and interpretation: Avelar, R. I. S.; Souza, D. C.; Resende, K. F. M; Mendes, M. H. A.; and Writing and editing: Avelar, R. I. S.; Resende, L. V.; Mendes, M. H. A.; Bittencourt, W. J. M.

References

Abdel Bar, F. M. et al. (2023). Genus *Lactuca* (*Asteraceae*): A comprehensive review. *Records of Natural Products*, 17(2):201-231.

Ahmad, K. et al. (2010). Taxonomic diversity in epidermal cells of some sub-tropical plant species. *International Journal of Agriculture & Biology*, *12*(1):115-118.

Altınordu, F. et al. (2016). A tool for the analysis of chromosomes: KaryoType. *Taxon*, *65*(3):586-592.

Alvares, C. A. et al. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, *22*(6):711-728.

Bahadur, S. et al. (2022). Leaf epidermal traits of selected *Euphorbiaceae* and *Phyllanthaceae* taxa of Hainan Island and their taxonomic relevance. *Diversity*, *14*(10):881.

Bahadur, S. et al. (2023). Foliar micromorphology with emphasis on the trichomes diversity and its taxonomic relevance in selected tribes of Asteraceae from Hainan Island. *Flora, 300*:152229.

Batista, D. S. et al. (2021). Organic fertilization in the production and chemical composition of non-conventional leafy vegetables. *Pesquisa Agropecuária Tropical*, *51*:e66508.

Bennett, M. D., & Leitch, I. J. (2011). Nuclear DNA amounts in angiosperms: Targets, trends and tomorrow. *Annals of botany*, *107*(3):467-590.

Butt, N. et al. (2020). Importance of species translocations under rapid climate change. *Conservation Biology*, *35*(3):775-783.

Castro, E. D., Pereira, F. J., & Paiva, R. (2009). *Histologia vegetal: Estrutura e função de órgãos vegetativos*. Lavras: Editora UFLA. 234p.

Chung, G. Y. et al. (2020). Chromosome numbers of 36 vascular plants in South Korea. *Journal of Asia-Pacific Biodiversity*, *13*(3):504-510.

Das, A. B., & Mallick, R. (1993). Karyotype diversity and interspecific 4C DNA variation in Bupleurum. *Biologia Plantarum*, *35*(3):355-363.

Santos, M. et al. (2022). Stomatal analysis in leaves of *Siparuna guianensis* Aubl. (Siparunaceae). *Research, Society and Development*, *11*(1):e17111124722.

Doležalová, I. et al. (2002). Variation in chromosome numbers and nuclear DNA contents in genetic resources of *Lactuca* L. species (*Asteraceae*). *Genetic Resources and Crop Evolution*, *49*:385-397.

Doležel, J., & Bartoš, J. A. N. (2005). Plant DNA flow cytometry and estimation of nuclear genome size. *Annals of Botany*, *95*(1):99-110.

Doležel, J. et al. (1998). Plant genome size estimation by flow cytometry: Inter-laboratory comparison. *Annals of Botany*, *82*:17-26.

El-Esawi, M. A., & Sammour, R. (2014). Karyological and phylogenetic studies in the genus *Lactuca* L. (*Asteraceae*). *Cytologia*, *79*(2):269-275.

Fernandes, L. et al. (2020). Karyotype and nuclear DNA content of four species of the genus *Costus* L.: A cytotaxonomic and evolutionary contribution. *Brazilian Journal of Development*, *6*(9):72196-72217.

Guerra, M. D. S. (1986). Reviewing the chromosome nomenclature of Levan et al. *Brazilian Journal of Genetics*, *9*(4):741-743.

Guerra, M. (1990). A situação da citotaxonomia de angiospermas nos trópicos e, em particular, no Brasil. *Acta Botanica Brasilica*, *4*:75-86

Guerra, M., & Souza, M. D. (2002). *Como observar cromossomos: Um guia de técnicas em citogenética vegetal, animal e humana*. Ribeirão Preto: FUNPEC, 131p.

Hassan, M. N. et al. (2021). Recent molecular and breeding strategies in lettuce (*Lactuca* spp.). *Genetic Resources and Crop Evolution*, *68*:3055-3079.

Hauser, T. P., Jørgensen, R. B., & Toneatto, F. (2012). Reduced sexual compatibility between cultivated and wild chicory and their F 1 hybrids. *Genetic Resources and Crop Evolution*, *59*(6):783-791.

Janaćković, P., Susanna, A., & Marin, P. D. (2019). Micromorphology and anatomy in systematics of *Asteraceae*. An old-fashioned approach?. *Biologica Nyssana*, *10*(2):77-85.

Jones, K. E. et al. (2018). Northern hemisphere disjunctions in *Lactuca* (Cichorieae, *Asteraceae*): Independent Eurasia to north America migrations and allopolyploidization. *Willdenowia*, *48*(2):259-284.

Kiers, A. M., van der Meijden, R., & Bachmann, K. (2000). A search for diagnostic AFLP markers in wild and cultivated *Cichorium* species. *Genome*, *43*(3):470-476.

Kilian, N., Gemeinholzer, B., & Lack, H. W. (2009). Tribe cichorieae. In V. A. E. A. Funk, (Ed.). *Systematics, evolution, and biogeography of the compositae*. Vienna: Int. Assoc. Plant: (pp. 343-383).

Kinupp, V. F. et al. (2021). *Plantas alimentícias não convencionais (PANC) no Brasil: Guia de identificação, aspectos nutricionais e receitas ilustrad*as. Nova Odessa-SP: Instituto Plantarum de Estudos da Flora. 789p.

Koopman, W. J. (2000). Identifying lettuce species (*Lactuca* subsect. *Lactuca*, *Asteraceae*): A practical application of flow cytometry. *Euphytica*, *116*:151-159.

Křístková, E. et al. (2008). Description of morphological characters of lettuce (*Lactuca sativa* L.) genetic resources. *Horticultural Science*, *35*(3):113-129.

Lebeda, A. et al. (2009). Wild Lactuca germplasm for lettuce breeding: Current status, gaps and challenges. *Euphytica*, *170*:15-34.

Lebeda, A. et al. (2012). Genetic polymorphism in *Lactuca aculeata* populations and occurrence of natural putative hybrids between *L. aculeata* and *L. serriola*. *Biochemical Systematics and Ecology*, *42*:113-123.

Lebeda, A. et al. (2019a). Research gaps and challenges in the conservation and use of North American wild lettuce germplasm. *Crop Science*, *59*(6):2337-2356.

Lebeda, A. et al. (2019b). Wild lactuca species in north America. In S. Greene. et al. (eds). *North American crop wild relatives.* Volume 2: Important Species, Springer, Cham. (pp. 131-194).

Lemos, V. D. O. T. et al. (2020). Ecological anatomy of *Myrcia guianensis* (Aubl.) DC. leaf in Restinga in Ceará state. *Ciência Florestal*, *30*(2):307-322.

Liberal, Â. et al. (2021). Chemical features and bioactivities of *Lactuca canadensis* L., an unconventional food plant from Brazilian Cerrado. *Agriculture*, *11*(8):734.

Marie, D., & Brown, S. C. (1993). A cytometric exercise in plant DNA histograms, with 2C values for 70 species. *Biology of the Cell*, *78*(1-2):41-51.

Martin, A. P., & Mort, M. E. (2023). *Vallisneria* (Hydrocharitaceae): Novel species, taxonomic revisions, and hybridization. *Aquatic Botany*, *188*:103669.

Matoba, H. et al. (2007). Chromosomal study of lettuce and its allied species (*Lactuca* spp., *Asteraceae*) by means of karyotype analysis and fluorescence in situ hybridization. *Hereditas*, *144*(6):235-243.

Monge, M. et al. (2016). Two new records of *Lactuca* L. (Cichorieae, *Asteraceae*) in South America. *Revista Brasileira de Biociencias*, *14*(2):117-123.

Moradi, M., Khaleghi, A., & Khadivi, A (2023). Morphological variability of wild-growing crown imperial (*Fritillaria imperialis* L.) germplasm in central region of Iran-implications for in-situ conservation initiatives. *BMC Plant Biology*, *23*:12.

Ossani, P. C. et al. (2020). Machine learning in classification and identification of nonconventional vegetables. *Journal of Food Science*, *85*(12):4194-4200.

Pallaoro, D. S. et al. (2020). Effects of humus and shading levels in the production of *Lactuca canadensis* L. seedlings. *Acta Agronómica*, *69*(1):32-37.

Paszko, B. (2006). A critical review and a new proposal of karyotype asymmetry indices. *Plant Systematics and Evolution*, *258*:39-48.

Pedrosa, M. W. (2012). *Hortaliças não convencionais: Alternativa de diversificação de alimentos e de renda para agricultores familiares de Minas Gerais*. EPAMIG- Centro Oeste, 22p.

Peruzzi, L., & Eroğlu, H. E. (2013). Karyotype asymmetry: Again, how to measure and what to measure? *Comparative Cytogenetics*, *7*(1):1-9.

Peruzzi, L. et al. (2009). Chromosome diversity and evolution in *Liliaceae*. *Annals of Botany*, *103*(3):459-475.

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. Avaliable in: https://www.R-project.org.

Ranieri, G. R. et al. (2017). *Guia prático sobre PANCs: Plantas alimentícias não convencionais*. São Paulo: Instituto Kairós. 44p.

Rivera, P. et al. (2019). Arquitectura y anatomía foliar de especies de *Asteraceae* en un matorral xerófilo de la Ciudad de México, México. *Acta Botanica Mexicana*, (126):e1515.

Santos, I. D., Silva, A. F., & Fonseca, M. C. M. (2016). Wild lettuce, dandelion, lamb's ears and commom sow thistle: Edible exotic plants. *Informe Agropecuario*, *37*(295):67-74.

Schwarzacher, T., Ambros, P., & Schweizer, D. (1980). Application of giemsa banding to orchid karyotype analysis. *Plant Systematics and Evolution*, *134*:293-297.

Silva, L. F. L. E. et al. (2017). Unconventional vegetables collected in Brazil: Chromosome number and description of nuclear DNA content. *Crop Breeding And Applied Biotechnology*, *17*(4):320-326.

Silva, L. F. L. E. et al. (2018). Nutritional evaluation of non-conventional vegetables in Brazil. *Anais da academia Brasileira de Ciências*, *90*:1775-1787.

Stebbins, G. L. (1971). *Chromosomal evolution in higher plants*. Hodder & Stoughton Educational. 220p.

Tomb, A. S. et al. (1978). Chromosome numbers in the compositae. XIV. Lactuceae. *American Journal of Botany*, 65(7):717-721.

Wei, Z. et al. (2017). Phylogenetic relationships within *Lactuca* L. (*Asteraceae*), including African species, based on chloroplast DNA sequence comparisons. *Genetic Resources and Crop Evolution*, *64*:55-71.

Widarmi, W. D. et al. (2020). Characterization of lettuce chromosomes based on condensation patterns and physical mapping of 45S and 5S rDNAs using fluorescence *in Situ* hybridization. *Cytologia*, *85*(1):49-55.

Yang, J. Y. et al. (2009). A karyotype analysis of *Lactuca* (Asteraceae) in Korea. *Korean Journal of Plant Taxonomy*, *39*(1):24-28.

Zarco, C. R. (1986). A new method for estimating karyotype asymmetry. *Taxon*, 35(3):526-530.