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MORPHOLOGICAL AND ANATOMICAL PROFILE OF *CASSIA OCCIDENTALIS* (L.) SPECIES GROWN IN THE REPUBLIC OF MOLDOVA

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Abstract: The current research deals with the morpho-anatomical characteristics of *Cassia occidentalis* (L.) (Coffee senna plant), grown in the steppe climate conditions of the Republic of Moldova. This study was performed according to investigated organ in vegetative periods of 2019-2021 years. Morphological and anatomical indices of organs with diagnostic role in identifying species and those with adaptive potential to the action of unfavorable growth factors, have been elucidated. These indices were evaluated in the light of data already reported by other researchers. This morpho-anatomical research has revealed new aspects to support some anatomical features already outlined, but less confirmed in the studies of the last decades and will serve as landmarks for a real clarity of the integral microstructural picture of the *C. occidentalis* species.

Keywords: *Cassia occidentalis*, *Senna occidentalis*, morphology, anatomy, Republic of Moldova

1. Introduction

Morphological and taxonomic data of *Cassia occidentalis* (L.) (synonym of *Senna occidentalis* (L.) Link) species is very confusing in the evaluated bibliography, sometimes even contradictory (Kumar, 2009; Begum et al., 2014; Fonkou et al., 2018). We agree, as taxonomic monographs are now considered incomplete without epidermal micromorphology (Rejdali, 1991) in particular, and in general without microscopic characteristics. We believe that microscopic studies can provide clarity in this aspect. In this regard, we refer to recent microscopic studies on some species of g. *Cassia* in general (Sahai, 2001; Ogundipe et al., 2009) and *S. occidentalis* in particular (Nassar et al., 2013; Begum et al., 2014; Doty et al., 2020). This species is known in English as Coffee senna, Coffeeweed, Piss-a-bed, Mogdad coffee, Negro-coffee, Senna coffee, Stephanie coffee, Sinkingweed or Styptic weed, but the most used English common name is Coffee senna (Allsopp, 1996). The current investigation concerns the morphology and anatomy of Coffee senna plants, grown in the climate conditions of the Republic of Moldova, situated in southeastern Europe, and characterized by a predominantly temperate steppe climate. The

species was introduced into Collection of Medicinal and Aromatic Plans of the Institute of Genetics, Physiology and Plant Protection (IGPPP) in 2017 and has been propagated from its own seeds in the following period. The morpho-anatomical structure of plant organs was performed in the mass flowering period of the plant during the growing season of 2020-2021 years.

The Coffee senna plants contain valuable chemical constituents like: sennosides and anthraquinones (pods); dianthronic heterosides (leaves); apigenin (pericarp); emodol (roots); betasitosterol (flowers); volatile oils (leaves, roots, seeds) used to combat illness and support the body's own defense to regain good health (Khare, 2004; Yadav et al., 2010; Vijayalakshmi et al., 2013; Ngombe et al., 2019). These metabolites are responsible for numerous pharmacological properties: antidiabetic, antimicrobial, antioxidant, anti-inflammatory, anticarcinogenic and antimalarial. The Coffee senna plant also possesses purgative, tonic, febrifugal, expectorant, and diuretic activities (Singh et al., 2016). However, one of the best solutions for securing the necessary raw materials for phytopharmaceuticals is to cultivate this species on a scientific basis.

Obviously, the morpho-anatomical data on the species *S. occidentalis* cultivated in the climatic conditions of the Republic of Moldova are of particular interest both for determining the potential structural adaptability to the action of unfavorable environmental factors and from the point of view of the possibility of cultivation and exploitation of this species for medicinal purposes.

2. Materials and methods

The anatomical structure of the plant organs was performed during the mass flowering period of the plant in the Collection of Medicinal and Aromatic Plants of IGPhPP during the growing season of 2020-2021 years. The microscopic study was carried out at the Department of Pharmacognosy and Pharmaceutical Botany of Nicolae Testemițanu State University of Medicine and Pharmacy.

The studied organs were: main root and main stem (cross-sections and superficial preparations of rhizodermis and epidermis), leaf (cross-sections through rachis and through leaflets in median region, superficial preparations of adaxial and abaxial epidermises), flower (superficial preparations of sepals and petals), fruit (cross-sections through pericarp and superficial preparations of outer and inner epidermises), seed (cross-section through seed coat and cotyledons). The micropreparations was carried out by applying classical techniques to obtain cross-sections and surface preparations from fresh, dried and clarified botanical material in hydrochloride solution. Selective staining reagents were applied to identify the chemical nature of some structures according to methodical recommendations (Nistreanu and Calalb, 2016; Calalb and Nistreanu, 2021).

Test for anthraquinones

2 ml of aqueous extract was boiled with 5 ml of 10% HCl for 3 minutes followed by further addition of 5 drops of 10% ammonia. A rose-reddish coloration indicates a positive result.

Test for lipids

A few drops of Sudan III solution were applied to the sections for 2-4 hours. The lipid globules are stained in red.

Test for cell wall

A few drops of Zn-Cl-I solution were applied to the sections. The lignified cell walls are colored in dark orange and cellulose one – in blue.

Methylene solution colors the cellulose cell walls in dark blue.

Test for starch grains

A few drops of Lugol solution was applied to the sections. The starch granules are stained purple-black.

Micropreparations were analyzed in *Mikos* optical microscope coupled with computer software.

3. Results and discussions

Morphology and biometry

Coffee senna (*Senna occidentalis* (L.) Link. or *S. occidentalis* (L.) Roxb.) belongs to the family Fabaceae. It is a pantropical plant species native to the tropical and subtropical regions of America (Colombia, Argentina, Ecuador, Brazil, Peru and Paraguay). The species was mentioned as invasive in parts of Kenya, Uganda and Tanzania (Global Invasive Species Database). Nowadays, Coffee senna is grown on extensive plantations for industrial purposes in India and Egypt (Sayed et al., 2016).

Coffee senna is a dicotyledonous, foetid, annual plant, which develops very strong and long root of tetrarch in nature. *S. occidentalis* is a pantropical plant species native to the Americas and under those conditions can be annual or undershrub (Rotton et al., 2021). Similarly, in many scientific works it is described as herb or undershrub (Kumar, 2009; Suma and Tanuja, 2014), but in the conditions of the Republic of Moldova, characterized with a temperate-steppe climate and differing from those of the native center of formation, the plant develops and grows as an annual. The stems are erect, green and brownish in color with a soft and smooth texture. Imparipinnate compound leaves with alternate arrangement on the stem are characterized by strong rachis and ovate to ovate-lanceolate leaflets. Fruits are pods, brown greenish in color, cylindrical, slightly curved with 30 or more brown to dark-olive green, hard, matt, and lenticular to cordate, slightly flattened seeds (**Fig. 1, 2**).

Coffee senna plant propagates through seeds and it is fast growing. Morphological description and biometric analysis of measurable and numerable parameters was applied to 50 plants. The diagnostic morphological characteristics of plant organs are specified in the **Table 1.**

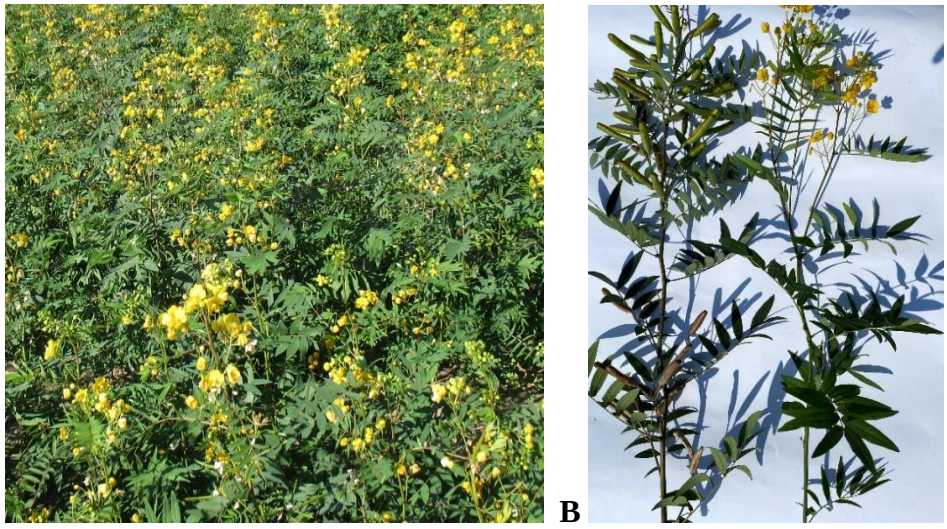

Fig.1. *Senna occidentalis* species: A – plants in the Collection of Medicinal and Aromatic of IGPhPP; B – morphology of plant

Fig. 2. Morphology of plant organs: A – roots; B – leaves; C – flowers; D – fruits and seeds

Table 1. Morphological characteristics of organs of *Senna occidentalis* species from IGPPP collection

Rows	Morphological parameters	Morphological characteristics
1.	Vegetative form of plant	Annual plant
2.	Root	Strong tap system
3.	Type of leaf	Imparipinnate compound with strong rachis
4.	Arrangement on the stem	Alternate
5.	Shape of rachis	Obovate, prominent wing type
6.	Shape o leaflet	Ovate to ovate-lanceolate
7.	Shape of leaflet apex	Slender acuminate
8.	Shape of leaflet base	Ovate, obtuse, sometimes asymmetric
9.	Venation of leaflets	Pinnate (mature leaflets with sunken veins)
10.	Shape of stipule	Triangular
11.	Flower	Yellow of papilionaceous type
12.	Type of inflorescence	Erect simple raceme
13.	Type of fruit	Pod with a rostrum at the apex, splits open along 2 thickened lines into 2 valves
14.	Shape of pod	Rounded (cylindrical) slightly curved
15.	Color of pod	Brown with a slightly greenish tinge
16.	Surface of pod	Matt with regular ribs perpendicular to the length of the pod
17.	Shape of seed	Lenticular to cordate, slightly flattened
18.	Color and surface of seed	Dark-olive green to brownish, mat, hard
19.	Smell of plant, especially of leaves	Foetid smell when damaged
20.	Usage Hazard	Toxic upon ingestion

We mentioned that the most of the morphological characteristics shown on plants in the Collection of Medicinal and Aromatic Plants of IGFPPl are consistent with morphological descriptions by other authors (Kumar, 2009; Naeem et al., 2009; Nassar et al., 2013; Fonkou et al., 2018).

The experimental data of *S. occidentalis* plantation over 5 years show that climatic conditions in the Republic of Moldova are favorable for their growth and development. Phenological observations denote that the plants develop healthy appearance and are not attacked by any pathogens. The plants being annuals go through the whole onto morphogenetic cycle of development during one vegetative period, which ends with fruit ripening and complete seed maturation. The biometric data results of the Coffee senna plants are shown in **Table 2**, which demonstrate the development peculiarities of the plants in the Collection of Medicinal and Aromatic Plants.

Anatomy of plant organs

The anatomical study was carried out on multiple micrographs, representing cross-sections or superficial views, and the highlighted structures were evaluated in the light of data from scientific papers already published by other authors and presented in **Table 3**.

Table 2. Biometric parameters of *Senna occidentalis* plants in the IGPhPP collection (2020-2021 years)

Rows	**Biometric parameters**	**Average value M±m**
1.	Height of plant (cm)	162.4±9.1
2.	Wide of the main stem: lower/ middle/ upper portions of plant	6.6±0.9/ 4.9±0.6/ 3.1±0.2
3.	Length of main root (cm)	56.6±4.1
4.	Number of leaves per axial twig	17.8±1.6
5.	Number of leaves plant	38.8±1.9
6.	Length of leaf (cm)	18.4±2.0
7.	Wide of leaf (cm)	12.5±1.3
8.	Length of leaflet (cm)	5.6±0.4
9.	Wide of leaflet (cm)	2.3±0.2
10.	Number of pair leaflets per leaf	5.2±0.8
11.	Number of inflorescences per plant	25.2±1.2
12.	Number of flowers per inflorescence	19.3±1.8
13.	Length of inflorescence (cm)	11.8±1.1
14.	Number of pods per plant	63.3±7.7
15.	Length of pod (cm)	6.5±0.5
16.	Wide of pod (cm)	0.9±0.1
17.	Number of seed per pod	32.5±2.1
18.	Length of seed (mm)	4.5±0.3
19.	Wide of seed (mm)	3.5±0.2
20.	1000 seed weight (g)	17.5±0.9

Table 3. Anatomical characteristics for *Sennia occidentalis* species in current research and literature data

Organs	**Anatomical parameters**	**Our results**	**Other researches**	
Root	Tetrarch type	+	+	Nassar et. al., 2011
	Diarch type		+	Kumar, 2009
	Druses in cortex and Pismatic crystals in the vascular sheath	+		
Stem	Single layered epidermis with thin cuticle		+	Suma and Tanuja, 2014
	Uni- and multicellular non-glandular trichomes	+		
	Glandular trichomes	+		
	Paracytic stomata	+		
	Druses and solitary prismatic crystals in cortex	+	+	Suma and Tanuja, 2014; Nassar et al., 2013
	Prismatic crystals in the vascular sheath	+		
	Druses in the pith		+	Nassar et al., 2013

Leaf	**Rachis**	Uniseriate epidermis of rectangular cells	+	+	Nassar et al., 2013
		Unicellular non-glandular trichomes	+	+	Nassar et al., 2013; Ogundipe et al., 2009
		Multicellular non-glandular trichomes	+	+	Nassar et al., 2013
		Glandular trichomes	+	+	Nassar et al., 2013; Doty et al., 2020; Amponsah et al., 2016
		Paracytic, anisocytic and anomocytic stomata	+		
		Druses		+	Nassar et al., 2013
		Prismatic crystals in the vascular sheath and druses	+		
	Blade leaflet	Dorsoventral mesophyll	+	+	Nassar et al., 2013; Suma and Tanuja, 2014; Amponsah et al., 2016
		Solitary crystals and druses	+	+	Nassar et al., 2013
		Prismatic crystals in the vascular sheath	+		
		Upper epidermis of thin-walled cells		+	Nassar et al., 2013; Suma and Tanuja, 2014
		Lower epidermis of radially elongated cells		+	Nassar et al., 2013
		Wavy epidermal cells	+	+	Amponsah et al., 2016; Kotresha and Seetharam, 2000
		Lower epidermis of rectangular cells		+	Suma and Tanuja, 2014
		Amphystomatic leaf	+	+	Nassar et al., 2013; Amponsah et al., 2016; Suma and Tanuja, 2014
		Paracytic stomata on both epidermises	+	+	Suma and Tanuja, 2014; Kotresha and Seetharam, 2000
		Anomocytic stomata	+	+	Amponsah et al., 2016
		Paracytic and anisocytic stomata	+	+	Ogundipe et al., 2009; Nassar et al., 2013
		Paracytic, anisocytic and tetracytic stomata	+	+	Begum et al., 2014
		Paracytic and anomocytic stomata		+	Kumar, 2009
		Horn like unicellular non-glandular trichomes	+	+	Suma and Tanuja, 2014; Amponsah et al., 2016; Kotresha and Seetharam, 2000
		Multicellular non-glandular trichomes	+		
		Only glandular trichomes		+	Begum et al., 2014; Doty et al., 2020; Kotresha, 2000
Flower	**Sepal**	Upper epidermis of a uniseriate layer of barrel-shaped cells		+	Nassar et al., 2013
		Upper epidermis with a thin cuticle layer		+	Nassar et al., 2013
		Paracytic and anisocytic stomata	+		
		Filiform formations	+		
		Glandular trichomes	+		
	Petal	2 epidermal layers with nearly square parenchyma cells		+	Nassar et al., 2013

		Mamelous protuberance	+		
		Paracytic stomata	+		
		Folded surface	+		
		Glandular trichomes	+		
Pericarp fruit		Exocarp consisting of epidermis only with uniseriate, barrel-shaped cells	+	+	Nassar et al., 2013
		Waxes deposition and thick layer of epidermis cuticle	+	+	Nassar et al., 2013
		Mesocarp consisting of 10 layers of irregular, thin-walled parenchyma cells, sometimes slightly lignified	+	+	Nassar et al., 2013
		Endocarp of uniseriate, cylindrical parenchyma cells	+	+	Nassar et al., 2013
		Druses	+		
		Non-glandular and glandular trichomes	+		
Seed		Coat formed from 4 distinct layers	+	+	Nassar et al., 2013
		Outermost layer of seed coat composed of waxy cuticle	+	+	Nassar et al., 2013
		Second layer is formed of thick-walled, elongated palisade cells called macrosclereids	+	+	Nassar et al., 2013
		Two cotyledons are more or less orbicular and occur parallel position to the long axis of the embryo	+	+	Nassar et al., 2013
		Lipidic globules in cotyledons	+		

Root anatomy (**Fig. 3**). Anatomical structure was investigated on cross-section of the mature main root. Secondary structure is established by secondary growth. On cross-section, the first histological zone is the rhizodermis, consisting of one layer of cells. We can also see the formation of peridermis, composed of several layers of phellem, phellogen (cork cambium) and phelloderm. The periderm provides safe protection for the internal root structures. In section, the periderm is followed in the interior by cortex with druses in some cells, and islands of primary phloem and clusters of sieve tubes with their accompanying cells, in addition to sclerenchyma fibers and phloem parenchyma, which form the secondary phloem, located opposite to the rows of secondary xylem. In the vascular bundles sheath, there are prismatic crystals. The vascular cambium is thin and consists of 3-4 layers of meristematic cells. A large secondary xylem space is mentioned, represented by vessels in radial rows, surrounded by sclerenchyma fibers and separated by parenchyma cells. The primary xylem occupies the central position of the root. The prismatic crystals of calcium oxalate are present in the vascular bundle sheath. Data on the anatomy of the main root in *C. occidentalis* are largely consistent with those described in other works (Kumar, 2009; Nassar et al., 2011). Confusion is found in the type of vascular bundle, where we describe it as tetrarch (4 patches of xylem alternate with similar number of phloem patches), which is in agreement with Nassar's (2011) description, but disagrees with Kumar (2009), who mentions it as diarchic. The roots give a reddish stain when tested for anthraquinones, being one of the best effects comparatively with other plant organs (**Fig. 4**).

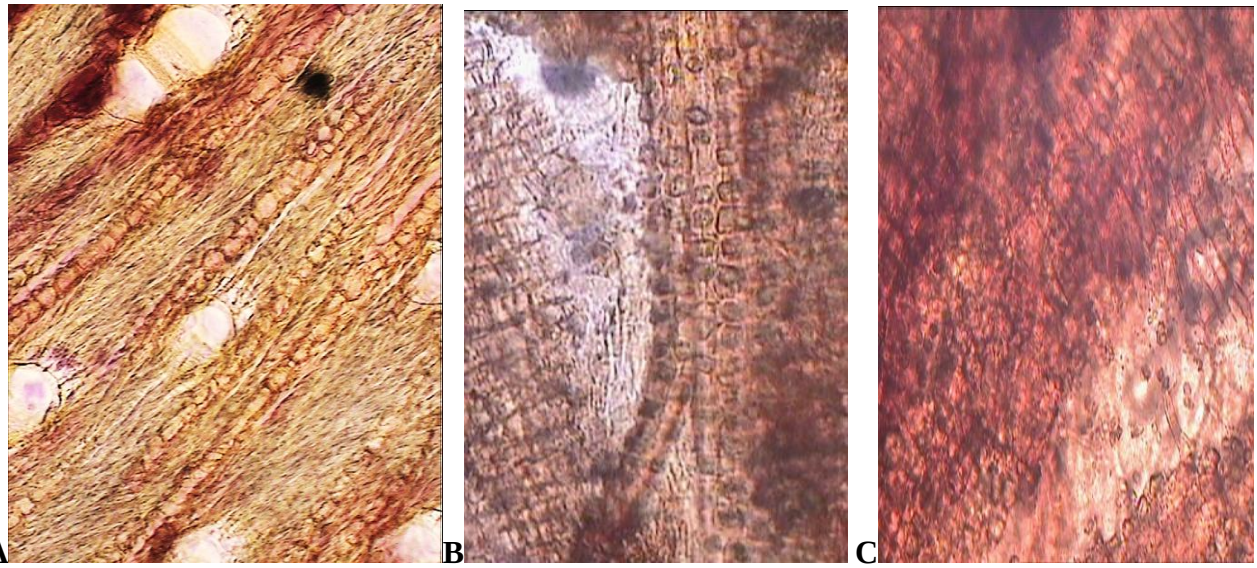

Fig. 3. Anatomy structure of root (40x): A – lignified rows of xylem (staining with Cl-Zn-I solution); B – prismatic crystals in vascular bundles sheath; C – reddish effect of anthraquinone test

Fig. 4. The effect of the anthraquinone test on plant organs: A – roots; B, C – stems; D – leaf rachis; E – leaflets; F – flowers; G – pods; H – fruit pericarp; I – seeds

Stem anatomy (**Fig. 5**). Anatomical structure was investigated on cross-section of the middle internode of the mature main stem. The cross-section configuration of the stem is circular, with attenuated and not prominent ribs. The anatomical structure is the result of secondary thickening, mainly expressed by the massive secondary xylem in the central cylinder. The outer layer of the stem is epidermis, composed of radially and tangentially elongated cells covered by a thick layer of cuticle. Paracytic stomata are present. Non-glandular unicellular and multicellular trichomes are found very rarely. Glandular trichomes, consisting from short stalk and multicellular glands with brownish content have a moderate frequency. The cortex composed of 6-12 layers is represented by 4-8 layers of collenchymatous cells and 2-3 layers of parenchymatous chlorenchymatous cells.

In epidermis and cortical cells, druses and solitary prismatic crystals were observed, but we note, that prismatic crystals are more characteristic for vascular bundles sheath.

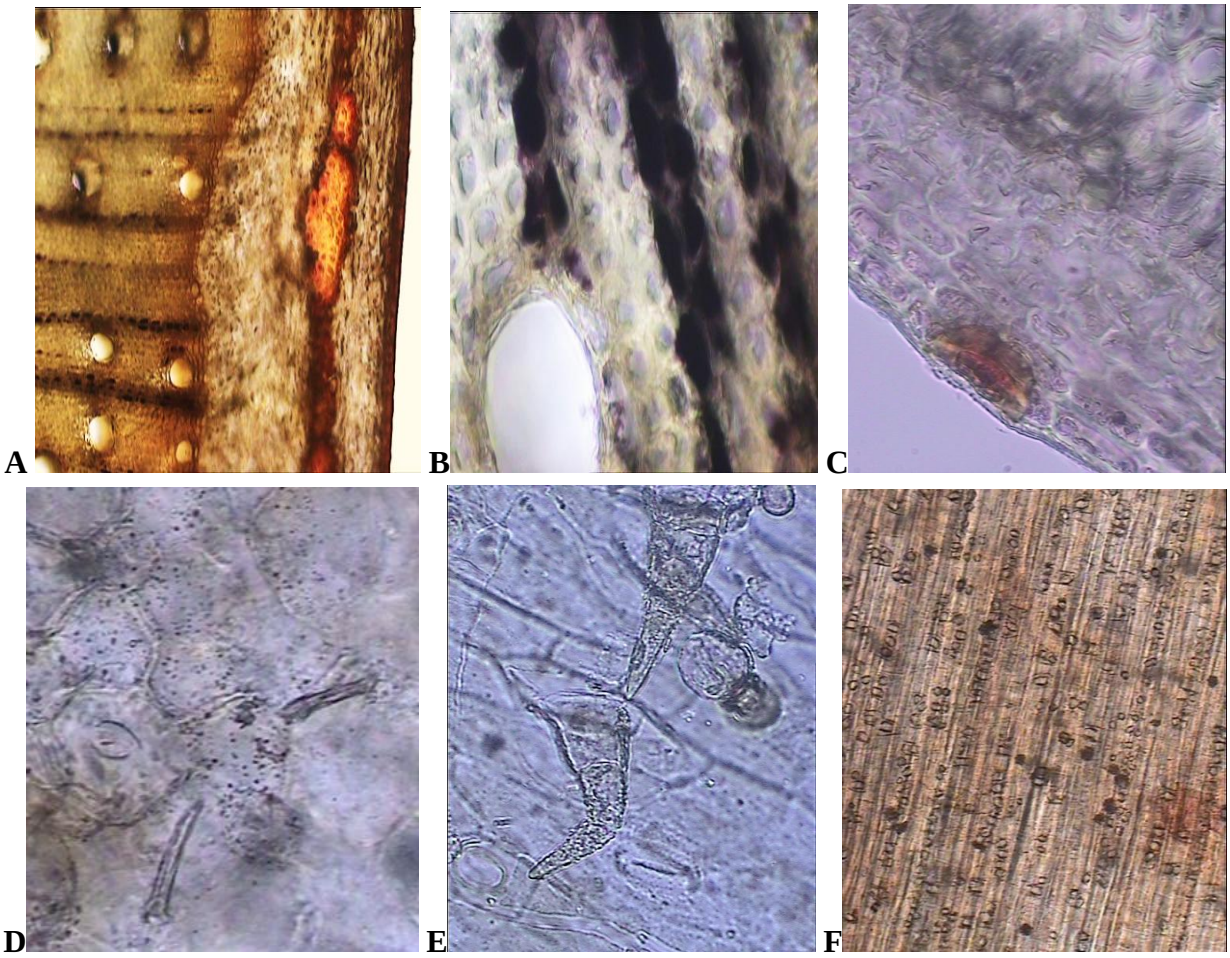

Fig. 5. Anatomy structure of stem (40x): A – histological zonality on cross-section: 1 – epidermis, 2 – cortex, 3 – sclerenchyma fibers, 4 – phloem, 5 – secondary xylem; B – parenchimal rows with starch grains (staining with Lugol solution); C – outerlayers of stem with brownish gland (cross-section); D – unicellular non-gladular trichomes (upper view); E – multicellular non-glandular trichomes (upper view); F – prismatic crystals

Other authors (Suma and Tanuja, 2014) indicated the presence of both prismatic crystals and druses (rosette) in cortical cells, and prismatic solitary crystals only in the stem endodermis.

The central cylinder is surrounded by an interrupted ring of sclerenchymatous fibers. In the central cylinder there are about 25 collateral open vascular bundles of various sizes, arranged in a ring shape. The secondary phloem consists of sieve tubes, companion cells, phloem parenchyma, while sclerenchymatous phloem fibers are not observed. The secondary xylem contains a lot of vessels of different diameters, sclerenchyma fibers and parenchyma cells. The primary xylem forms a thin, pressed layer attached to the pith. Medullary rays from lignified parenchyma cells separate the vascular bundles. The pith, consisting of polygonal parenchymatous cells, occurs the central part of the stem. The druses were mentioned like other authors (Nassar et al., 2013).

Leaf. *Rachis anatomy* (**Fig. 6**)**.** In cross-section, the petiole is cordate in shape, with a groove protruding inward and two prominent notches on the adaxial side. The petiole is surrounded on the outside by a uniseriate epidermis, covered by the cuticle. In the

epidermis, the paracytic (each guard cell is accompanied by 2 cells, whose longitudinal axes are parallel to those of the guard cells and the aperture), anysocytic (guard cells are surrounded by 3 subsidiary cells of unequal size) and anomocytic (guard cells are surrounded by 5-6 cells with radial position) stomata are observed.

Rarely, non-glandular, unicellular trichomes are found. Also, the glandular trichomes with multicellular, brownish glands were observed, mentioned early in other works (Nassar et al., 2013; Amponsah et al., 2016; Doty et al., 2020).

In general, data on the presence of different types of trichomes in *S. occidentalis* rachis leaf are contradictory. Some anatomical studies (Begum et al., 2014; Doty et al., 2020) describe only multicellular glandular trichomes, while other studies (Ogundipe et al., 2009; Saheed and Illoh, 2010) mentioned only the presence of unicellular non-glandular trichomes. Another anatomical research (Nassar et al., 2013) describes the presence of both unicellular and multicellular non-glandular trichomes, although the latter with a rare frequency. Below the epidermis, there is cortex composed of chlorenchyma (most on the adaxial side) and collenchyma.

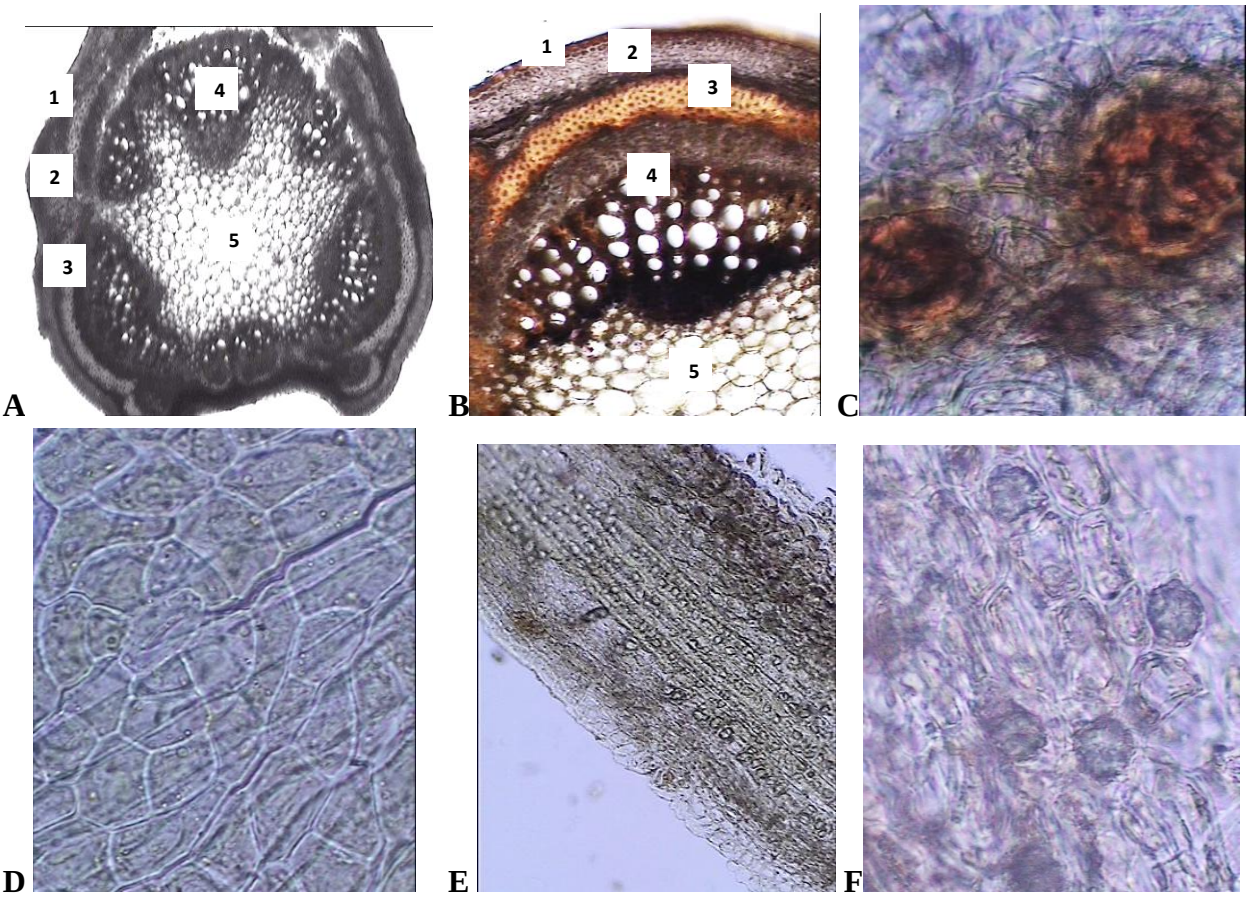

Fig. 6. Anatomy aspects of leaf rachis: A (10x), B (staining with Zn-Cl-I solution, 40x) – cross-section of rachis: 1 – epidermis, 2 – chlorenchyma, 3 – collenchyma, 4 – collateral vascular bundle, 5 – mesophyll; C – glandular trichomes with brownish content (upper view, 40x); D – anomocytic stomata (upper view, 40x); E – sheath of vascular bundles with prismatic crystals (10x); F – druses in parenchyma cells (40x)

Marginally, on the central part of the petiole there are 7 collateral conducting bundles (3 dossals, 2 laterals and 2 ventrals) with well-developed sclerenchyma sheath, especially on external side, which induces stringency for rachis, the pith is wide and is represented by assimilating parenchyma. There are 2 ridge bundles. The druses and prismatic crystals are mentioned. The presence of druses only, has been mentioned in another research (Nassar et al., 2013).

The leaflet blade anatomy (**Fig. 7**). According to the imparipinnate compound type of the leaf, the anatomical study was carried out on leaflets from the central region of the mature leaf. The anatomical type of the leaf is the dorsoventral mesophyll, in which the palisade tissue is located on the adaxial side and the spongy tissue on the abaxial side of the blade. The adaxial epidermis is composed of thin walled, tangentially elongated cells covered by a layer of cuticles. The abaxial epidermis consists of thin walled, radially elongated cells and is thinner than the adaxial epidermis. This data concerning the anatomy type of leaf is in harmony with results found in some articles (Kumar, 2009; Nassar et al., 2013).

It is an amphistomatous leaf, as stomata are found on both epidermises, but there are more numerous on the abaxial than on the adaxial side. Note, that the paracytic type of stomata was found on both types of epidermis, the anisocytic type and tetracytic (guard cells are surrounded by 4 subsidiary cells) ones, only on the abaxial one. These data are consistent with those reported in other articles (Begum et al., 2014) and differ from those described in another papers (Ogundipe et al., 2009; Nassar et al., 2013) which indicate the presence of 2 types of stomata (paracytic and anisocytic) on the abaxial epidermis and only the paracytic one on the adaxial side, while in another paper (Kotresha and Seetharam, 2000; Suma and Tanuja, 2014) it is described only the paracytic type, but on the both leaf epidermises, however in this research (Amponsah et al., 2016) – only anomocytic stomata on both sides of the leaf.

Both epidermises develop short, non-glandular, horn like unicellular trichomes, but more on the abaxial epidermis, particularly along the nerves and in the basal part of the leaflet, and multicellular non-glandular ones only on the abaxial side. The morphology of horn non-glandular trichomes is similar to that described in this scientific paper (Suma and Tanuja, 2014). The multicellular glandular trichomes with brownish content observed on both epidermises of leaflet, but frequently on the abaxial one was pointed by other scientific works (Kotresha and Seetharam, 2000; Begum et al., 2014).

Chlorenchymatous palisade tissue consists of 1-2 layers and occurs a half of mesophyll. Another half of mesophyll occurs the spongy tissue consisted of lobed chlorenchymatous cells with large intercellular spaces. The blade is perforated by vascular bundles, one principal, large, located in the midrib and others of much smaller diameter, distributed in the sides of the blade. The mechanical tissue, represented by the collenchyma is mentioned on the side of principal vascular bundle and is prominent on the abaxial side of the blade.

The sclerenchymatous cells surround as a continuous ring the midrib, which is thicker on the abaxial side. Thus, the main vascular bundle is not directly embedded by the mesophyll as are the other side small vascular bundles. The vascular bundles are collateral, the xylem represented by vessels is located on the adaxial side and phloem – by sieve tubes, companion cells and parenchyma, are found on the abaxial side. Solitary crystals and druses of calcium oxalate are distributed throughout in the mesophyll, but only prismatic ones are in rows in the sheath of the vascular bundles.

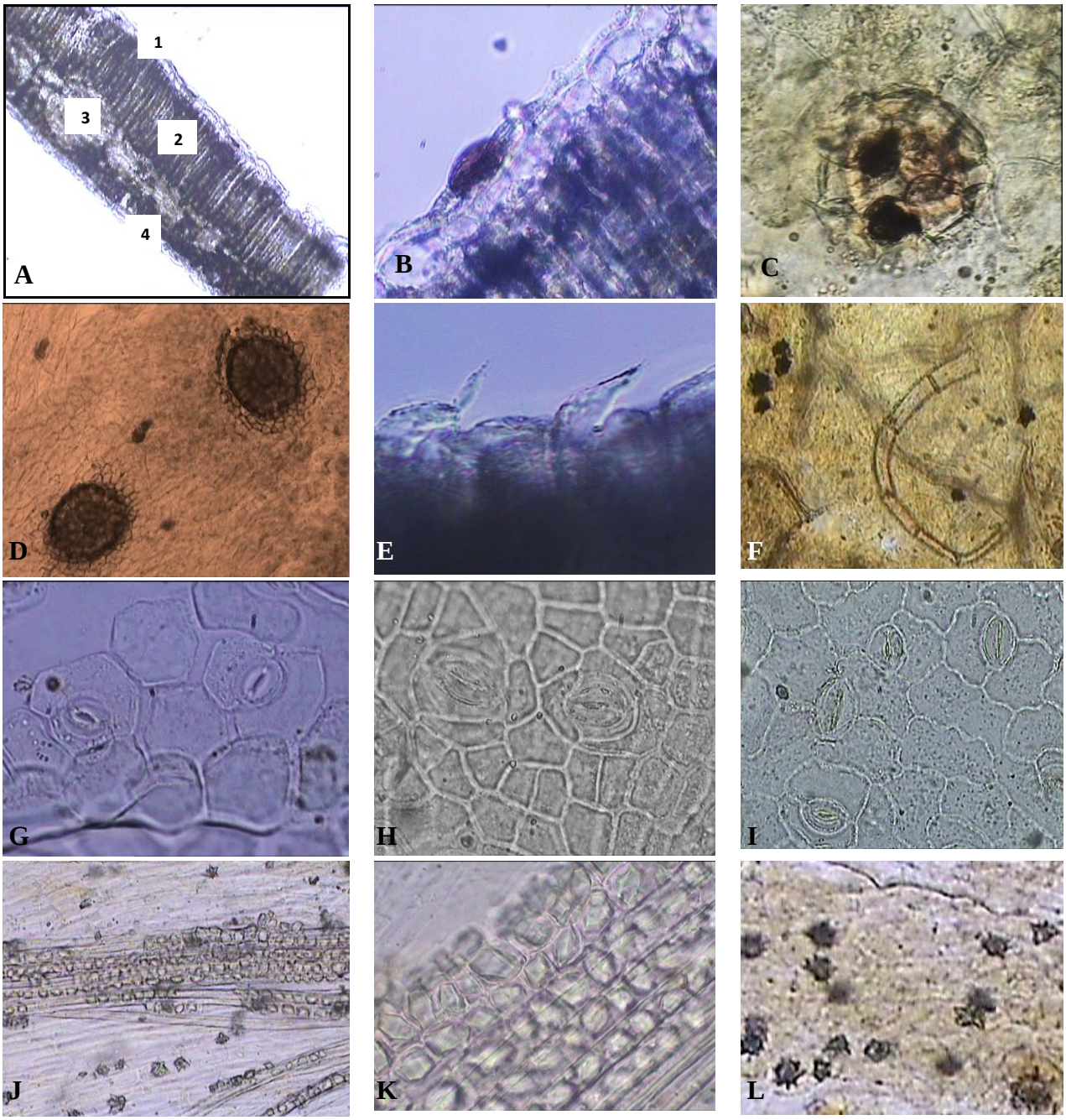

Fig. 7. Anatomy aspects of leaflet: A – dorsoventral type of mesophyll on cross-section (10x): 1 – adaxial epidermis, 2 – palisade mesophyll, 3 – spongy mesophyll, 4 – abaxial epidermis; B – uniseriate epidermis with glandular trichome in cross-section (40x); C – glandular trichome with brownish content (upper view, 40x); D – glandular trichome with brownish content (upper view, 10x); E – short unicellular non-glandular trichomes (40x); F – multicellular non-glandular trichomes (10x); G – paracytic stomata (upper view, 40x); H – anisocytic stomata (upper view, 40x); I – paracytic, anisocytic and anomocytic stomata (upper view, 40x); J – druses and prismatic crystals (upper view, 10x); K – prismatic crystals in the sheath of vascular bundles (40x); L – druses in mesophyll (40x).

Leaf anatomy regarding the species *S. occidentalis* is generally consistent with a study of Nassar et al. (2013), except for calcium oxalate crystals, mentioned as solitary and druses.

Leaves give the lowest expression of the staining gradient for anthraquinone testing compared to fruits, seeds, and roots.

Flower anatomy (**Fig. 8, 9**). Investigation of flower anatomy was carried out on sepals and petals. Two marginal layers of uniseriate epidermis, covered by a thin layer of cuticles, can be distinguished on cross-section of the sepals. The epidermises contain paracytic and rarely anisocytic stomata of rounded shape protruding from the level of the epidermis, from which radially arranged folds can be seen. Several layers of chlorenchymatous cells with large intercellular spaces, perforated by small vascular bundles, fill the space between the epidermis.

Two very thin layers of epidermis, made up of square, tightly packed cells, can also be seen in cross-section of the petals. On the upper epidermis there are mamelous protuberances, especially along the veins. The folding of the epidermis surfaces is radial around the rounded paracytic stomata. On the base of the petals, there are developed a few small and thin non-glandular trichomes.

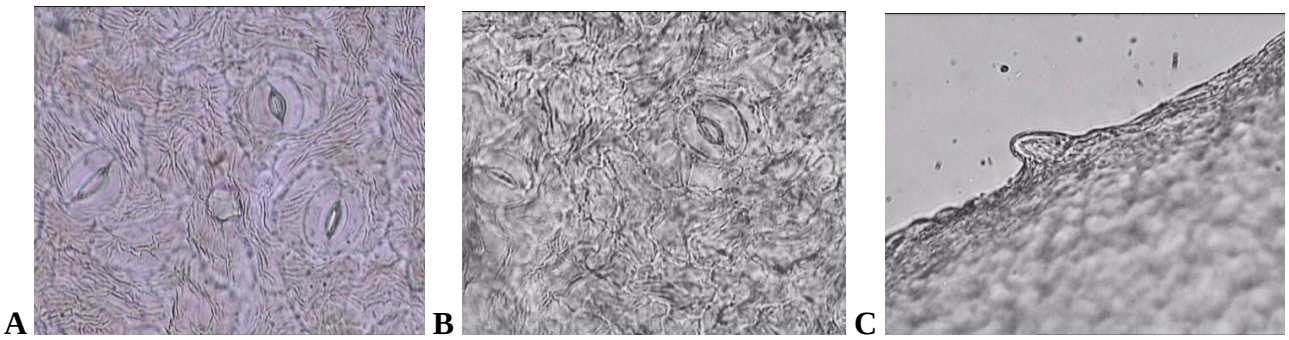

Fig. 8. Sepals anatomy: A – rounded paracytic stomata on adaxial epidermis (40x); B – rounded paracityc stomata on abaxial epidermis (40x); C – short non-glandular trichome on abaxial epidermis (cross-section, 40x)

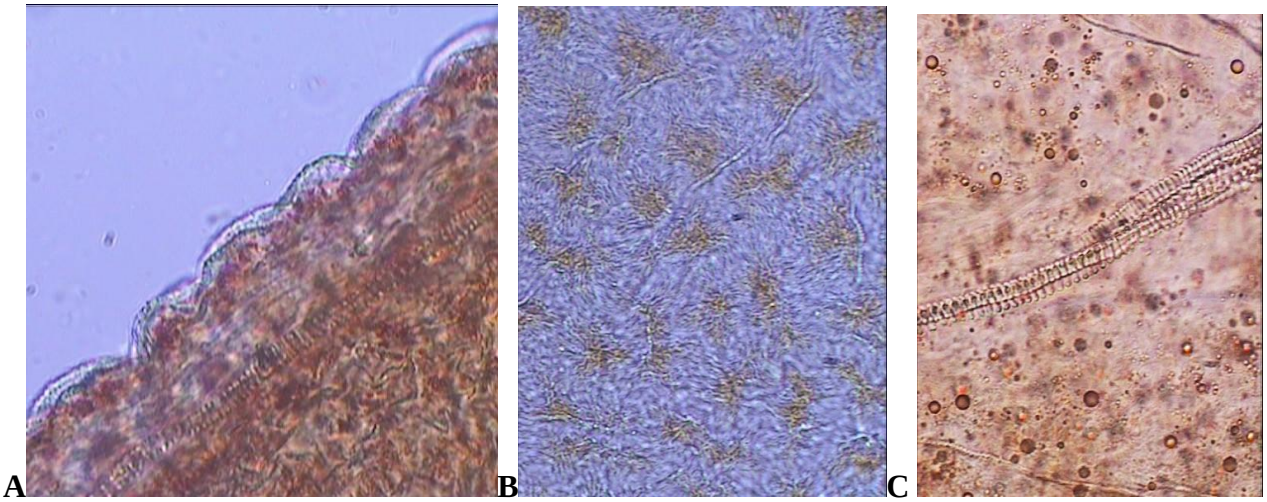

Fig. 9. Petals anatomy: A – mamelous protuberances on cross section (40x); B – mamelous protuberances (upper view, 40x); C – lipidic globules (staining with Sudan III, 40x)

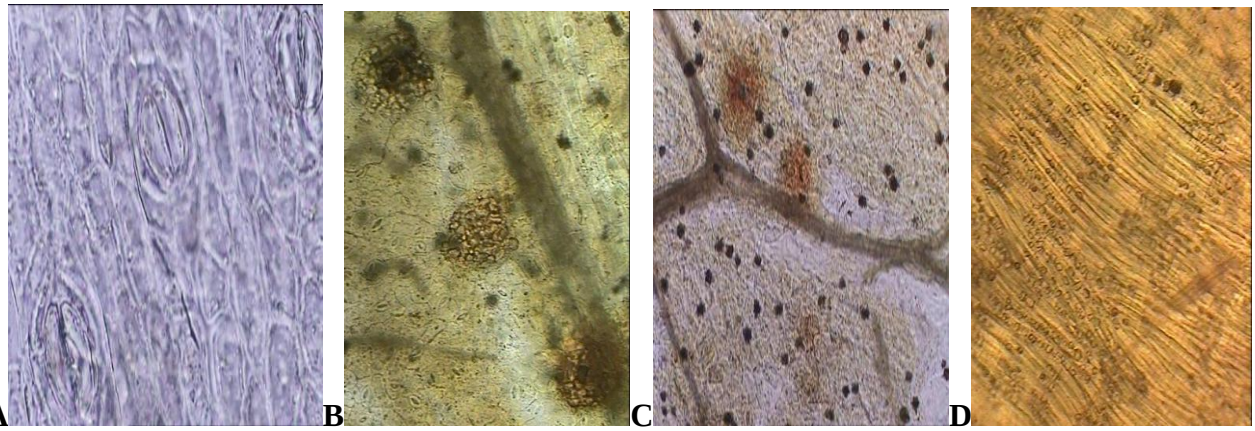

Fig. 10. Outer epidermis of pericarp (upper view): A – paracytic stomata (40x); B – glandular trichomes with multicellular, brownish glands (10x); C – druses (10x); D – prismatic crystals (10x)

Fig. 11. Anatomy of seed: A – seed coat (cross-section, 10x); B – compact arrangement of epidermis cells (upper view, 10x); C – lipidic globules in cotyledons (staining with Sudan, 40x)

Between the epidermis, there is the parenchyma consisting of 3-5 layers of cells, frequently developing lipid-containing globules (red staining when Sudan III reagent is applied). Sepals and petals give a reddish stain when tested for anthraquinones.

Fruit pericarp anatomy (**Fig. 10**). Three histological zones can be distinguished on cross-section of the pericarp in the middle area of the pod: exocarp, consisting only of a layer of tangentially elongated outer epidermal cells, with thickened and slightly lignified cell walls, compact, rarely separated by paracytic stomata and very rarely by unicellular non-glandular trichomes, covered by a relatively thick cuticle; mesocarp is composed of 8-10 layers of parenchymal cells, irregular in shape, in some of which there are druses; the mesocarp is perforated by collateral vascular bundles, surrounded by the fibrous sclerenchymatous sheath; endocarp, represented by the inner uniseriate epidermis, consisting by cells of the same shape, is compactly arranged.

Seed anatomy (**Fig. 11**). The seed consists of seed coat and embryo. The cross-section of the seed coat shows: the outer layer is composed of thick waxy cuticle, next there is epidermis, consisting of thick-walled, tangentially elongated cells with a very compact arrangement, followed by several layers of osteosclereids, and the last inner layer is represented by the parenchyma, consisting of thin walled, tangentially elongated cells. The embryo includes 2 cotyledons, extending parallel to the long axis, formed by the radical, hypocotyl, epicotyl and plumule. The

cotyledons are covered by epidermis and the internal mesophyll consists of parenchymatous cells with gelatinous content and small intercellular spaces. The anatomy description of seed structure of *S. occidentalis* agrees with the work of Nassar et al. (2013) and another study on species of *Cassia* genus (Sahai, 2001).

Our results show that, even though full sun and moderate water are required for the care and propagation of *S. occidentalis*, however, under the conditions of the steppe climate of the Republic of Moldova, with moisture deficit practically all year round, the plants in the Collection of Medicinal and Aromatic Plants have developed very well, both underground and above ground parts.

Notwithstanding the different and sometimes contradictory morphological and anatomical data in the literature, we were nevertheless able to evaluate the data obtained in the current study through the prism of those already presented by other scientific researchers (Ogundipe et al., 2009; Saheed and Illoh, 2010; Nassar et al., 2013; Doty et al., 2020) and to establish specific structural indices for each organ of Coffe senna plants. Anatomical study and consultation of literature shows that most conflicting data refer to the type of stomata on the aerial parts of Coffe seena plants: paracytic type only (Suma and Tanuja, 2014); paracytic and anisocytic type (Ogundipe et al., 2009; Nassar et al., 2013); paracytic, anisocytic and tetracytic type (Begum et al., 2014); anomocytic only (Amponsah et al., 2016); paracytic and anomocytic type (Kumar, 2009). We found on aerial parts of plants paracytic, anisocytic and anomocytic type, but with different frequency and distribution, however, most encountered being the paracytic type.

It is known that anatomical structures were established during evolution and represent specific structures of species. Anatomical structures are stable and conservative, depending on the growing conditions of the plant, only the quantitative aspects of the structures can change. The slightly different information on the anatomical structures of sp. *S. occidentalis* in the literature requires some explanation. We could assume that microscopic studies were carried out at different stages of ontogenetic development of the plant, but it was not specified in the paper, or the elucidation of all structures certainly depends on the complexity and insistence of analysis of the applied microscopic techniques. As an example, non-glandular trichomes occur very rarely, only on the rachis and the basal part of the leaf blade and could be identified by analysis of micropreparations of cross-sections on fresh material and of leaf surfaces obtained from clarified botanical material in hydrochloride solution.

The taxonomic value of anatomical characters, especially the epidermal ones of leaves, has a decisive weight in taxonomic monographs (Rejdali, 1991). In this context, microscopic studies to evaluate and highlight those with diagnostic role in the identification with certainty the species and in determination of structural indices with adaptive role under conditions of global climate change are very necessary. For example, the physiological role of calcium oxalate crystals in plants is still debatable. A role of these crystals against herbivores is known, but lately they are increasingly thought to play a key role in several biological processes in plants, such as: oxalate functions in metal tolerance, ion balance and defense against pathogenic bacteria, viruses, fungi, and insects (Franceschi and Nakata (2005); Foster et al., 2016). However, recently, more and more authors attribute the role of calcium oxalate crystals in plant adaptation and protection under adverse climatic factors especially related to the evolutionary adaptation of species to drought

stress conditions (Leon-Martinez and Ortiz-Hernandez, 2022).

In summary, this morpho-anatomical research will reveal new aspects, will contribute to support some anatomical features already reported but less confirmed in the studies of the last decades on the species *S. occidentalis* and will serve as landmarks for a real clarity of the integral microstructural picture.

Conclusions

1. The present study shows that microscopic research on the species *S. occidentalis* is very welcome and important, as in scientific research there are often conflicting and confusing data. Anatomical research is needed to highlight diagnostic microscopic characters specific to each plant organ, to identify with certainty the taxonomy of species *S. occidentalis* and the relationships between members of the genus *Senna*, considering that many species are potentially toxic.
2. Specific microscopic parameters for *S. occidentalis* species are: tetrarch root type; secondary anatomical structure of root and stem; unicellular non-glandular trichomes on stem, rachis and leaflet base; glandular trichomes with brownish content on stem, leaves and floral elements; paracytic, anysocytic and anomocytic stomata on aerial part of plants; calcium oxalate in the form of prismatic crystals, usually in the sheath of vascular bundles and druses in the parenchymatic cells of roots, stems and leaves; presence of lipid globules in petals and cotyledons of seeds; presence of anthraquinones with different gradation (decreasing) – roots, pods, seeds, flowers, leaves, stems.

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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