

COMPARATIVE STUDIES ON THE ANATOMICAL STRUCTURE OF STEM AND LEAF OF THREE GENERA OF *LAMIACEA*

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ABSTRACT

Plants of marjoram, basil and sage all of the family *Lamiaceae* were grown during 1998/ 99 season for studying their anatomical features. Results indicated that stem primary structure in the three genera as the stem layout is quadrangular . Cortex showed cells of different types in corners and edges. The procambial ring is well defined with a considerable number of well differentiated and lignified xylem vessels. The phloem is embedded in a sheath -like ring of small polygonal cells. This sheath has smaller cells as compared with those of the contacting cortical layers. The pith is relatively wide as compared to whole stem diameter in marjoram and sage. It comprises round to oval cells with variable size. Stem secondary structure proved that average thickness of the cortex of the three studied genera is relatively thicker. A complete cylinder of well lignified vascular vessels are found in the three genera. As well as, leaf anatomy of the three studied genera shows some differences in their components. Stomata in the three studied genera are anomocytic which comprises no subsidiary cells. Stomata number and size are varied between the upper and lower epidermis of leaf blade and between the three studied genera. Non-glandular uniseriate hairs of varying length with swollen basal cells are commonly found. Glandular hairs are found in the three studied genera. Though, both marjoram and basil showed glandular heads of 4 cells and short stalks, while heads of 8 cells with long stalks are found in sage.

INTRODUCTION

The family *Lamiaceae* includes plants with a considerable variety of habit forms. Sweet marjoram (*Majorana hortensis* Moench.), sweet basil (*Ocimum basilicum* L.) and sage (*Salvia officinails* L.) plants were chosen for this histological study as they are considered the most common cultivated aromatic and medicinal herbaceous prennial plants in Egypt. Some confusion exists regarding the identity of many plants belonging to *Lamiaceae*. It is substantial to mention that, while many studies of the morphology and anatomy of the main genera of *lamiaceae* have been made in the past, yet, few have been found upon the three studied genera. Moreover, the comparative anatomy attended to this study covered the gap found concerning the anatomy of the primary structure of the stem as well as the stomata types in the leaf and their structure. Many workers, suggested that taxonomic relationships be based upon all fields of plant science, Mullan (1932), Foster (1941), Bailey (1969) and Metcalfe and Chalk (1979). Therefore, this anatomical study has been undertaken to give more information about the anatomy of the three studied genera belonging to *Lamiaceae* which could be adopted in differentiating between the three genera.

MATERIALS AND METHODS

Seeds of marjoram (*Majorana hortensis* Moench), basil (*Ocimum basilicum* L.) and sage (*Salvia officinalis* L) were secured through Legume Research Section, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. Seeds were sown on mid of October during the season 1998 / 99 in hot beds containing a mixture of 1:1:1 clay: sand: peatmoss for germination; seedlings were transplanted on the first of March into 3×3 m² experimental plots. All common agricultural procedures for growing marjoram, basil and sage were followed. Random samples of 5 plants from each genus were collected on 30 day intervals after sowing for anatomical studies. The first sample was taken after 30 days from sowing for studying the primary structure of the stem. The secondary structure was studied in 120 day old samples. Specimens were taken from the middle of the second internode just below the shoot apex, and from the 5th internode for the primary and secondary structures, respectively. Samples were killed, fixed in (F. A. A) and dehydrated in a series of alcohol's before being embedded in paraffin wax according to Sass (1951). Sections were cut to thickness of 12μ and stained by crystal violet Erothrosin combination and mounted in Canada balsam (Jackson, 1926). Slides were microscopically examined and measurements were taken as averages of 10 readings from 5 slides. The epidermis impressions of the lower and upper epidermis were prepared by pressing the leaf (taken from the 5th node at the age of 120 days) against a drop of strong liquid glue (Amir Alpha glue) on a glass slide for approximately 30 seconds, then removing the leaf.

RESULTS

1-Stem structure

A- Primary structure

Stem anatomical features of the three studied genera are presented in Table (1) and Figures (1- a, b and c)

I-Marjoram

Stem structure is rectangular in out line with four convexed corners and four edges. The average thickness of the apical internode in the transverse section is 4798 X5908. The epidermal cells are square with dense cytoplasm. No stomata are recognized in the epidermis of the apical internode, while it bears a wide range of trichomes. However, the average thickness of the epidermis is 22μ. The cortex is comparatively wide with about 5-8 layers of round parenchyma cells, and the thickness is ranging between 260 to 280μ at edges and corners respectively. At this stage of growth no collenchyma or chlorenchyma are detected at the corners of the stem. Four bundles of vascular elements are found. The number of rows of vessels is 4-6. The average dimensions of each bundle averages 175x 220 μ and average vessel diameter is 36 μ (Fig. 1-a). The phloem is embedded in a sheath -like ring of 3-5 layers of small polygonal cells. This sheath has

smaller cells as compared with those in contact with cortical layers. The pith is relatively wide compared to whole stem diameter. It comprises round to oval cells with variable size. The average pith radius is 1860 μ and the average pith cell diameter is 130 μ .

II- Sage

Stem structure, as shown in the transverse sections of the internode, is presented in (Table1 and Figure 1-b). It is obvious that the stem outline is quadrangular with four convex angles. The epidermal cells are square in shape with thin wall and dense cytoplasm. Stomata are found in the epidermis of the apical internode, and it bears numerous monocellular and multicellular hairs. The average thickness of the epidermis cell is 18 μ . The cortex is comparatively wide with about 7-10 layers of round parenchymatous cells. The transaction illustrates that cortex cells in the corners are collenchyma with relatively small round cells with small intercellular spaces. The cortex thickness is ranging between 188 to 295 μ at edges and corners, respectively.

Table (1): Means of certain histological counts and measurements (μ) of the primary structure as shown in cross sections of the second internode just below the shoot tip of the three studied genera.

Plants/ characters	Marjoram	Sage	Basil
Epidermis thickness(μ)	22	18	19
Range of Cortex thickness(μ)			
Edges	260	188	210
Corners	280	295	242
Average number of cortex rows	5-8	7-10	7-9
Average diameter of the stem(μ)			
Edges	4798	4310	4480
Corners	5908	4840	4720
Average number of vascular bundles	4	4	4
Average dimensions of vascular bundle	175 X220	133X228	140X245
Average vessel diameter (μ)	36	29	28
Average number of vessels rows	4-6	5-7	3-5
Average number of phloem layers	3-5	3-4	3-4
Average number of cells across pith	14	21	12
Average pith radius (μ)	1860	1640	1125
Average pith cell diameter (μ)	130	80	93

At early stage of growth the vascular elements are four bundles located at angles of the stem, later to this stage a complete cambial ring appears in cross sections (Fig 1-b). Each bundle assumes the c-shaped configuration and contains 5-7 rows of vessels. The vascular bundle dimensions are 133 μ \times 228 μ . However, the average vessel diameter is 29 μ . Phloem elements are consisted of 3-4 rows and are accompanied with comparatively high amount of parenchyma cells, of variable size, and thin walls and remarkable large intercellular spaces. The average pith radius is 1640 μ and the average diameter of pith cell is 80 μ .

III –Basil

Stem structure as shown in Table 1 and figure (1-c) is considerably different than that of both marjoram and / or sage. The epidermal cells are square in cross section. Numerous glandular scales and non- glandular hairs are present along the internode. The cortex consists of 7 to 9 layers of small oval to round parenchyma cells with small intercellular spaces. The cortex thickness is ranging between 210 μ to 242 μ at out edges and corners, respectively. No fibers are found towards the innermost layer of the cortex as small colenchyma cells with well developed wall are present. The average number of vascular bundles are four bundles found at the four corners of the transverse section. The average dimensions of the vascular bundle is 140X245 μ and the average diameter of the vessel is 28 μ . Number of rows of vessels 3-5. The phloem ranges between 3-4 rows. The pith is relatively narrow and its radius is 1125 μ , while the total number of cells across the pith are 12 cells, with an average diameter averages 93 μ .

B-Secondary structure

I-Marjoram

Anatomical features in transverse sections of mature marjoram stem are presented in Table (2) and Figure (2- a). It is obvious that the average thickness of the epidermis is 22 μ . The cortex ranges between 290 to 420 μ and is comparatively wider as compared with the cortex of the primary structure. It consists of 7-10 layers of small parenchyma cells. At this stage of growth a complete cylinder of well lignified vessels ranges from 11-20 vessels per row. The average vessel diameter is 64 μ . The secondary phloem is fully differentiated where a considerable number of sieve elements dispersed opposite to the outer side of xylem vessels. The phloem is embedded in a sheath – like ring of 8 - 10 layers of small fibers cells. The pith is relatively narrow to whole stem thickness, it comprises a round to oval cells with variable size. The average pith thickness is 2050 μ and the average pith cell diameter is 157.5 μ .

II- Sage

Stem anatomical secondary structure as shown in transverse section is presented in Table (2) and Figure (2-b). The average stem thickness ranges from 6616 to 6881 μ . The epidermal cells are small sized and square in shape. The average thickness of the epidermis cell is 18 μ . The cortex is 7 to 10 layers with small round parenchyma cells. Most cortex cells in the corners are collenchyma with relatively small round intercellular spaces. However, cortex thickness is ranging between 260 to 370 μ at edges and corners, respectively. It is also noticed that, in cross section, isolated strands of fibres present on the inside part of cortex and around the phloem. The thickness of the complete cylinder of vascular vessels, the average vessel diameter and the average number of vessels per row are 1804 μ , 42 μ and from 12 -19 , respectively. Where, a sheath like ring of 7-9 layers of phloem elements embedded with small fibers cells are found. The pith is comprises round cells with variable size; thin walled parenchyma cells with remarkable large intercellular spaces. The average pith diameter is 2465 μ . While, the average diameter of pith cell is 112.2 μ .

III-Basil

It is realized from Table 2 and figure (2 c) that the epidermal cells are square in cross section with average thickness of 19 μ . Numerous glandular scales and non-glandular hairs are present along the internode. The cortex is relatively narrower (250 to 316 μ) as compared with both marjoram and sage. The cortex exhibited 7 to 9 layers of small oval to round parenchyma cells with small intercellular spaces. No fibrous tissues are found towards the innermost layer of the cortex, while small collenchyma cells are found at the corners of the stem. A complete cylinder of vascular vessels of 2481 μ in thickness and average vessel diameter of 54 μ are found. The average number of vessels ranges from 9- 16 vessel per row. The secondary phloem consists of a ring of 6-8 layers . However, The average pith diameter is 2100 μ . The total number of parenchyma cells across the pith is 17 cells with an average diameter of 123.5 μ .

Table (2): Means of certain histological counts and measurements (μ) of secondary structure as shown in cross sections of the fifth basal internode of the three studied genera.

Plants/ characters	Marjoram	Sage	Basil
Epidermis thickness (μ)	22	18	19
Cortex thickness (μ)	Edges	290	260
	Corners	420	370
Average number of cortex rows	7-10	7-10	7-9
Average diameter of the section (μ)	Edges	7734	6616
	Corners	7980	6881
Average number of vessels/ row	11-20	12-19	9-16
Average number of phloem rows	8-10	7- 9	6-8
Average thickness of vascular cylinder(μ)	2506	1804	2481
Average vessel diameter (μ)	64	42	54
Average number of cells across pith.	13	22	17
Average pith diameter (μ)	2050	2465	2100
Average pith cell diameter (μ)	157.6	112.2	123.5

3- Leaf anatomy

Leaf anatomy of the three studied genera shows some essential differences in their components (Table 3, and Figure 3 a, b ,c).The average lamina thicknesses are 570, 450 and 337 μ for marjoram, sage and basil, respectively. The cuticle has moderate thicknesses and both upper and lower epidermis are composed of one layer of oval to rectangular cells with thin wall. The upper epidermis thickness are 19, 13, and 9 μ for marjoram, sage and basil, respectively. While, the corresponding values for the lower epidermis are 13, 10 and 8 μ in the same order. The mesophyll consists of a palisade tissue of one row of elongated cells with small intercellular spaces occupying nearly the half or the third of blade thickness being 247, 171 and

114 μ for marjoram, sage and basil, respectively. The spongy tissue consists of 6 layers of round loosely arranged cells in marjoram, while it consists of 8 layers of spongy cells in both basil and sage. The spongy tissue thickness is comparatively wide in marjoram; 315 μ as compared to both basil ; 203 and sage 268 μ . The average spongy cell diameters are 52, 38 and 19 μ for marjoram sage and basil , respectively. Although the midrib bundle of the three studied genera showed no difference in their components , yet , the midrib bundle of sage is remarkably larger in dimensions , 230 x 340 as compared with marjoram and basil , being 100 x 120 μ and 130 x 165 μ , respectively.

Table (3): Means of certain histological counts and measurements (μ) as shown in cross sections of the leaf blade of the three studied genera.

Plants/ characters	Marjoram	Sage	Basil
Blade thickness (μ)	570	450	337
Epidermis thickness	Upper	19	13
		Lower	13
Number of palisade rows	1	1	1
Mesophyll thickness (μ)	562	439	317
Palisade tissue thickness (μ)	247	171	114
Average number of spongy rows	6	8	8
Average spongy tissue thickness (μ)	315	268	203
Average spongy cell diameter (μ)	52	38	19
Dimensions of midrib bundle	Length,(μ)	100	230
	Width,(μ)	120	340
Number of stomata per microscopic field			
(upper epidermis)	13	9	7
(Lower epidermis)	18	13	12

Stomata and trichomes

It is realized that stomata are confined to the lower and upper epidermis of the lamina for the three studied genera as well as the trichomes as shown in Figure (4 a, b &c). The stomata are anomocytic with no subsidiary cells. Several ordinary epidermal cells irregularly surround the stomata. The guard cells are kidney shaped and contain abundant amount of chloroplasts. Stomata generally are randomly scattered in the epidermis in the upper epidermis and the average number of stomata /microscopic field are 13, 9 and 7 stomata for marjoram, sage and basil, respectively. However, the corresponding values at the lower epidermis are 18, 13 and 12 stomata for the three studied genera in the same order. Different types of trichomes are found on both surfaces of leaf blade. The density of trichomes is more abundant at lower surface in the three studied genera, and shown in Fig. (5 a, b, c, d &e) : A- Non-glandular uniseriate hairs of varying length with swollen basal cells are commonly found, while a considerable number of branched or straight multicellular trichomes with short basal and long terminal cells are observed in the lower epidermis of marjoram plants only. B- The

most frequent glandular hairs of the three studied genera vary in the number of cells in the head ,and length of the stalks. Marjoram and basil show glandular lobed heads of 4 cells and short stalks, while heads of 8 cells, with long stalks, 3 to 4 cells are found in sage.

DISCUSSION

Primary structure of the stem:

It is evident from the study of the primary structure that marjoram gave the highest measurements in most of the studied characters. This included epidermis thickness, cortex thickness (although it gave the least average number of cortex rows), average diameter of the stem , average dimensions of vascular bundles, average vessel diameter and average pith radius and cell diameter. The other two genera ; sage and basil did not show much difference between each others . The three genera have quadrangular stem with 4 bundles. This is in accordance with those stated by Metcalf and Chalk (1979), Saad (1981) and Sabbour (1983).

Secondary structure of the stem:

The epidermis thickness was not different from that of the primary structure in the three genera . Other tissues were increased in thickness than in the primary structure , i.e cortex , average diameter of the section, vascular cylinder and pith diameter.

It is evident that most of the previous measurements of marjoram and basil were almost similar in this stage , and both were higher than sage in most measurements, except in pith diameter and number of cells across the pith.

The secondary growth in marjoram, basil and sage forms a vascular cylinder of almost similar thickness . Similar results were indicated by Holm (1946), Hayward (1948), Metcalf and Chalk (1979) and Sabbour (1983).

Leaf structure:

The study of leaf anatomy revealed that marjoram – in general- gave the highest measurements of blade thickness, palisade tissue thickness, average thickness of spongy tissue, although it gave the least number of spongy rows, average diameter of the spongy cell and number of stomata per microscopic field in the upper and lower epidermis.

Basil gave the least measurements, while sage was intermediate between marjoram and basil. Stomata were anomocytic with no subsidiary cell in the three studied genera. Different types of trichomes were found on both surfaces of the blade, and were more abundant on the lower one in the three genera. The midrib bundle of the 3 genera showed no difference in their components , although in sage it was remarkably larger than the other 2 genera. Similar results were obtained by Metcalf and Chalk (1979).

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- تم إجراء هذه التجربة بغرض دراسة التركيب التشريحي لثلاثة أنواع نباتية تابعة لثلاثة أجناس من نباتات الفصيلة الشفوية ذات الأهمية الاقتصادية كنباتات طبية و عطرية و ذلك خلال موسم 1999/98. و كانت أهم النتائج المتحصل عليها كالاتي:
- أظهر التركيب التشريحي الابتدائي فى القشرة اختلافات فى شكل و عدد أنواع الخلايا خاصة فى الأركان والحواف
 - كما أظهر التركيب التشريحي للاثلاثة البردقوش و الريحان والسالفيا أنها رباعية الاركان مع وجود عدد 4 حزم وعائية فى مقابل الاركان .
 - كانت حلقة الكامبيوم الأولى واضحة مع وجود عدد ملحوظ من الأوعية ذات الجدر الملجننة. أما الخشب فكان به أوعية مبعثرة ملجننة بدرجات متفاوتة مع وجود أشرطة لحائية على شكل حرف C متكونة من خلايا لحائية عديدة الأضلاع .
 - كانت خلايا النخاع دائرية ألي بيضاوية الشكل ومنتسعة وكانت أكثر اتساعا فى البردقوش و الريحان مقارنة بالسالفيا.
 - أظهر التركيب التشريحي الثانوى أن متوسط سمك القشرة فى الأجناس الثلاثة نسبيا أكثر اتساعا عنه فى التركيب التشريحي الابتدائي.
 - التركيب التشريحي للورقة أظهر وجود اختلافات فى المحتوى التشريحي سواء فى العرق الوسطى أو منطقة النصل.
 - كانت الثغور من طراز Anomocytic التى تتميز بعدم وجود خلايا مساعدة فى الأنواع الثلاثة للأجناس المدروسة. وكان هناك اختلافات فى حجم و عدد الثغور فى كلا من البشرة العليا والسفلى لنصل الورقة.
 - ووجدت شعيرات غير غدية وحيده الخلية مختلفة الأطوال وذات قاعدة منتفخة. بالإضافة ألي شعيرات عديدة الخلايا غير غدية . و لوحظ وجود شعيرات غدية لها رأس غدي من 4 خلايا مع وجود عنق قصيرة فى كلا من البردقوش و الريحان بينما كانت هناك شعيرات غدية ذات رأس من 8 خلايا و عنق طويل فى السالفيا.