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Research Article

Botanical and Genetic Characteristics of *Lobularia libyca* (viv). C.F.W. Meissn. (Brassicaceae)

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ABSTRACT

Lobularia libyca (viv). C.F.W. Meissn. is an annual herbaceous desert plant native to North Africa belonging to Family Brassicaceae (Cruciferae). Study of the botanical features was carried out for the root, the young and old stems, leaf, flower and seed of the plant. L. libyca was characterized by the presence of non-glandular branched unicellular two armed hair in the stem, leaf and flower while the root shows narrow cork and xylem occupies about 80% of the diameter of the root. The flower is characterized by hairy sepals and spherical pollen grains with three germ pores and three germ furrows. The pericarp is characterized by hairy epicarp and presence of fibrosclerides as innermost layer of the mesocarp. The septum shows pitted lignified parenchyma. The seed shows accumbent embryo and the testa is characterized by the presence of pigment layer with reddish brown content. The DNA of the plant was extracted from leaf samples and analyzed using eleven random decamer primers. A total of 89 random amplified polymorphic DNA (RAPD) markers were identified. Both the botanical study and the DNA fingerprint helped in the identification of the plant.

Keywords: Lobularia libyca, botanical study, DNA fingerprint.

INTRODUCTION

Family Brassicaceae (Cruciferae) is one of the largest families in the plant kingdom. It is known for its richness in medicinal plants. It consists of about 338 genera and 3700 species with a wide distribution especially in the Mediterranean region to central Asia, and North West America^{1, 2}. Members of this family are mostly herbs, rarely shrubs or subshrubs.

Lobularia is one of the members of that family^{1, 3}. It consists of four species native to Cape Verde, Canary Islands and Mediterranean region to Arabian desert³.

Members of this family are characterized by the presence of simple, alternate, exstipulate leaves which are often varying from entire to palmate or pinnatifid. The flower is hermaphrodite, hypogenous, actinomorphic with four sepals, four petals and tetradynamous stamens. The fruit is a siliqua or a silicula, rarely indehiscent and often flattened. The septum of the fruit remains on the plant when the seeds fall. The seeds are arranged in one or two rows, winged or not, with the embryo folded, accumbent or incumbent, conduplicate or very rarely straight^{1, 3, 4}. Plants belonging to this family possess characteristic secretory cells whose essential constituent is myrosin, consequently termed myrosin cells. Hairs are usually unicellular, sometimes simple unbranched or branched^{5, 6}. In a previous work, we isolated a novel quercetin glycoside identified quercetin 3-O- -L-

rhamnopyranosyl (1''' 4'')- -D-galactopyranoside-7-O- -L-rhamnopyranoside, along with kaempferol and quercetin from the n-butanol and ethyl acetate extracts of this plant respectively. Moreover, different plant extractives and the new compound showed a significant antioxidant and hepatoprotective activities *in-vitro*⁷.

A literature survey indicated that no data are available on the anatomical features of *L. libyca*. The present work includes a study of the morphological and anatomical features, as well as the DNA fingerprint of the plant to facilitate its identification both botanically and genetically.

EXPERIMENTAL

Plant material: Fresh herbs of *Lobularia libyca* (viv) C.F.W. Meissn. (Brassicaceae) were collected in March (2011) from the northern coast (Alexandria-Marsa Matrouh Road) and identified by Dr. Abdel-Halim Abdel-Mogaly, Herbarium of Horticultural Research Institute, Agricultural Research Centre, Dokki, Cairo, Egypt. The plant was identified according to T ckholm (1974) and Boulos (1999)^{1, 3}. The collected plant is visually matched against herbarium specimens and voucher specimens were deposited there. Samples of the plant were examined either fresh or after being kept in 70% ethanol containing 5% glycerol, as well as samples used after being air-dried and reduced to fine powder.

Botanical profiling

Macroscopical studies: Macroscopic studies were carried out by using organoleptic evaluation method. The shape, size, color, odour, taste and texture of different organs of *L. libyca* were observed.

Micromorphological studies: The transverse sections were prepared by the gliding technique using rotary gliding microtome. The sections were then dehydrated by transferring them into series of ethyl alcohol concentrations and stained by flooding them with safranin and light green stains, mounted in a drop of Canada balsam, covered with a cover slip and left to dry in an oven adjusted at 60° C for at least three days. The surface preparations were prepared by free hand mounting technique. The isolated elements were stained with phluroglucinol / conc. hydrochloric acid, Ruthenium red, iodine and Sudan III reagents. The slides were examined and photographed using Leica DMLB Image microscope equipped with leica Q.550 lw image and peripheral software version 2.1.

Genetic profiling

DNA fingerprinting: Fresh leaves of the plant under investigation were freeze-dried and ground to fine powder under liquid nitrogen.

DNA extraction: DNA was extracted from 10 g of leaf tissue in 1.5 ml microcentrifuge tubes using the DNA extraction method described by Williams *et al.*, 1990 ⁸.

Oligonucleotide primers: A total of 11 random decamer oligonucleotide primers (Operon Technologies Inc.) were used to amplify Lobularia libyca DNA. They have the following sequence: A-04: AATCGGGCTG, C-01: TTCGAGCCAG, C-12: TGTCATCCCC, C-16: CACACTCCAG. C-17: TTCCCCCCAG. D-05: TGAGCGGACA, A-17: GACCGCTTGT, G-19: GTCAGGGCAA, O-14: AGCATGGCTC, Z-19: GTGCGAGCAA, E-08: TCACCACGGT.

Polymerase chain reaction (PCR): PCR amplification was conducted using 25 μ l of reaction mixture containing 1% Triton 10-X reaction buffer (100 mM Tris–HCl (pH= 8.3), 500 mM KCl, 0.01% (w/v) gelatin), 2.0 μ l MgCl₂ (25 mM), 2.5 μ l of each dNTP (2 mM), 3 μ l primer, 0.3 μ l of Taq polymerase (Promega), and 2.5 μ l of genomic DNA, completed to volume with distilled water. The reaction mixture was overlaid with 2 drops of mineral oil. The amplification reaction was carried out in a Thermocycler Perkin Elmer Cetus 480 (Warrington, UK).

The thermocycler was programmed for 1 cycle of 5 min initial strand separation at 94 °C and for 40 cycles each 1 min at 94 °C for denaturation, 1 min primer annealing at 36 °C, a 7 min primer elongation at 72 °C, followed by 1 cycle of final primer extension at 72 °C for 10 min.

Gel electrophoresis and staining: PCR products were separated in 1.5% agarose gel by electrophoresis in TE buffer (10 mM Tris-HC1, 1.0 mM EDTA, pH =8.0) with a constant power of 95 volts for about 3 h. The products were stained with 0.5 μ g/ml ethidium bromide and then visualized and photographed under UV light using Polaroid camera type 57 (ASA 3000).

RESULTS AND DISCUSSION

Botanical profiling

Macromorphological characters: *Lobularia libyca* (Fig. 1 A & B) is an annual herbaceous greyish green to greyish brown desert shrub native to the Mediterranean coast and North Africa. It reaches 20-40 cm in height. The plant is tender and wholly covered with appressed tiny whitish hairs. The stem is erect and densely branched. The flowering period starts in January and ends at the beginning of March while the fruiting period ends in May.

The root is yellowish white to bright yellow in color. It shows lateral branching (Fig. 2A) where the main root is very tough and harder than the smaller branches. The surface usually shows numerous wrinkles. The fracture of root is fibrous on the inner part and smooth on the outer part. The main root measures 0.2-0.6 cm in diameter, while the lateral branches measure 0.1-0.3 cm in diameter and extends 5-20 cm laterally in the soil. The root possesses a characteristic odour and a slight pungent taste.

The young stem is erect, herbaceous and cylindrical (Fig 2B). It is green sometimes with violet tinge, showing a solid bright yellow interior. It measures 0.1 to 0.2 cm in diameter. The surface is rough covered with appressed whitish hairs. The lateral buds are very tiny, 0.05 to 0.1 cm in length, green and also covered with tiny whitish hairs. The young stem has a short, splintery fracture, characteristic odour and slight pungent taste.

The old stem is greenish purple in colour, hard, rough with sympodial branching (Fig. 2C). It measures 0.2 to 0.4 cm in diameter. It is also covered with appressed whitish hairs. It breaks with a fibrous fracture. There is no

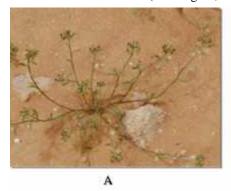




Fig. 1: Photographs of the young and old shrubs of Lobularia libyca (viv) C.F.W. Meissn., (A) The young shrub. (X=0.24), (B) The old shrub. (X=0.09).

Fig. 2: Photographs of young and old stem and leaves of Lobularia libyca (viv.) C.F.W. Meissn. (A) The root (X= 0.55), (B) Young stem (X= 1.1), (C) Old stem (X=1.4), (D) The upper surface of leaves (X= 1.8), (E) The lower surface of leaves (X= 1.6).

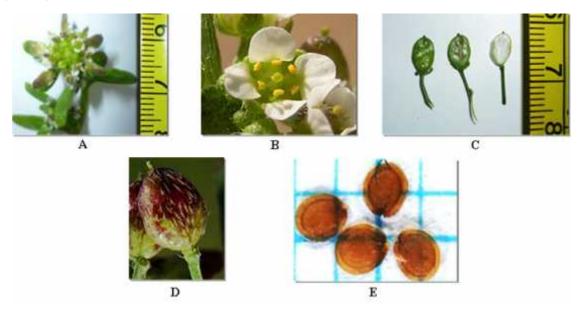


Fig 3: Photographs of flowers, fruits and seeds f Lobularia libyca (viv.) C.F.W. Meissn. (A) Flowers in inflorescence (X=1.7), (B) The flower showing its components (X=12.4),

(C) The fruits and the septum (X=1.85), (D) Epicarp with violet tinge and covered with hairs (X=2.6), (E) Seeds (X=15),

cork. The old stem is odourless and has a slightly pungent taste.

The leaves are alternate, sessile and exstipulate. The lamina is simple, linear oblong in shape with obtuse sometimes acute apex (Fig 2 D & E). The upper and lower surfaces are dark green to greyish green, sometimes with a purplish tinge near the apex only (Fig 2 D & E). The surface is rough and covered with longitudinally

appressed fine whitish hairs parallel to the midrib; as a result venation is indistinct. The midrib is prominent to the lower surface. The leaf has a slightly fleshy texture. It measures 0.5-2 cm in length and 0.1-0.5 cm in width. The leaf has a characteristic odour and a slightly pungent taste.

The flower is very small ranging from 0.2 to 0.3 cm in length and 0.25 to 0.35 cm in width (Fig. 3 A & B). The

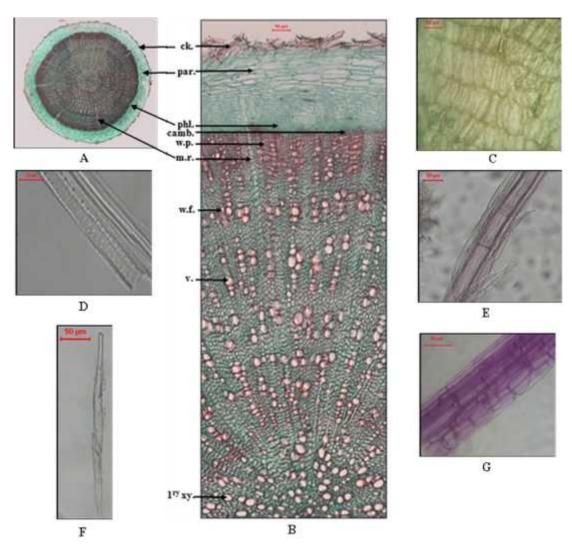


Fig. 4: Micromorphology of root of L. libyca (A) Photograph of the T.S. (X=10), (B) Detailed T.S. sector (X=104), (C) Cork cells (X=100), (D) Xylem vessels (X=415), (E) Tracheids (X=120), (F) Wood fibers (X=180). (G) Medullary rays (X=166), 1ry xy., primary xylem; camb., cambium; ck., cork; m.r., medullary rays; par., parenchyma; phl., phloem; v., vessels; w.f., wood fibers; w.p., wood parenchyma.

flowers are bisexual actinomorphic, and hypogenous. The calyx is composed of four free sepals in two decussate pairs while the corolla has four free white coloured petals alternating with the sepals. The androecium has six tetradynamous stamens

(four long and two short) while the gynaecium shows superior ovary of two united carpels, short style and capitate stigma. The inflorescence is raceme type.

The fruit is true, simple, dry and dehiscent siliqula which opens with two flat or convex valves, rounded to ovate in shape, more or less obtuse with a distinct style, attached to a pedicel that measures 0.4-0.7 cm in length (Fig. 3C). The surface of the fruit is rough covered with closely appressed fine whitish hairs. The pericarp is pale green to green, sometimes with a violet tinge (Fig. 3D). It is thin with the epicarp, mesocarp and endocarp being indistinguishable with a naked eye. The fruit is derived from superior bicarpellary bilocular ovary with a very thin membranous finely veined septum between the two locules. The septum may remain attached to the fruit pedicel after the seeds and pericarp fall off. The fruit

contains about 4-5 seeds attached to a parietal placentation where the seeds are attached to the junction between the two valves of the fruit. It measures 0.4-0.6 cm in length, 0.3-0.5 cm in width and 0.1-0.2 cm in thickness. The fruit possesses a faint characteristic odour and slightly pungent taste.

The seed is circular in shape, flattened, with a transparent membranous wing (Fig. 3E). It measures 0.05-0.1 cm excluding the wing which reaches 0.02-0.03 cm along the circumference of the seed. The testa is thin, brittle, orange brown to dark reddish brown in colour, glabrous with a fine reticulated surface. The raphe is hardly distinct as a small groove near one edge of the seed separating the position of radicle from that of the cotyledons. The seed is albuminous, derived from anatropus ovule, with an accumbent embryo and two thin pale cotyledons. The seed possesses a characteristic pungent odour and a mucilaginous pungent taste.

Micromorphological characters

The root A transverse section (T. S.) in the old root (Fig. 4 A & B) is circular in outline showing an outer narrow

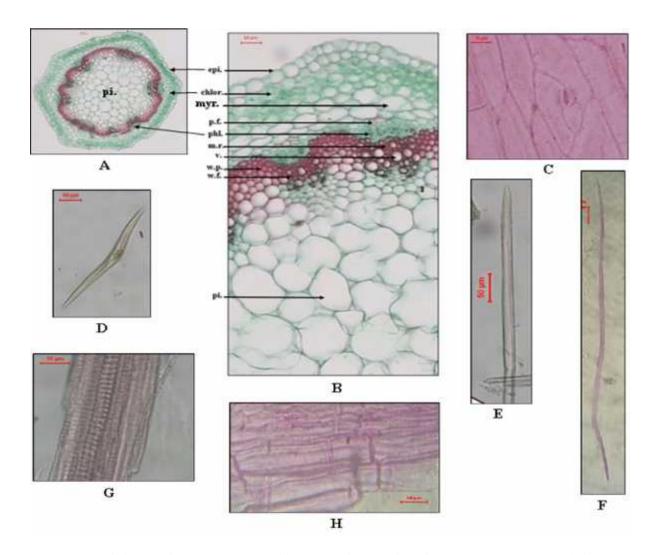


Fig. 5: Micromrphology of the young stem of L. libyca, (A) Photograph of the T.S. (X=7.5), (B) Detailed T.S. sector (X=100), (C) Surface preparation showing anisocytic stomata (X=160) (D) Non-glandular hair (X=110), (E) Pericyclic fiber (X=162), (F) Wood fiber (X=100), (G) Vessels (X=233.3), (H) Medullary rays (X=130). chlor., chlorenchyma; epi., epidermis; m.r., medullary rays; myr., myrosin cells; p.f., pericyclic fibers; phl., phloem; pi., pith; v., vessel; w.f., wood fibers; w.p., wood parenchyma.

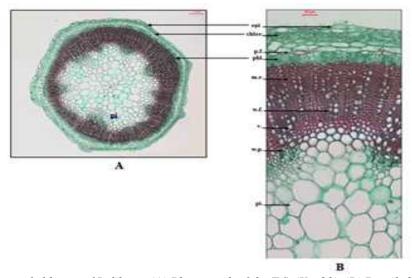


Fig 6: Micromorphology of old stem of L. libyca. (A) Photograph of the T.S. (X=23), (B) Detailed T.S. sector (X=92)

cork, followed by parenchymatous cortex. Endodermis and pericycle are indistinct. The vascular system is relatively wide with a comparatively narrow phloem and a wide central wood which occupies about 80% of the diameter of the root. The xylem is traversed by narrow medullary rays.

The cork cells are tangentially elongated tabular cells with cellulosic walls. They are radially arranged in several rows that may reach 5-10 rows where some rows are collapsed (Fig. 4C).

The cortex consists of several rows of somewhat tangentially elongated cells with thickened cellulosic walls and showing few scattered myrosin cells.

The vascular tissue is composed of several vascular bundles. The phloem is formed of continuous band of several rows of delicate cellulosic elements traversed by narrow, lignified medullary rays of 1-3 cells wide. The starch granules are absent. The cambium is distinct and formed of 2-3 rows of thin walled radially arranged cambiform cells. The xylem consists of lignified radially arranged elements. The vessels are mostly pitted (Fig. 4D). Also there are few fusiform tracheids, having blunt apices and pitted walls (Fig.4E). Wood fibers are fusiform, with straight or undulating lignified walls, narrow or wide lumina and acute apices (Fig. 4F). Wood parenchyma consists of rectangular elongated cells with lignified walls. The outer region of xylem representing the secondary xylem contains less vessels and more wood

fibers, while the inner region forming the primary xylem shows wider vessels. The medullary rays are wavy, uni-, bi-, or triseriate and are formed of rectangular polygonal cells with lignified walls (Fig. 4G).

The powder is yellow in color having a characteristic odour and a slightly pungent taste. Microscopically it is characterized by the presence of fragments of cork cells which are polygonal cells with cellulosic walls, lignified wood fibers with straight or undulating lignified walls, narrow or wide lumina and acute apices, lignified pitted xylem vessels, and fusiform pitted tracheids in addition to lignified medullary rays.

The young stem

A transverse section in the young stem (Fig. 5 A & B) is more or less circular in outline, showing 8 ridges.

The epidermis is composed of one row of polygonal, axially elongated with straight beaded anticlinal walls and covered with thick smooth cuticle (Fig. 5C). The epidermal cells contain mucilage (stained red with Ruthenium red reagent). The stomata are few of anisocytic type. Hairs are abundant, leaving a cicatrices when falling down (Fig. 5D). They are non-glandular, branched, unicellular, T-shaped having the lower part embedded in between the epidermal cells. The upper part is spreading showing distinctly thick nippled cuticle and thick walls. The hairs are arranged in a form that their long axis is parallel to the main axis of the stem.

The cortex is narrow and occupies about 1/8 the diameter

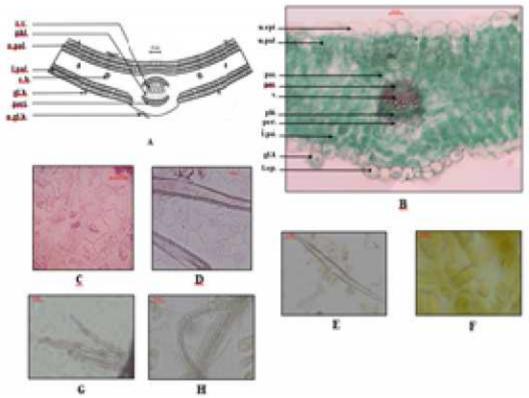


Fig. 7: Micromorphology of the leaf of L. libyca. (A) Diagram of T.S. (X=70), (B) Detailed T.S. sector (X=90), (C) Lower epidermis (X=130), (D) Upper epidermis (X=130), (E) Non-glandular hair (X=80), (F) Palisade cells (X=450), (G) Pericyclic fibers (X=80), (H) Xylem vessel (X=150). gl.h., glandular hair; l.epi., lower epidermis; l.pal., lower palisade; par., parenchyma; peri., pericycle; phl., phloem; u.epi., upper epidermis; u.pal., upper palisade; v., vessel; x.v., xylem vessel.

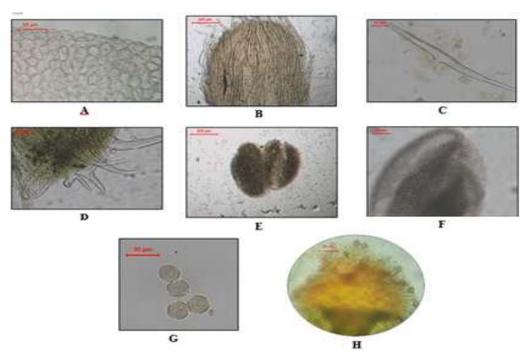


Fig. 8: Micromorphology of the flower of L. libyca. (A) Papillosed epidermis of petal (X= 190), (B) Hairy sepal (X= 45), (C) Non-glandular hair with warty cuticle (X= 102), (D) Y-Shaped hair with smooth cuticle (X= 80), (E) Anther (X= 44.5), (F) Fibrous layer of anther (X= 102), (G) Pollen grains (X= 356.6), (H) Papillosed stigma (X= 124).

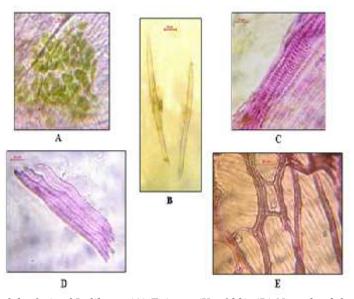


Fig. 9: Micromorphology of the fruit of L. libyca. (A) Epicarp (X=100), (B) Non-glandular hair (X=83), (C) Spiral xylem vessels (X=340), (D) Fibro-sclerenchyma of mesocarp (X=150), (E) Parenchyma of septum overlapped by a layer of liginified parenchyma (X=450).

of the stem, and consists of three to four rows of subepidermal rounded chlorenchymatous cells followed by two to four layers of nearly rounded, thin walled parenchymatous cells showing few scattered myrosin cells. The endodermis is indistinct containing few starch granules (stained blue with iodine solution). The cortex is devoid of calcium oxalate and secretory cells.

The pericycle consists of thin walled, parenchymatous cells interrupted by very few groups of thin walled slightly lignified fibers (Fig. 5E).

The vascular tissue is formed of several collateral vascular bundles forming a complete ring. Each has a

narrow phloem capping a layer of wood elements which is formed of fibers, vessels, wood parenchyma and traversed by medullary rays (Fig. 5 F, G & H). The tissue between the vascular bundles is formed mainly of lignified wood parenchyma and fibers. The phloem consists of thin walled cellulosic phloem elements. The phloem is devoid of fibers and calcium oxalate. The xylem is lignified, formed of pitted and spiral vessels. The wood fibers are numerous, having moderately thick lignified walls, wide or narrow lumina. The wood parenchyma consists of polygonal axially elongated cells with slightly thickened pitted and lignified walls. The

Table 1: Microscopical measurements of different organs of L. libyca

| Item | | | | | | |
|----------------------|---------------------------|--------------------------------|---------------------------|-----------------|--|--|
| | L | W | Н | D | | |
| Young stem | | | | | | |
| Epidermal cells | 122 <u>144</u> 174 | 30 <u>37</u> 45 | 22 <u>35</u> 44 | | | |
| Non-glandular hair | 353 <u>410</u> 529 | 50 <u>62</u> 75 | | | | |
| Myrosin cell | 90 <u>95</u> 100 | 43 <u>48</u> 54 | | | | |
| Wood fiber | 800 <u>834</u> 900 | 50 <u>68</u> 75 | | | | |
| Pericyclic fiber | 692 <u>718</u> 750 | 14 <u>17</u> 19 | | | | |
| Medullary rays cell | 120 <u>132</u> 175 | 40 <u>47</u> 55 | | | | |
| Vessels | | | | 12 <u>19</u> 28 | | |
| Pith cells | | | | 50 111 167 | | |
| Leaf | | | | | | |
| Upper epidermis | 35 45 80 | 30 <u>45</u> 68 | 24 34 39 | | | |
| Lower epidermis | 30 <u>45</u> 57 | $\frac{1}{22} \frac{1}{33} 40$ | $21 \ \overline{30} \ 50$ | | | |
| Stomata | 20 24 30 | 14 21 25 | | | | |
| Glandular hair | | | | 25 <u>31</u> 40 | | |
| Non-glandular hair | 340 <u>412</u> 550 | 45 <u>52</u> 72 | | _ | | |
| Palisade cells | 25 <u>30</u> 40 | 5 <u>8</u> 11 | | | | |
| Vessels | | _ | | 10 <u>14</u> 16 | | |
| Pericyclic fibers | 380 406 445 | 10 <u>13</u> 16 | | <u> </u> | | |
| Root | | · <u></u> | | | | |
| Cork cells | 65 90 100 | 28 35 40 | 13 20 27 | | | |
| Wood fibers | 300 329 410 | 13 <u>15</u> 17 | <u> </u> | | | |
| Medullary rays cell | 70 <u>83</u> 90 | $\frac{22}{30}$ 35 | | | | |
| Vessels | · · · <u></u> · · | | | 18 <u>35</u> 45 | | |
| Seed | | | | <u></u> | | |
| Epidermis | 36 45 51 | 30 36 42 | 32 35 40 | | | |
| Pigment layer | $14 \ \overline{20} \ 29$ | 7 <u>10</u> 14 | $10 \ \frac{1}{12} \ 14$ | | | |
| Aleurone layer cells | $16 \ \overline{20} \ 23$ | 10 <u>13</u> 14 | 9 <u>10</u> 12 | | | |
| Endosperm | 20 <u>35</u> 45 | 15 <u>25</u> 35 | 7 <u>14</u> 16 | | | |
| Embryo | 27 <u>33</u> 45 | 12 17 15 | 6 <u>12</u> 20 | | | |
| Myrosin cell | 28 <u>32</u> 36 | 20 <u>24</u> 27 | | | | |

L: Length

W: Width

medullary rays consisting of radially elongated cells which are cellulosic, thin walled in phloem region and thick walled lignified in xylem region.

The pith is wide and consists of more or less large, rounded parenchymatous cells occupying about ½ the diameter. The cells of the pith are usually narrow in the periphery and getting wider towards the center.

The powder of the young stem:

The powder (Fig. 5) is greenish yellow in color having a characteristic odour and a slightly pungent taste. Microscopically, it is characterized by the presence of:

- Fragments of elongated epidermal cells with straight beaded anticlinal walls, anisocytic stomata, nonglandular hairs or their cicatrices, and covered by smooth cuticle.
- Non-glandular unicellular, T-shaped trichomes, the upper part of which is covered by nippled granular cuticle.
- Fragments of long pericyclic fibers with moderately thick slightly lignified walls and wide lumina and acute, tapering apices.

H: Height D: Diameter

- Fragments of wood fibers with a thick lignified walls with wide or narrow lumina and tapering, occasionally pointed tips
- Fragments of lignified vessels, having pitted or spiral thickening.
- Fragments of lignified pitted wood parenchyma and medullary rays.
- Fragments of pitted lignified tracheids.

The old stem

A transverse section in the old stem (Fig. 6 A & B) is more or less similar to that of the young stem except that it is more circular and the ridges are more prominent. The vascular tissue is wider with narrower pith, while the endodermis is more indistinct and pith cells are more lignified near the vascular bundles.

The leaf

A transverse section in the leaf (Fig. 7 A & B) shows that the midrib is slightly prominent on the lower surface and straight on the upper surface with thick lamina. It also shows an isobilateral mesophyll, collateral vascular bundles and fibrous pericycle.

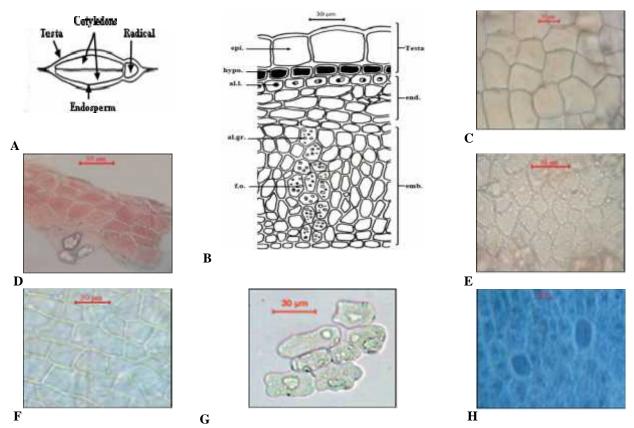


Fig. 10: Micromorphology of the seed of L. libyca (A) Diagram of transverse cut (X=35), (B) Detailed T. S. sector (X=433.3), (C) Epidermis (X=166.6), (D) Pigment layer (X=273.3), (E) Aleurone layer (X=310), (F) Polygonal endosperm (X=283.3), (G) Embryo (X=350). al.l., (H) Myrosin cells (X=246.6). aleurone layer; al.gr., aleurone grain; emb., embryo; end., endosperm; epi., epidermis; f.o., fixed oil;

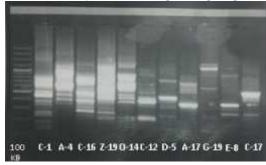


Fig. 11: The RAPD-PCR products of Lobularia libyca using eleven primers.

The upper and lower epidermises are nearly similar (Fig. 7 C & D). They show polygonal isodiametric or slightly axially elongated cells with straight anticlinal walls in the upper epidermis and slightly wavy anticlinal walls in the lower epidermis. They are covered with thin smooth cuticle and show anisocytic stomata. The upper and lower epidermal cells contain mucilage (stained red with Ruthenium red reagent). Hairs are abundant covering both surfaces. They are non-glandular unicellular calcified two-armed medifexed hairs covered with warty nippled cuticle (Fig. 7E). Their arrangement is parallel to the main axis of the leaf. Few glandular hairs are present, they have unicellular stalk and unicellular head, covered with smooth cuticle.

The palisade tissue consists of two or three rows of columnar closely packed cells, extended continuously on the upper surface only, being shorter on the lower surface (Fig. 7 B & F). The spongy tissue is narrow formed of irregular shaped parenchyma cells. Small vascular bundles are embedded within the spongy tissue.

The cortical tissue of the midrib consists of three or four rows of irregular shaped thin walled parenchyma beneath the upper palisade and lower epidermis.

The pericycle is composed from fibers with relatively thin lignified walls and wide lumina (Fig. 7G), present above and beneath the vascular bundle forming two arches (Fig. 7B).

The vascular tissue is composed of collateral vascular bundle containing phloem patches beneath the xylem vessels. The xylem vessels show spiral thickening (Fig. 7H). The cambium is formed of two rows of radially arranged small thin cellulosic cambiform cells.

The powder is pale green in color, with a characteristic odour and slightly pungent taste. Microscopically, it is characterized by the presence of the following:

• Fragments of upper and lower epidermises with polygonal isodiametric or slightly elongated cells having straight anticlinal walls in the upper epidermis and slightly wavy anticlinal walls in the lower epidermis. They are covered with thin smooth cuticle and showing anisocytic stomata.

Table 2: Molecular size in base pair of amplified DNA fragments produced by eleven decamer primers in *Lobularia librea*

| libyca. Molecular | | | | | | | | | | | C- |
|-------------------|-----|-----|------|------|------|------|-----|------|------|-----|----|
| size (bp) | C-1 | A-4 | C-16 | Z-19 | O-14 | C-12 | D-5 | A-17 | G-19 | E-8 | 17 |
| 100 | - | - | - | - | - | - | - | - | + | - | - |
| 150 | + | - | - | _ | - | - | + | - | - | - | - |
| 170 | + | - | - | - | - | - | - | - | - | - | - |
| 200 | - | - | - | + | - | - | - | - | + | + | - |
| 220 | - | - | - | - | - | + | - | - | - | - | - |
| 230 | - | - | - | - | - | - | + | - | - | - | - |
| 250 | + | - | | + | - | | | | | | + |
| 270 | - | - | - | - | - | - | | - | + | + | - |
| 280 | + | - | + | - | - | - | - | - | - | - | - |
| 290 | - | - | - | + | - | - | | - | - | - | - |
| 300 | - | - | + | - | - | - | + | + | - | + | + |
| 330 | - | - | + | - | - | - | - | - | - | - | - |
| 340 | - | - | - | - | - | - | + | - | - | - | - |
| 350 | + | - | - | - | - | + | - | - | - | - | - |
| 360 | - | - | + | + | - | - | - | - | - | - | - |
| 370 | - | - | - | - | + | - | - | - | - | - | + |
| 380 | + | + | - | - | - | + | - | - | - | - | - |
| 400 | - | - | - | - | + | - | + | - | - | - | - |
| 410 | - | + | + | - | - | - | - | - | - | - | - |
| 420 | - | - | - | + | - | - | - | - | - | - | - |
| 430 | - | - | - | - | + | - | - | - | - | - | - |
| 450 | + | + | + | - | - | + | - | - | - | - | + |
| 460 | - | - | - | - | + | - | - | - | - | - | - |
| 470 | - | - | + | + | - | - | - | - | - | - | - |
| 500 | + | - | - | + | - | - | - | + | + | - | - |
| 520 | - | - | + | - | - | - | - | - | - | - | - |
| 530 | - | + | - | - | - | - | - | - | - | - | - |
| 550 | - | - | - | + | - | - | - | + | - | - | - |
| 580 | - | - | - | - | + | - | - | - | - | - | - |
| 590 | + | - | - | - | - | - | - | - | - | - | - |
| 600 | - | - | - | - | - | + | - | - | - | - | - |
| 620 | - | - | - | - | - | - | - | + | - | - | - |
| 630 | + | - | - | - | - | - | - | - | - | - | - |
| 650 | - | + | + | + | + | - | - | - | - | - | - |
| 670 | - | - | - | - | - | - | - | + | - | - | - |
| 700 | + | - | + | - | - | - | + | - | - | - | + |
| 750 | - | - | - | + | - | - | - | - | - | - | - |
| 780 | + | _ | - | _ | _ | _ | - | - | - | - | - |

| Table 2: Molecular size in base pair of amplified DNA | A fragments produced by eleven | decamer primers in Lobularia |
|---|--------------------------------|------------------------------|
| libyca. | | |

| Molecular size (bp) | C-1 | A-4 | C-16 | Z-19 | O-14 | C-12 | D-5 | A-17 | G-19 | E-8 | C-17 |
|------------------------|-----|-----|------|------|------|------|-----|------|------|-----|------|
| 800 | - | - | - | - | + | + | - | - | + | - | + |
| 850 | - | - | - | - | + | = | - | - | = | + | - |
| 900 | - | - | - | + | - | - | - | - | - | - | - |
| 950 | - | + | - | - | - | + | - | - | - | - | - |
| 1000 | - | + | - | + | - | - | - | - | - | - | - |
| 1050 | - | - | - | - | - | - | - | - | - | + | - |
| 1080 | - | + | - | - | - | - | - | - | - | - | - |
| 1100 | - | - | + | - | + | - | - | - | + | + | - |
| 1200 | - | - | - | - | - | - | - | - | + | - | - |
| Total | 12 | 8 | 11 | 12 | 9 | 7 | 6 | 5 | 7 | 6 | 6 |

(+) = Presence of bands

(-) = Absence of bands

- Fragments of columnar, thin walled palisade cells containing green plastids.
- Numerous scattered non-glandular two armed hairs and few glandular hairs with unicellular stalk and unicellular head with smooth cuticle.
- Fragments of pericylic fibers with slightly lignified walls and wide lumina.
- Fragments of lignified xylem vessels with spiral thickening.

The flower

The powder is pale green in color having a characteristic odour and a slight pungent taste. Microscopically it is characterized by the presence of the following:

- Fragments showing papillosed epidermis of petal (Fig. 8A).
- Fragments showing epidermis of sepal densely covered with non-glandular unicellular calcified two-armed medifexed hairs covered with warty nippled cuticle (Fig. 8B).
- Fragments showing non-glandular unicellular calcified two-armed medifexed hairs covered with warty nippled cuticle (Fig. 8C).
- Fragments showing non-glandular unicellular branched hair covered with smooth cuticle (Fig. 8D).
- Fragments showing the anther (Fig. 8E).
- Fragments showing the fibrous layer of anther (Fig. 8F).
- Fragments showing spherical smooth pollen grain with three germ pores, three germ furrows and smooth exine (Fig. 8G).
- Fragments showing papillosed epidermis of stigma (Fig. 8H).

The fruit

The powder is pale green to brownish green in color, with slight pungent taste and odour. Microscopically it is characterized by presence of polygonal green cells of epicarp due to their chlorophyll content (Fig. 9A), nonglandular two armed hair covered with a smooth cuticle (Fig. 9B), spiral lignified xylem vessels (Fig. 9C), and elongated pitted fibro-sclerenchymatous cells of the inner layer of mesocarp (Fig. 9D). Moreover, the false septum

shows pitted lignified large parenchymatous cells accompanied by a layer of elongated thin walled parenchyma (Fig. 9E).

The seed

A transverse section in the seed (Fig. 10 A & B) shows the testa which is narrow and composed of an epidermis and hypodermis. The testa is followed by endosperm and embryo.

The epidermis is formed of one row of large polygonal isodiametric cells with beaded cellulosic anticlinal walls covered with thick smooth cuticle. The epidermal cells contain mucilage (stained red with Ruthenium red reagent) (Fig. 10C)

The pigment layer is formed of one row of smaller tabular cells being polygonal isodiametric in top view with thick straight cellulosic anticlinal walls showing reddish brown content (Fig. 10D).

The endosperm shows an aleurone layer (Fig. 10E) followed by 3-5 rows of isodiametric or slightly axially elongated thick-walled polygonal parenchymatous cells. It contains numerous fixed oil droplets (stained red with Sudan III reagent) (Fig. 10F).

The cotyledon is formed of several rows of small isodiametric or slightly elongated thin-walled parenchyma cells with slight wavy anticlinal walls filled with oil droplets (stained red with Sudan III reagent) and protein masses (stained yellow with picric acid reagent) (Fig. 10G). The cotyledon shows well distinct scattered myrosin cells (Fig. 10 H)

The powder

The powdered seed is pale orange brown in colour with pungent odour and oily pungent mucilaginous taste.

Microscopically it is characterized by the presence of the following:

- Fragments of polygonal isodiametric cellulosic epidermal cells containing stratified mucilage.
- Fragments of polygonal isodiametric cells of the pigment layer with thick straight cellulosic anticlinal walls showing reddish brown content.
- Fragments of endosperm showing thick walled polygonal cells and numerous oil droplets.

• Fragments of isodiametric or slightly elongated thinwalled embryo cells with slightly wavy anticlinal walls filled with oil droplets and protein masses.

Microscopical measurements

The dimensions of different cells (e.g. parenchyma, fibers, sclerides, hairs, pollen grains, etc.) were measured according to the magnification bar provided by the software (mentioned in section 2.2.2.) and listed in table (1).

DNA fingerprint

The DNA fingerprint of *Lobularia libyca* was carried out as a contribution to the macro- and micromorphological identification and characterization of the plant. In this study the extracted

DNA of the plant was amplified using eleven decamer primers. The RAPD electrophoretic profile of the DNA sample showed distinguishable bands and generated 89 fragment patterns.

The obtained banding profiles produced by the primers used in the RAPD analysis were represented in (Fig 11). The distribution of these bands is illustrated in Table 2.

The eleven primers of arbitrary sequences produced multiple band profiles with a number of amplified DNA fragments ranging from 12 when C-1 and Z-19 were used to the least number of fragments being 5 when A-17 was used. The primers C-1 and Z-19 were found to be the most effective in the selective discrimination of *Lobularia libyca* (viv) C.F.W. Meissn by the production of 12 amplified DNA fragments each, followed by C-16 producing 11 DNA fragments.

However, the primer A-17 produced 5 amplified DNA fragments and D-5, E-8 and C-17 produced 6 amplified DNA fragments each. Therefore, they can be considered of less contribution to the identification of *Lobularia libyca*

CONCLUSION

Lobularia libyca (viv). C.F.W. Meissn. is an annual cruciferous herbaceous desert plant native to North Africa. This study aimed to characterize the plant on both the botanical and the genetic levels. L. libyca is characterized by presence of non-glandular branched unicellular two armed hair in the stem, leaf and flower while the rootis normal with narrow cork. The leaf also characterized by presence of lignified pericyclic fibers.

The DNA of *L. libyca* was amplified using eleven decamer primers to reveal RAPD fragments. The results suggest the use of primers C-01 and Z-19 for the selective discrimination of *Lobularia libyca* (viv). C.F.W. Meissn.

REFERENCES

- 1. Tackholm V, Students' Flora of Egypt. Cooperative Printing Co., Beirut, Lebanon, 1974.
- 2. Al-Shehbaz IA, Beilstein MA, Kellogg E. Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. *Plant Systematics and Evolution* 2006; 259: 89-120.
- 3. Boulos L, Flora of Egypt. Al-Hadara Publishing, Cairo, Egypt, 1999.
- 4. Bailey LH, The Standard Cyclopedia of Horticulture. The Macmillan Company, New York, 1953, 36.
- Solreder H, Systematics Anatomy of the Dicotyledons. The Clarendon Press, Oxford, London, 1908, 58-67.
- 6. Metcalfe CR, Chalk L, Anatomy of Dicotyledons. The Clarendon Press, Oxford, London, 1950, 79-87.
- 7. Ayoub N, Nematallah KA, Al-Gendy AA, Zaghloul SS, Novel quercetin glycoside with promising hepatoprotective activity isolated from *Lobularia libyca* (viv). C.f.W. (Brassicaceae). *European Scientific Journal* 2013; 9: 177-193.
- 8. Williams JKG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphism amplified by arbirtrary primers are useful as genetic markers. *Nucleic Acids Research* 1990; 18: 6531-6535.