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Original Article

Anatomical and histochemical analysis of *Dysphania ambrosioides* supported by light and electron microscopy

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ABSTRACT

Dysphania ambrosioides (L.) Mosyakin & Clemants (syn: *Chenopodium ambrosoides* L.), Amaranthaceae, popularly known as "mastruz", is an herb widely used in Brazil as anthelmintic. To contribute to the knowledge about medicinal plants, a microscopic analysis was accomplished to describe the main anatomical characters of root, stem, petiole and leaf blade of *D. ambrosioides* and histochemical tests were performed on the leaf blade. Cross-sections were obtained, by hand, for microscopic analysis of root, stem, petiole and leaf blade; to the leaf blade were still made paradermal sections, scanning electron microscopy analysis, maceration and histochemical tests. The main characters useful in the identification of the plant were: anomalous secondary thickening in the root and stem; presence of idioblasts containing crystal sand in the root, stem, petiole and leaf blade; in these there are also idioblasts with druses; presence of non-glandular and glandular trichomes in the stem, petiole and leaf blade; stomata on the stem, petiole and leaf blade, identified in these as anomocytic and anisocytic; dorsiventral mesophyll and collateral vascular bundles. Maceration revealed that the vessel elements are helical type. Through the histochemical tests, it was evidenced the presence of lipophilic substances, essential oils, oleoresins, phenolic compounds, starch, lignin and calcium oxalate crystals. This work provides support to the quality control of the species.

Introduction

The family Chenopodiaceae was comprised about 102 genera and 1400 species distributed worldwide (Joly, 2002). Recent molecular phylogenetic studies include representatives of the family Chenopodiaceae in the family Amaranthaceae. Some species of the genus *Chenopodium* were transferred to the genus *Dysphania*, as *Chenopodium ambrosioides* L., which is currently known as *Dysphania ambrosioides* (L.) Mosyakin & Clemants (Fuentes-Bazan et al., 2012a,b; Senna, 2016).

D. ambrosioides is popularly known as epazote, Mexican tea, American wormseed, paico, mastruz and erva-de-Santa-Maria (Kliks, 1985; Albuquerque et al., 2009). The species is native to Central and South America, originated, probably, from Mexico. It has spontaneous growth in tropical and subtropical regions (mainly America and Africa) and also in temperate zones (from the Mediterranean to Central Europe) (Kismann, 1991). In Brazil, its distribution is extensive, occurring in almost all the territory (Sousa et al., 2004; Lima et al., 2006).

It is an herb that reaches up to 1 m high, being highly branched. The leaves are alternate, elongated, with jagged edges, acute apex, hairy and have different sizes, where the smaller are located on top of the plant and are sessile; the largest stand at the bottom and have short petiole. They have a strong and characteristic smell. The inflorescence is the racemosa type, presenting small flowers green colored. The seeds are numerous, spherical and have black color (Cruz, 1995; Lorenzi and Matos, 2002; Matos, 2007; Lorenzi, 2008).

The plant is considered by the World Health Organization as one of the most used among traditional medicines in the world (Lorenzi and Matos, 2002). The leaves are the part of the plant most often used in folk medicine as anthelmintic and also as antifungal (Taylor, 2005; Fenner et al., 2006; Neiva et al., 2011), for digestive disorders, muscle pains and bone fractures (Santayana et al., 2005; Garcia et al., 2010). In the Northeast of Brazil, where the species is widely used, the leaves are mixed in a blender with milk for flu treatments (Morais et al., 2005). In endemic areas of leishmaniasis, the population often uses its leaves in the topical treatment of ulcers caused by the disease (França et al., 1996).

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Phytochemical studies have identified polyphenols and terpenes as the main constituents of *D. ambrosioides* (Jorge et al., 1986; Jain et al., 1990; Paré et al., 1993; Kiuchi et al., 2002; Hegazy and Farrag, 2007; Hallala et al., 2010; Jardim et al., 2010; Neiva et al., 2011; Okhale et al., 2012; Barros et al., 2013; Sá, 2013). The essential oil of the leaves is mainly composed of monoterpenes, but the literature shows that there is wide variation both in its composition and in its percentage of constituents (Onocha et al., 1999; Gupta et al., 2002; Cavalli et al., 2004; Jardim et al., 2010; Sá et al., 2014).

Several biological activities have been reported for *D. ambrosioides* (Sá et al., 2015), such as antitumor (Nascimento et al., 2006; Barros et al., 2013), antipyretic, analgesic, anti-inflammatory, antinociceptive (Hallala et al., 2010; Trivellato Grassi et al., 2013), antifungal (Jardim et al., 2010), anthelmintic (Guimaraes et al., 2001; Neiva et al., 2011) and antiprotozoal (Monzote et al., 2007; Patrício et al., 2008; Monzote et al., 2014).

Given the recognized popular use and the pharmacological studies that have demonstrated the therapeutic potential, *D. ambrosioides* is one of 71 species of plants that arouse the Brazilian government's interest for the production of phytotherapics, being present in the National Relation Medicinal Plants of Interest to Unified Health System (MS, 2009).

Considering that the knowledge of the microscopic characteristics is fundamental for the standardization of plants used as medicines, this study aims to describe the main anatomical characters of the root, stem, petiole and leaf blade of *D. ambrosioides* and perform histochemical tests on the leaf blade.

Materials and methods

Plant material

Several adult specimens of *Dysphania ambrosioides* (L.) Mosyakin & Clemants (syn: *Chenopodium ambrosioides* L.), Amaranthaceae, cultivated under full sun, were collected in the garden of the Laboratório de Fitoterapia, in the company Pernambuco Participações e Investimentos S/A, located in Recife at the Pernambuco State in Brazil. The voucher specimen was deposited in the Herbarium UFP-Geraldo Mariz, of the Universidade Federal de Pernambuco, Brazil, under registration number 69718.

Anatomical characterization

For their structural characterization, various cross-sections at the middle region of the root, stem, petiole and leaf blade fixed in FAA 50% (Johansen, 1940) were obtained by hand, using a common razor blade. For leaf blade were also performed paradermal sections on the adaxial and abaxial faces. All sections were clarified in sodium hypochlorite solution (50%) (Kraus and Arduin, 1997). Posteriorly, cross-sections were stained with safranin and astra blue (Bukatsch, 1972) and paradermal sections were stained with methylene blue (1%) (Krauter, 1985). Subsequently, semipermanent histological slides were prepared containing cross-sections and paradermal sections of botanical material, following usual plant anatomy procedures (Johansen, 1940; Sass, 1951). Analyses were performed on images in software (Toup View Image), obtained by digital camera coupled to a light microscope (Alltion).

Maceration

The maceration was performed using leaf blade fragments that were disintegrated with the mixture of 10% nitric acid and 10% chromic acid (1:1), according to the method of Jeffrey (Johansen, 1940). Analyses were carried out on images in software (Toup View Image), obtained by digital camera coupled to a light microscope (Alltion).

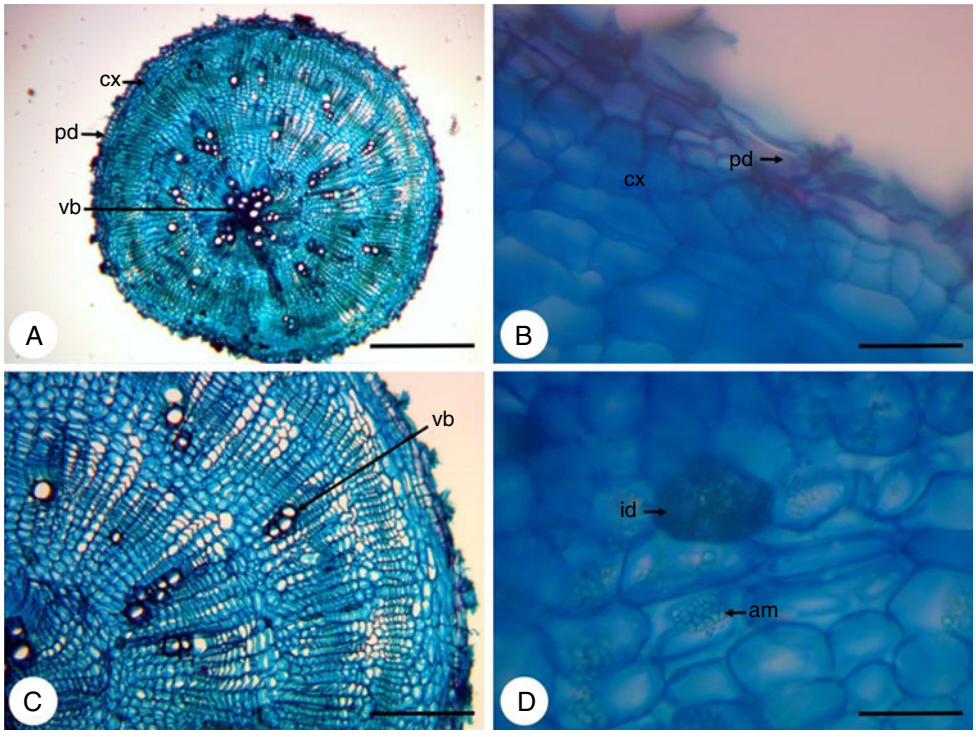

Fig. 1. *Dysphania ambrosioides* (L.) Mosyakin & Clemants, cross-sections of the root. (A) General aspect, showing peridermis (pd), cortex (cx) and vascular bundle (vb); (B) Detail of periderm (pd) and cortex (cx); (C) Secondary growth with vascular bundles (vb) irregularly distributed; (D) Detail of amyloplast (am) and idioblast (id) with crystal sand. Bars: A = 500 μm; B, D = 50 μm; C = 200 μm.

Histochemical characterization

Histochemical tests were made on cross-sections of fresh leaf blades obtained by the same method used in anatomical study. The following specific reagents were used to show the secretion sites and/or accumulation of substances: Sudan III for lipophilic substances (Sass, 1951); Nadi reagent for essential oils and oleoresins (David and Carde, 1964); potassium dichromate (10%) for phenolic compounds (Gabe, 1968); Lugol's iodine reagent for starch (Johansen, 1940); phloroglucinol for lignin (Johansen, 1940); Dragendorff's reagent for detecting alkaloids (Farmacopeia Brasileira, 2010); antimony trichloride for triterpenes and steroids (Mace et al., 1974); vanillin chloridric for tannins (Mace and Howell, 1974) and hydrochloric acid (10%) to establish the nature of the crystals (Jensen, 1962).

Controls were performed in parallel with the tests and semipermanent histological slides were prepared containing the cross-sections (Johansen, 1940; Sass, 1951). Analyses were performed on images in software (Toup View Image), obtained by digital camera coupled to a light microscope (Alltion).

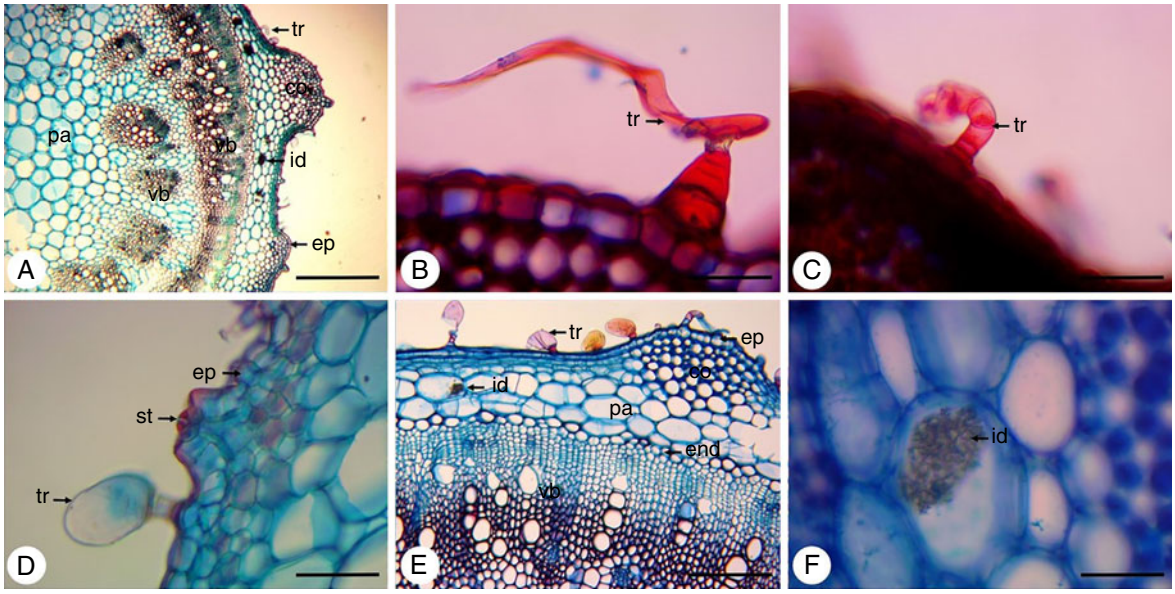

Fig. 2. *Dysphania ambrosioides* (L.) Mosyakin & Clemants, cross-sections of the stem. (A) General aspect, showing epidermis (ep), trichome (tr), collenchyma (co), parenchyma (pa), vascular bundles (vb) and idioblast (id) with crystal sand; (B) Detail of non-glandular trichome (tr), multicellular and uniseriate, with enlarged cells at the base and an elongated apical cell; (C) Detail of non-capitate glandular trichome (tr) with short uniseriate stalk and a small globoid apex and bents toward the epidermis; (D) Detail of capitate glandular trichome (tr) with a short pedicel and a large unicellular head, stomata (st) and epidermis (ep); (E) Detail of epidermis (ep), trichome (tr), collenchyma (co), parenchyma (pa), endodermis (end), vascular bundles (vb) and idioblast (id) with crystal sand. (F) Detail of idioblast (id) with crystal sand. Bars: A = 500 μm; B, C, D, F = 50 μm; E = 200 μm.

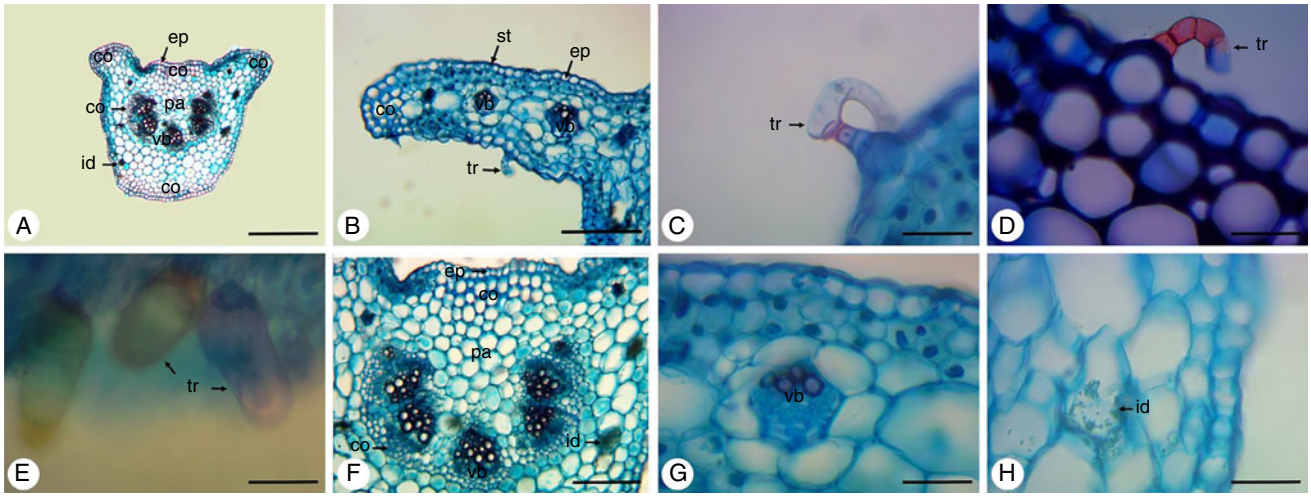

Fig. 3. *Dysphania ambrosioides* (L.) Mosyakin & Clemants, cross-sections of the petiole. (A) General aspect, showing epidermis (ep), collenchyma (co), parenchyma (pa), vascular bundles (vb) and idioblast (id) with crystal sand; (B) Lateral extremity, showing uniseriate epidermis (ep) with stomata (st) and trichome (tr), collenchyma (co), parenchyma (pa) and vascular bundles (vb); (C) Detail of non-glandular trichome (tr), multicellular and uniseriate, with enlarged cells at the base and an elongated apical cell; (D) Detail of non-capitate glandular trichome (tr) with short uniseriate stalk and a small globoid apex and bents toward the epidermis; (E) Detail of capitate glandular trichomes (tr) with a short pedicel and a large unicellular head; (F) Central region, showing epidermis (ep), collenchyma (co), parenchyma (pa), idioblast (id) with crystal sand and collateral vascular bundles (vb) arranged in a semicircle; (G) Detail of a vascular bundle (vb) located in lateral extremity; (H) Detail of idioblast (id) with crystal sand. Bars: A = 500 μm; B, F = 200 μm; C, D, E = 50 μm.

Scanning electron microscopy (SEM)

Leaf blades samples were fixed in 2.5% glutaraldehyde (buffered with 0.1 M sodium cacodylate) and post fixed in 2% osmium solution (buffered with 0.1 M sodium cacodylate). After dehydration in ethanol series, the material was submitted to critical point drying (Bal-Tec CPD 030). Suitable portions were mounted onto SEM stubs using double-sided adhesive tape and sputter-coated with gold (Leica EM SCD 500) (Silveira, 1989). Both adaxial and abaxial surfaces were examined with a QUANTA 200

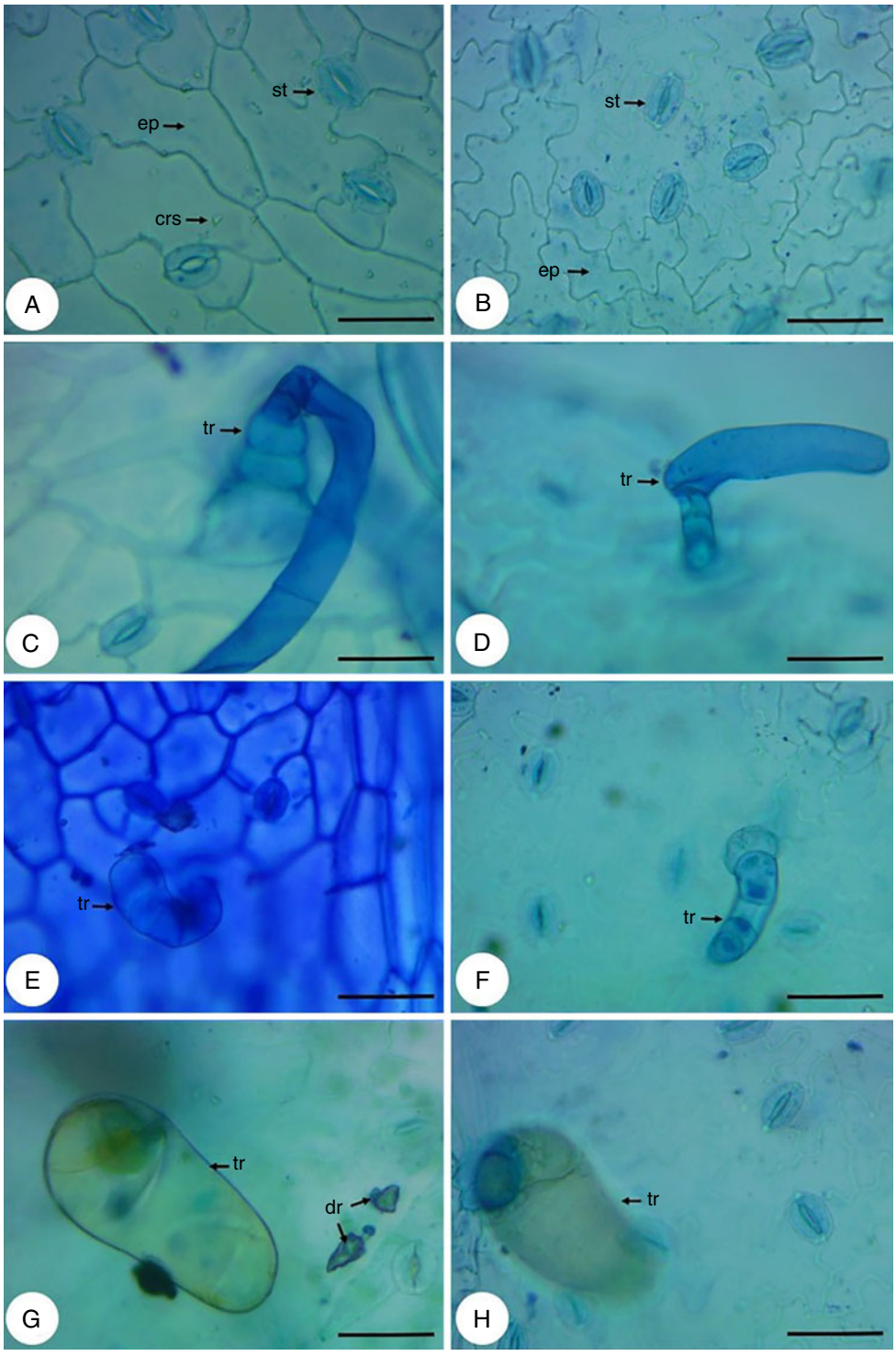

Fig. 4. *Dysphania ambrosioides* (L.) Mosyakin & Clemants, frontal view of the leaf blade. (A) View of the adaxial face showing epidermal (ep) cells with straight or slightly sinuous walls, stomata (st) anomocytic and anisocytic, and idioblast with crystal sand (crs); (B) View of the abaxial face showing epidermal (ep) cells with sinuous walls and stomata (st) anomocytic and anisocytic; (C) Detail of non-glandular trichome (tr), multicellular and uniseriate, with enlarged cells at the base and an elongated apical cell on the adaxial face; (D) Detail of non-glandular trichome (tr), multicellular and uniseriate, with enlarged cells at the base and an elongated apical cell on the abaxial face; (E) Detail of non-capitate glandular trichome (tr) with short uniseriate stalk and a small globoid apex and bents toward the epidermis on the adaxial face; (F) Detail of non-capitate glandular trichome (tr) with short uniseriate stalk and a small globoid apex and bents toward the epidermis on the abaxial face; (G) Detail of capitate glandular trichome (tr) with a short pedicel and a large unicellular head on the adaxial face and idioblasts with druses (dr); (H) Detail of capitate glandular trichome (tr) with a short pedicel and a large unicellular head on the abaxial face. Bars: A–H = 50 μm.

FEG scanning electron microscope in the Centro de Tecnologias Estratégicas do Nordeste (CETENE).

Results

Root

In cross-section the root has a circular shape (Fig. 1A) and presents periderm and a very small cortical region (Fig. 1B), due to the development of anomalous secondary thickening, characterized by a concentric zone of collateral vascular bundles irregularly distributed, that arise from a succession of arcs of cambium (Fig. 1C). It is observed in all root several cells containing starch and idioblasts with crystal sand (Fig. 1D).

Stem

In cross-section, the stem has a polygonal shape, with regions more prominent (Fig. 2A). Trichomes are located throughout its extension (Fig. 2A and D), which are non-glandular trichome, multicellular and uniseriate, with enlarged cells at the base and an elongated apical cell (Fig. 2B) and also glandular trichomes. These can be of two types: non-capitate glandular trichome with short uniseriate stalk and a small globoid apex and bents toward the epidermis (Fig. 2C), and the capitate glandular trichome, with a short pedicel and a large unicellular head (Fig. 2D).

The epidermis consists of a single layer of cells coated with a thin cuticle (Fig. 2A, D and E). Stomata are inserted above the level of the epidermal cells (Fig. 2D). In less prominent regions of the stem, adjacent to the epidermis, is found a layer of cells that may be part of the epidermis, making it multilayered, or may constitute a hypodermis. Already in more prominent regions, the angular collenchyma is located beneath the epidermis, being composed of four to nine layers of cells (Fig. 2A and E). In the cortical parenchyma are present idioblasts with crystal sand (Fig. 2A, E and F). The endodermis is seen as a last cortical layer (Fig. 2E). As well as the root, the stem also exhibits anomalous secondary thickening. It is distinguished two different zones of vascular bundles: a zone closer to the endodermis, in which the collateral bundles are distributed forming a continuous ring; and other zone closest to the medullary region, in which the collateral bundles are distributed discontinuously, separated from each other by parenchyma (Fig. 2A and E).

Petiole

The petiole, in cross-section, has concave–convex shape, with two lateral extremities (Fig. 3A). The epidermis is composed of a single layer of rounded cells and covered with a smooth and thin cuticle (Fig. 3A and B). Stomata are inserted in the same level of epidermal cells (Fig. 3B). As epidermal attachments, are present non-glandular trichome, multicellular and uniseriate, with enlarged cells at the base and an elongated apical cell (Fig. 3C), besides non-capitate glandular trichome with short uniseriate stalk and a small globoid apex and bents toward the epidermis (Fig. 3D) and capitate glandular trichome, with a short pedicel and a large unicellular head (Fig. 3E). The collenchyma of the angular type is observed below the epidermis in the central region of the petiole (Fig. 3A and F), being formed by two or three layers of cells. This tissue is also present in the lateral extremities and adjacent to phloem (Fig. 3A, B and F). The vascular bundles are collateral and are arranged in a semicircle in the central region of the petiole (Fig. 3A and F). Smaller vascular bundles are displayed in the lateral extremities (Fig. 3B and G). In the parenchyma are observed idioblasts containing crystal sand (Fig. 3A, F and H).

Leaf blade

The epidermis, in frontal view, consists of cells with straight or slightly sinuous walls on the adaxial face (Fig. 4A) and of cells with walls more sinuous on the abaxial face (Fig. 4B). The stomata, that are anomocytic and anisocytic, are present on both sides (Fig. 4A and B). Non-glandular trichomes (Fig. 4C and D) and glandular trichomes (Fig. 4E–H) occur both on the adaxial face (Fig. 4E and G) as on the abaxial face (Fig. 4F and H) and are of the same type previously described in the stem and petiole. However, the glandular trichomes occur in greater number on the adaxial face, among the two types of glandular trichomes found, predominate the capitate glandular trichome. In this cut is still possible to view idioblasts with crystal sand (Fig. 4A) and druses (Fig. 4G).

In the leaf blade analysis in SEM it was possible observed with more detail the largest sinuosity of the epidermal cells walls on the abaxial face (Fig. 5A and B) and that on this face the stomata are situated on the same level or slightly above of epidermal cells (Fig. 5B), while on the adaxial face they are located on the same level of epidermal cells (Fig. 5C). It was also observed the non-glandular (Fig. 5A) and glandular trichomes (Fig. 5A–C).

In cross-section of the leaf blade were observed all the trichomes (Fig. 6A–F) viewed in the paradermal cut and in SEM, also ratifying the fact that the glandular trichomes are predominant in the abaxial face. The epidermis is uniseriate, coated with a thin cuticle layer and is made of rounded cells or slightly elongated (Fig. 6G). The mesophyll has organization dorsiventral. It consists of one or two layers of palisade parenchyma and two to four layers of dense spongy parenchyma (Fig. 6G). Secretory cavities (Fig. 6G) and idioblasts with crystal sand and druses (Fig. 6G and H) are found in the mesophyll.

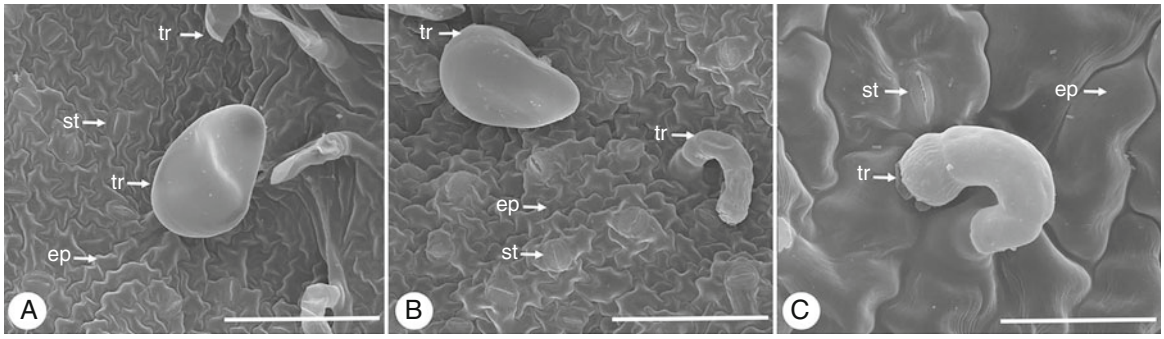

Fig. 5. *Dysphania ambrosioides* (L.) Mosyakin & Clemants, SEM of the leaf blade. (A) View of the abaxial face showing epidermal (ep) cells with sinuous walls, stomata (st) and non-glandular and glandular trichomes (tr); (B) View of the abaxial face showing epidermal (ep) cells with sinuous walls, stomata (st) and glandular trichomes (tr); (C) View of the adaxial face showing epidermal (ep) cells with slightly sinuous walls, stomata (st) and glandular trichome (tr). Bars: A, B = 100 μm; C = 40 μm.

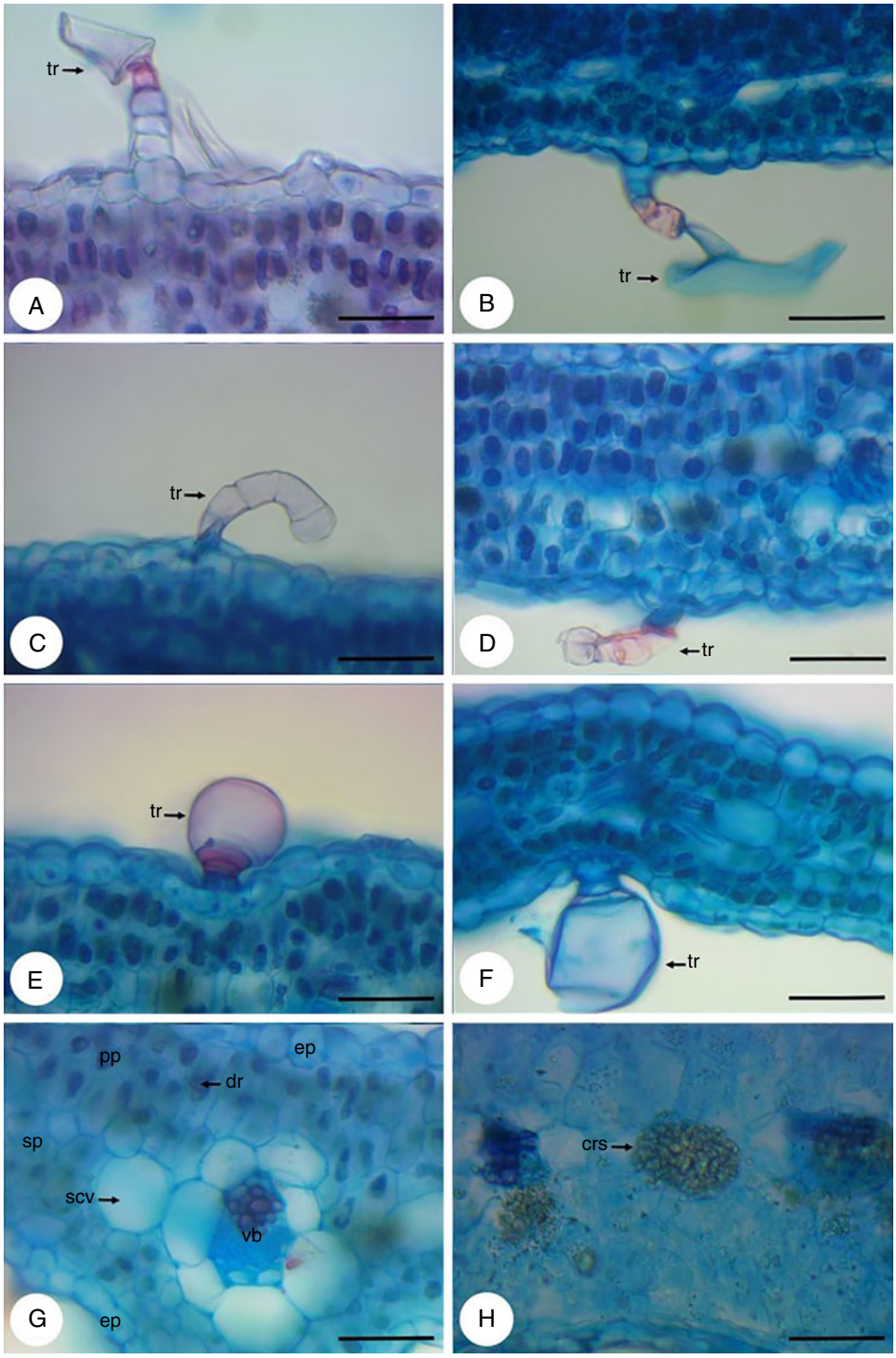

Fig. 6. *Dysphania ambrosioides* (L.) Mosyakin & Clemants, cross-sections of the leaf blade. (A) Detail of non-glandular trichome (tr), multicellular and uniseriate, with enlarged cells at the base and an elongated apical cell on the adaxial face; (B) Detail of non-glandular trichome (tr), multicellular and uniseriate, with enlarged cells at the base and an elongated apical cell on the abaxial face; (C) Detail of non-capitate glandular trichome (tr) with short uniseriate stalk and a small globoid apex and bents toward the epidermis on the adaxial face; (D) Detail of non-capitate glandular trichome (tr) with short uniseriate stalk and a small globoid apex and bents toward the epidermis on the abaxial face; (E) Detail of capitate glandular trichome (tr) with a short pedicel and a large unicellular head on the adaxial face; (F) Detail of capitate glandular trichome (tr) with a short pedicel and a large unicellular head on the abaxial face; (G) Detail of the epidermis (ep), palisade parenchyma (pp), spongy parenchyma (sp), secretory cavity (scv), vascular bundle (vb) and idioblast with druse (dr); (H) Detail of idioblast with crystal sand (crs). Bars: A–H = 50 μm.

The midrib has biconvex cross-section (Fig. 7A). It presents similar epidermis to which is situated in the mesophyll (Fig. 7B). Furthermore, all three types of trichomes visualized in the mesophyll are also present in the midrib (Fig. 7C–E). Angular collenchyma appears in subepidermal position, formed by two to three layers of cells (Fig. 7A and B), and also adjacent to phloem (Fig. 7A and F). In this tissue and in the parenchyma are observed idioblasts with crystal sand and druses (Fig. 7B and F). The vascular system is composed of collateral bundles arranged in a circle in the center of midrib (Fig. 7F).

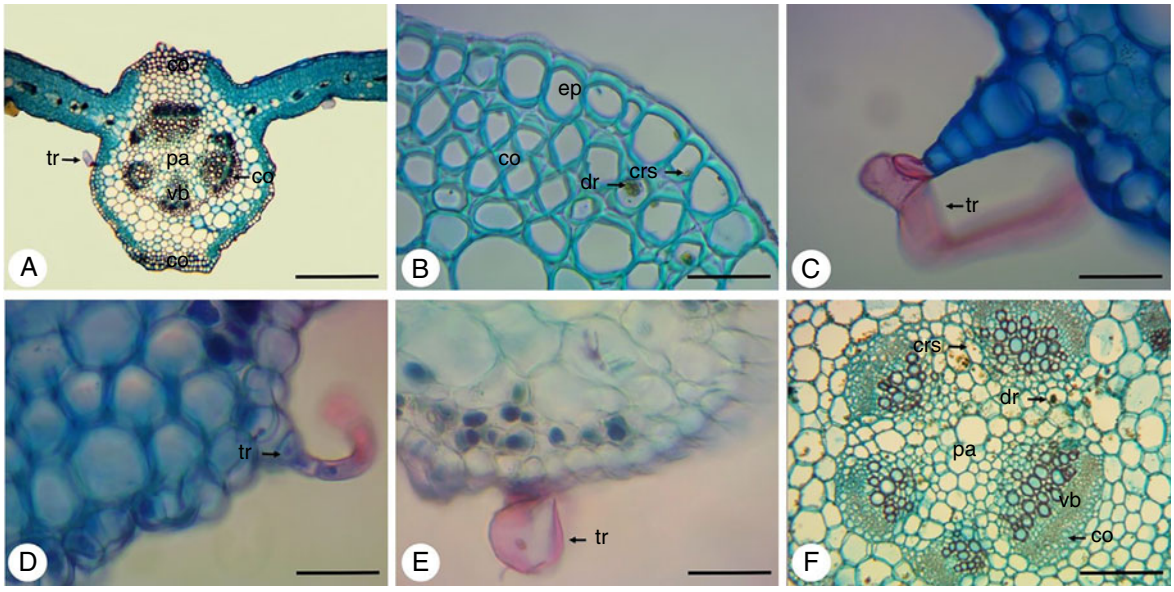

Fig. 7. *Dysphania ambrosioides* (L.) Mosyakin & Clemants, cross-sections of midri(B) (A) General aspect, showing collenchyma (co), parenchyma (pa), vascular bundles (vb) and trichome (tr); (B) Detail of the epidermis (ep), collenchyma (co) and idioblasts with crystal sand (crs) and druse (dr); (C) Detail of non-glandular trichome (tr), multicellular and uniseriate, with enlarged cells at the base and an elongated apical cell; (D) Detail of non-capitate glandular trichome (tr) with short uniseriate stalk and a small globoid apex and bents toward the epidermis; (E) Detail of capitate glandular trichome (tr) with a short pedicel and a large unicellular head; (F) Detail of the vascular bundles (vb) located in the parenchyma (pa), collenchyma (co) adjacent to phloem and idioblasts with crystal sand (crs) and druse (dr). Bars: A = 500 μm; B, C, D, E = 50 μm; F = 200 μm.

The leaf of *D. ambrosioides*, after maceration, presents epidermal cells, stomata (Fig. 8A), vessel elements of the helical type (Fig. 8B) and idioblasts with druses (Fig. 8C). The three types of trichomes viewed in transverse and paradermal sections and in SEM are also found in macerated leaf (Fig. 8D–F).

Fig. 9A–D shows cross-sections of fresh leaf blades without addition of reagent.

After using Sudan III, lipophilic substances were found in the cuticle covering the adaxial and abaxial faces (Fig. 10A), and are also present within capitate glandular trichomes with unicellular head (Fig. 10B). In these trichomes, lipophilic substances may be those which constitute the essential oil and oleoresins of *D. ambrosioides*, considering that, with the Nadi reagent, these compounds exhibit blue and pink colorations, respectively (Fig. 10C and D). Oleoresins were also found in the parenchyma cells of the midrib (Fig. 10E) and in the upper epidermis cells next to the mesophyll (Fig. 10F).

Potassium dichromate (10%) revealed the presence of phenolic compounds in the adaxial epidermal cells (Fig. 11A and B), as well as inside the capitate glandular trichome with unicellular head (Fig. 11C). Starch is present in the epidermal cells and in the mesophyll (Fig. 11D), as also in the parenchyma of the midrib (Fig. 11E). The phloroglucinol evidenced lignification of xylematic vessel in midrib (Fig. 11F). Fig. 11G and H show, respectively, the presence

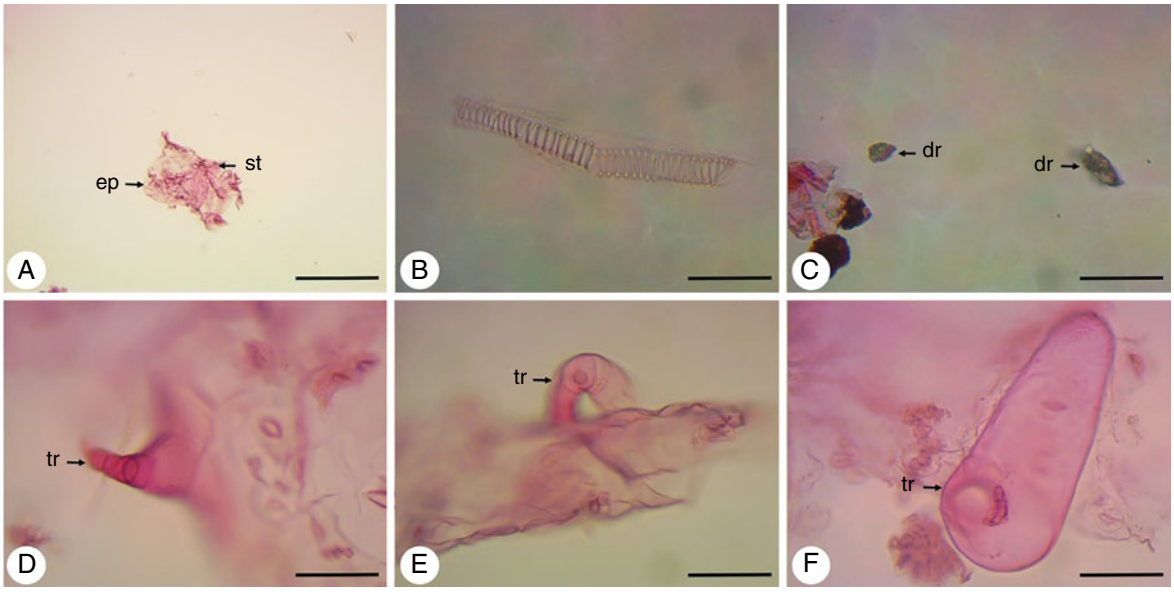

Fig. 8. *Dysphania ambrosioides* (L.) Mosyakin & Clemants, maceration of the leaf. (A) Epidermis (ep) and stomata (st); (B) Vessel element of the helical type; (C) Idioblasts with druses (dr); (D) Non-glandular trichome (tr), multicellular and uniseriate, with enlarged cells at the base and an elongated apical cell; (E) Non-capitate glandular trichome (tr) with short uniseriate stalk and a small globoid apex and bents toward the epidermis; (F) Capitate glandular trichome (tr) with a short pedicel and a large unicellular head. Bars: A = 200 μm; B–F = 50 μm.

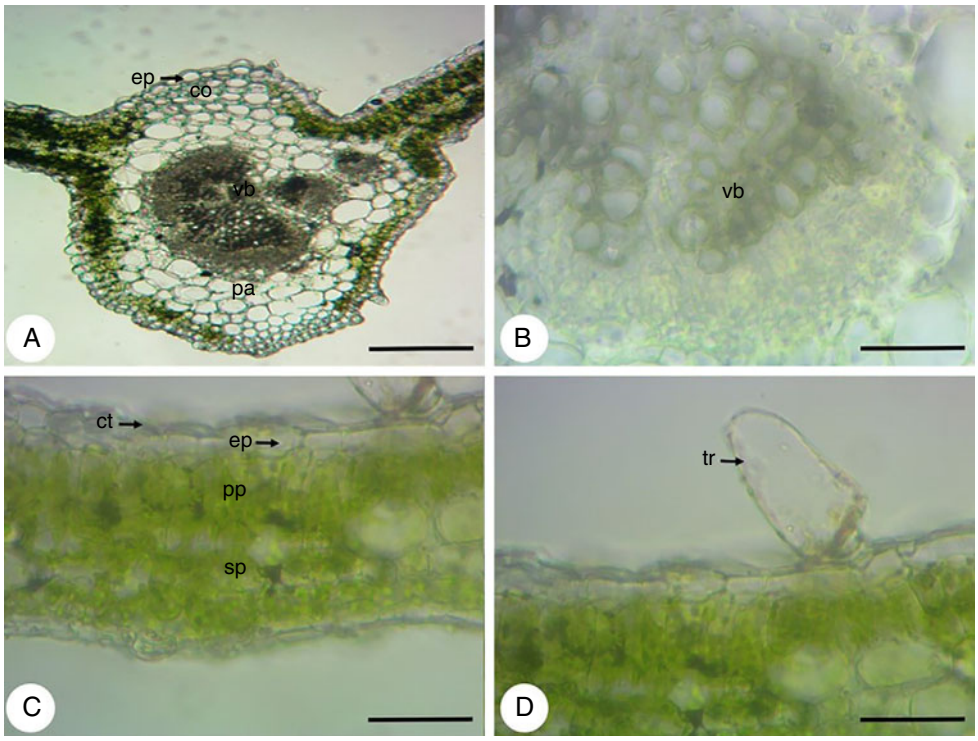

Fig. 9. *Dysphania ambrosioides* (L.) Mosyakin & Clemants, cross-sections of the leaf blade without adding reagents. (A) Midrib showing epidermis (ep), collenchyma (co), parenchyma (pa) and vascular bundles (vb); (B) Detail of vascular bundle (vb); (C) Details of cuticle (ct), epidermis (ep), palisade parenchyma (pp) and spongy parenchyma (sp); (D) Detail of capitate glandular trichome (tr) with a short pedicel and a large unicellular head. Bars: A = 200 μm; B–D = 50 μm.

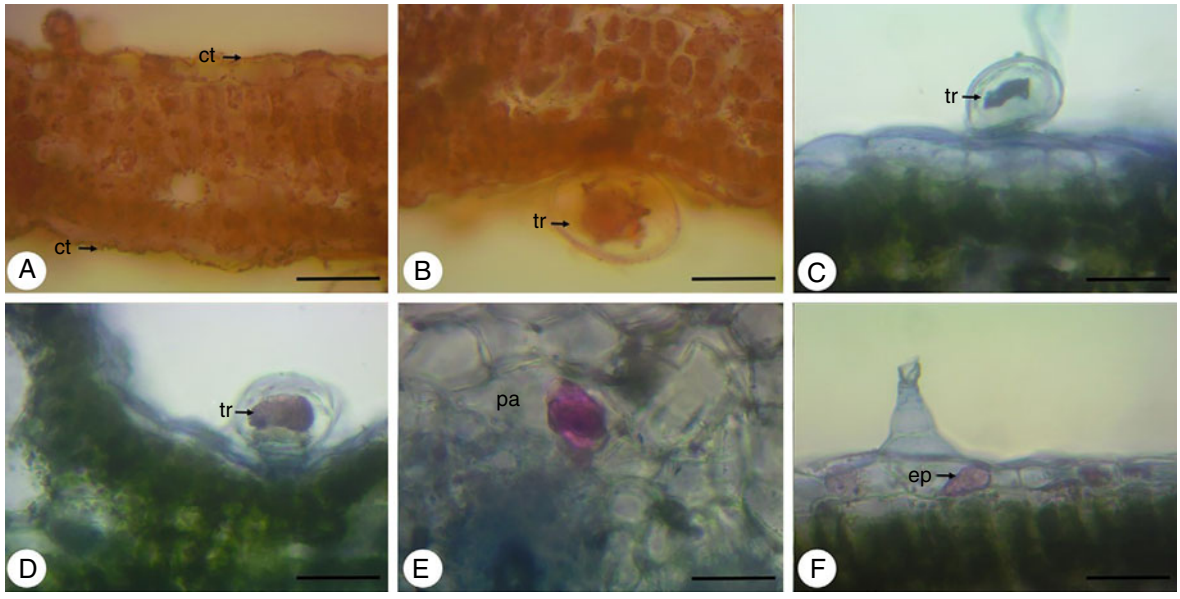

Fig. 10. *Dysphania ambrosioides* (L.) Mosyakin & Clemants, cross-sections of the leaf blade after reaction with Sudan III and Nadi reagent. (A) Lipophilic substances in the cuticle (ct) lining the epidermis; (B) Lipophilic substances inside capitate glandular trichome (tr); (C) Essential oil inside capitate glandular trichome (tr); (D) Oleoresin inside capitate glandular trichome (tr); (E) Oleoresin in parenchyma (pa); (F) Oleoresin in epidermal (ep) cells. Bars: A–F = 50 μm.

of crystals in leaf of *D. ambrosioides* and their dissolution with the test of hydrochloric acid (10%), confirming that they are of calcium oxalate. Tests with Dragendorff's reagent, antimony trichloride and vanillin chloridric were negative.

Discussion

The transition of some species of the family Chenopodiaceae to the family Amaranthaceae is recent. Although the modification, the literature still treats *D. ambrosioides* as *C. ambrosioides*. For this reason, the discussion of this work took into consideration the aspects of the family Chenopodiaceae.

According to Metcalfe and Chalk (1972), the family Chenopodiaceae has many anatomical points in common with other families, such as the Amaranthaceae. One of these points is the cambium variation, or anomalous secondary thickening (Wilson, 1924; Joshi, 1937; Balfour, 1965). The formation of successive cambia is known in 34 species of dicotyledons, being that in lianas this phenomenon

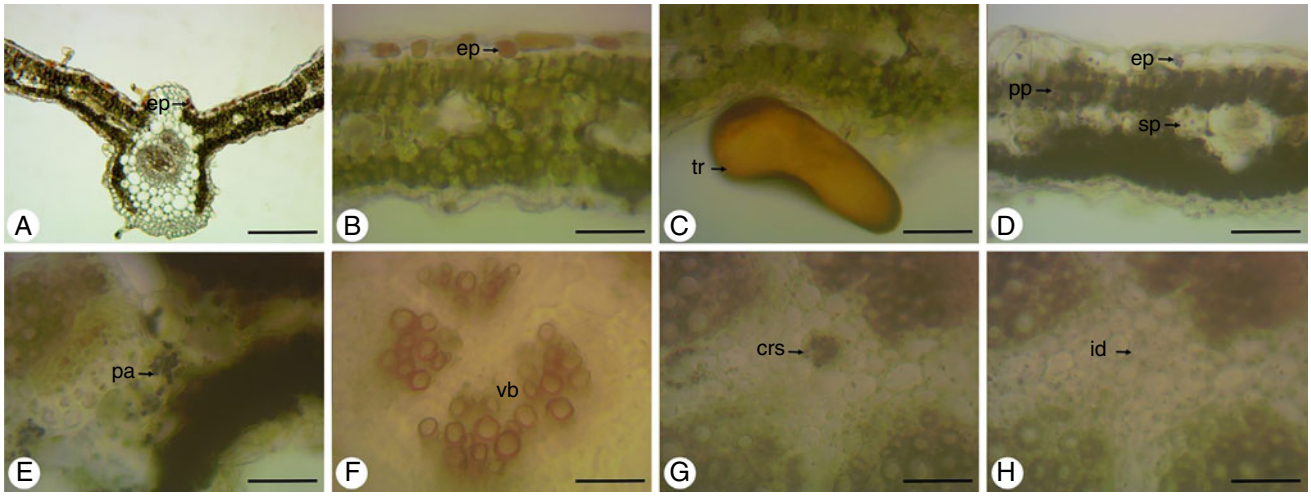

Fig. 11. *Dysphania ambrosioides* (L.) Mosyakin & Clemants, cross-sections of the leaf blade after reaction with potassium dichromate (10%), Lugol's iodine reagent, phloroglucinol and hydrochloric acid (10%). (A) View of the leaf showing phenolic compounds in epidermal (ep) cells; (B) Detail of epidermal (ep) cells containing phenolic compounds; (C) Detail of phenolic compounds inside capitate glandular trichome (tr); (D) Starch in epidermal (ep) cells, palisade parenchyma (pp) and spongy parenchyma (sp); (E) Detail of starch in parenchyma (pa); (F) View of vascular bundles (vb) with lignified walls; (G) Detail of idioblast with crystal sand (crs) before reaction with hydrochloric acid (10%); (H) Detail of idioblast (id) after reaction with hydrochloric acid (10%). Bars: A = 200 μm; B–H = 50 μm.

occurs more frequently (Metcalfe and Chalk, 1972; Carlquist, 2007). In Chenopodiaceae, the secondary growth starts from a cambium in a normal position. Subsequently, the other cambia emerge furthest from the first, producing xylem to inside and phloem to outside (Esau, 1997). The successive cambia and the vascular bundles are embedded in parenchymal tissue (Metcalfe and Chalk, 1972).

With respect yet to the secondary growth of the plant, it is observed the periderm formation in roots, but not in stems. The absence of periderm in stem was already observed in 12 species of *Chenopodium*, including *D. ambrosioides* (Bonzani et al., 2003), as well as in other species of Chenopodiaceae, such as *Atriplex halimus*, *Atriplex cristata*, *Atriplex oestophora* (Fahn and Zimmermann, 1982; Jáuregui et al., 2014); *Allenrolfea patagonica*, *Heterostachys olivascens*, *Heterostachys ritteriana* (Cuadra and Hermann, 2014); and in *Salsola kali* subsp. *Ruthenica* (Bercu and Bavaru, 2004).

The polygonal shape of the stem with the presence of regions more prominent formed by collenchymatic tissue is common in Chenopodiaceae (Fahn and Zimmermann, 1982; Bonzani et al., 2003; Bercu and Bavaru, 2004; Jáuregui et al., 2014). Bonzani et al. (2003) observed that in *Chenopodium burkartii* and *Chenopodium retusum* the collenchyma becomes lignified when these species are in secondary growth. Already in other species of *Chenopodium* studied, including *D. ambrosioides*, the collenchyma remains composed of cellulosic walls in secondary growth.

Stomata were found in the stem, petiole and leaf blade of the plant. Amphistomatic leaves are characteristics of the Chenopodiaceae family (Metcalfe and Chalk, 1972; Moris et al., 1996; Bonzani et al., 2003; Cuadra and Hermann, 2014; Jáuregui et al., 2014). Anomocytic stomata are the most frequent, but are also found anisocytic stomata in *Chenopodium chilense*, tetracytic in *Chenopodium multifidum* (Bonzani et al., 2003) and paracytic in *Salsola kali* subsp. *ruthenica* (Bercu and Bavaru, 2004). Previous studies with *D. ambrosioides* only affirmed the presence of anomocytic stomata, different from the results found in this study, which are described stomata anomocytic and anisocytic (Jorge et al., 1986; Costa and Tavares, 2006).

The presence of different types of trichomes in Chenopodiaceae was reported by some authors (Holm, 1923; Metcalfe and Chalk, 1972; Silva Filho et al., 1992; Bonzani et al., 2003). According to Metcalfe and Chalk (1972), in the family are present trichomes uniseriate, branched, stellate and capitate glandular trichome. In the genus *Chenopodium* are most common the non-glandular trichome uniseriate and the capitate glandular trichome. Both were observed in the stem, petiole and leaf blade of *D. ambrosioides*, but it was also found the glandular trichome uniseriate with body bent toward epidermis and with secretory distal cell rounded. This latter type of trichome is not described by Metcalfe and Chalk (1972), however, in a detailed study of the trichomes of the stem and leaves of *Chenopodium* species, Bonzani et al. (2003) cited all kinds of trichomes found in this work.

There is still controversy regarding the presence of glandular trichomes on the faces of the leaf blade of *D. ambrosioides*. As are seen in this study, the presence on both faces was also identified by Holm (1923), Jorge et al. (1986) and Bonzani et al. (2003) in *D. ambrosioides*. Jorge et al. (1986) affirmed that the glandular trichomes predominate in the adaxial face, in agreement with the results described in this paper and differing from Costa and Tavares (2006), who reported these trichomes restricted to abaxial face.

Studies suggest that the density and the change in the proportion of non-glandular and glandular trichomes can be influenced by environmental conditions, including herbivory and water availability (Rautio et al., 2002; Gonzales et al., 2008). Since the species is found in tropical, subtropical and temperate zones (Kismann, 1991), it can be expected that these variations occur in *D. ambrosioides*.

Most species of Chenopodiaceae have dorsiventral mesophyll (Metcalfe and Chalk, 1972). There are some exceptions, such as *C. retusum*, *Chenopodium oblanceolatum* (Bonzani et al., 2003) and *Salsola* sp. (Metcalfe and Chalk, 1972) that present isobilateral mesophyll.

Idioblasts containing crystal sand were often found in all plant parts examined. Only in the leaf blade were observed druses. The confirmation, by testing with hydrochloric acid that the crystals are of calcium oxalate corroborates the result of Costa and Tavares (2006). According Metcalfe and Chalk (1972), the occurrence of these crystals gives important diagnostic value for the species, since they are restricted between dicotyledonous.

The maceration of plant tissue is used to reveal some peculiarities of the nature of the cells that compose them. It is a technique recommended by the Brazilian Pharmacopeia for microscopic analysis of the plant material (Farmacopeia Brasileira, 2010) and a requirement for the registration of phytotherapic and traditional phytotherapic products (Anvisa, 2014). It is also important when the plant raw materials are marketed crushed or powdered,

not being possible performing sections to the anatomical study (Farmacopeia Brasileira, 2010).

They were detected in the leaf blades lipophilic and hydrophilic substances, evidencing lipids, essential oils, oleoresins and phenolic compounds. These data corroborate the findings in the literature for the species, known for producing various compounds of metabolism used in the defense and adaptation of plants to the environment and also in medicine (Sá et al., 2015). Most plants store starch as a reserve. This carbohydrate, as well as being related as an energy source is also an adaptive strategy of the species to adverse environmental conditions (Oliveira and Marquis, 2002). The lignin present in the wall of the xylem vessels is responsible for sustaining the plant and also provides defense for the plant, because it is considered as a substance resistant to pathogens, hidering their colonization (Silva et al., 2005).

Conclusion

Through the different microscopy techniques it was possible to establish anatomical features that are useful in the identification of *D. ambrosioides*, which were: anomalous secondary thickening in the root and stem; presence of idioblasts containing crystal sand in the root, stem, petiole and leaf blade; in these there are also idioblasts with druses; presence of non-glandular and glandular trichomes in the stem, petiole and leaf blade; stomata on the stem, petiole and leaf blade, identified in that as anomocytic and anisocytic; dorsiventral mesophyll and collateral vascular bundles. The maceration showed to be a useful tool when it cannot make cuts to the anatomical study. In addition to contributing significantly to the knowledge of the anatomy, it was also possible to see the histolocalization of some groups of metabolites present in the leaf blade of the species. Thus, the work provides quality parameters for the species studied, since it does not have appropriate monograph in current official codes.

Authors' contributions

RS contributed in collecting plant sample and identification, confection of herbarium, running the laboratory work, analysis of the data and drafted the paper. AS contributed in the preparation of semi-permanent slides. FS and LS contributed to critical reading of the manuscript. KR designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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