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# Characterization of Some Fig Cultivars by Anatomical Traits on Both Leaves and Stems

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**Abstract:** The present study included anatomical studies of the stems and leaves of 7 cultivars of *Ficus carica* were investigated. The anatomical examination of the cross sections of the above mentioned stems and leaves revealed diagnostic characters among species. Data of comparative characters reached 14 couplet characters and 2 quantitative characters, data matrix were organized on the basis of variations to obtain a classification using sequential indented key and cluster analyses. Data matrix included the anatomical description and features of the epidermis, cortex, pericycle, vascular bundles and pith for stem anatomy and epidermis, mesophyll, midrib region and vascular bundles for leaf anatomy. These results were used in distinguishing the 7 cultivars of *Ficus carica* by indented key and statistical analyses (MVSP) to showed similarity between matrix and dendrogram. Such type of results could be valuable for further studies which will employ these results to interpret abiotic stress, where there is a strong relationship between anatomical structure and abiotic stresses tolerance in plants.

**Key words:** Anatomical studies · Fig (*Ficus carica*) · Leaf anatomy · Stem anatomy · Indented key

## INTRODUCTION

Fig (*Ficus carica* L.) is a fruit crop species, that is particularly well suited for the different environmental conditions of the Mediterranean basin countries and more 600 cultivars are locally grown and called varieties [1]. These cultivars selected for their edible fruits and the trees are clonally propagated through cuttings. Fig genotypes are preserved in the Mediterranean basin countries since many centuries for many different purposes. For instance, figs are consumed either fresh or dried or used for jam or spirit beverage production [2]. Fig cultivation on the coastal region of the Mediterranean basin has a long history and a promising future [3, 4]. Fresh fig production, although still environmentally sensitive and appears to be less demanding in terms of climate characteristics [5]. Many studies were based on the morphological characterization of fig cultivars and proved the sustainability of the use of morphological parameters to evaluate and to identify the genotypes [6-9]. The most discriminate parameters were those related to leaves and fruits [10]. On the other side, several of cultivars have been characterized by pomological and chemical traits [11, 12]. Also, characteristics related to the internal structure of plants have been employed for systematic purposes for over 150 years and they are useful in both practical identification and determination of phylogenetic relationships. Data based on the use of morphological and pomological traits, particularly those concerning the fruits, have been reported and involved the main cultivars that are locally predominant [8, 9, 13, 14]. However, these characters are either sensitive to the environmental conditions or limited to the fruit production season. The question arises to whether, differences in anatomical characters of both leaves and stems traits, could be employed with high accuracy to distinguish cultivars. This information could be useful in developing selection criteria for abiotic stress tolerance programs and identifying the most tolerant cultivars for these stresses.

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The present work aims to investigate the anatomical variations between some local and some imported fig cultivars to use these anatomical variations as taxonomic characters to identify cultivars in this study.

## MATERIALS AND METHODS

Seven Fig cultivars (Aboudi, Black mission, Conadria, Gizy, Katoda, Sultany and White Adcy) were collected during the year of 2010 from different regions in Egypt and planted in the Experimental Station of National Research Center, Nobaryia District, Beheira Governorate, Egypt.

**Sections of Stem and Leaf:** Fresh stem and leaf samples were taken from young parts of Fig cultivars seedlings (age one year) to study the anatomical features of young stems and leaves. One cm long from the middle part of the technical length of the stem and 1 $cm^2$ from leaf were taken. Samples were fixed in formalin-acetic acid-ethyl alcohol for 48 hours, then dehydrated in a series of solutions of ascending concentrations of ethyl alcohol varying from 50% to 100% ethyl alcohol. The samples then embedded in paraffin wax [m.p. 58-61C] using xylol as a solvent. By using rotary microtome, sections were cut at the thickness of 15 $\mu$m and then mounted on slides with the aid of egg albumin as an adhesive. Wax dissolved in xylol and the slides were passed through descending series of ethyl alcohol solutions varying from 100% to 50% ethyl alcohol concentrations in descending order. The sections on the slides were stained with safranin and light green and then the colored sections were kept as permanent preparations on the slides with canada balsam as mounting medium [15] and examined by light microscope Carl Zeiss then photographed by eye piece digital camera (Hirocam 5).

**Statistical Analysis:** Cluster analysis by multi-variate statistical package (MVSP) is consider as a techniques used in numerical classification.

## RESULTS AND DISCUSSION

The present results included 14 qualitative and 2 quantitative characters from leaves and stems. The data were comparatively recorded in the data matrix (Tables 1&2). All the 16 anatomical characters were belonging to 7 fig cultivars.

**Stem Anatomical Characters:** Data presented in Table 1 showed the differences on stem anatomical structure of the 7 fig cultivars. From stem cross-section it could be observed that, the epidermis consists of more than one layer (multiple epidermis) as observed in all examined cultivars, which were differed in their number as presented in Table 1. Three epidermal cell layers were recorded in White adcy, Aboudi and Black mission cultivars; the largest number of multiple epidermal layers (4-layers) was recorded in Conadria cultivar. While, the lowest number of multiple epidermal layers (2-layers) was recorded in Katoda, Gizy and Sultany cultivars. Increasing thickness of epidermal and multi-epidermal layers could be an important factor against both biotic and abiotic stresses. The stem cortical layer consists of two types of cortical cells as presented in Figs. 1-6. Cortical cells which were very smaller in shape and size as collenchymatous cells were observed below multiple epidermal layers. While, the other types of cortical cells were larger in shape and size as parenchymatous cells were observed on the inner zone of cortical layer. Secretory canals were recorded on stem cortical layers of White adcy, Black mission, Katoda, Gizy and Sultany cultivars as presented in Fig. 2. However, stem cortical layers were devoid from secretory canals as recorded on Aboudi and Conadria cultivars (Fig. 3). Stem xylem vascular system were consists from well differentiated arms and clusters as recorded in White adcy, Aboudi, Black mission, Katoda, Gizy and Sultany, while, it was very slightly differentiated in Conadria (Fig. 3).

Numbers of xylem vessels cluster on stem cross-section were varied according to the studied cultivars (Table 1). More number of xylem vessels clusters was recorded in White adcy cultivar (9). Whereas, the lowest number of xylem vessels clusters (3) was recorded in Aboudi cultivar. Also Katoda and Gizy cultivars were identified by 5 and 4 xylem vessels clusters, respectively as presented in Table 1. While, Black mission and Sultany cultivars characterized by 7 xylem vessels clusters on stem cross-sections. More number of xylem vessels on stem cross–section could be facilitate translocation and absorption of physiological active substances from sink to source, which affected growth, development and yield of economic plants. Sclerenchymatous cells were recorded in medullary rays of White adcy, Black mission, Gizy and Sultany cultivars (Fig. 4). However, the medullary rays were consists from parenchyma cells without sclerenchymatous cells as presented in Aboudi, Katoda and Conadria cultivars (Table 1).

Secretory canals in medullary ray were recorded only in Sultany cultivar. Whereas, secretory canals in phloem tissue were recorded in Gizy and Sultany cultivars. The secretory canals in pith were presented in Sultany

Table 1: Stem anatomical characteristics of 7 cultivars belonging to *Ficus carica* of *Moraceae* with 8 qualitative characters and two quantitative characters.

| Cultivars | | | | | | | |
|---|---|---|---|---|---|---|---|
| Characters | Aboudi | Black mission | Conadria | Gizy | Katoda | Sultany | White adcy |
| Secretory canals in cortex present +/ absent- | - | + | - | + | + | + | + |
| Sclerenchymatous cells in medullary ray presented+/ absent - | - | + | - | + | - | + | + |
| Secretory canals in medullary ray present +/ absent - | - | - | - | - | - | + | - |
| Xylem formed from arms only + / arms and clusters - | - | - | + | - | - | - | - |
| Secretory canals in phloem present +/ absent- | - | - | - | + | - | + | - |
| Druses crystals in pith +/ absent- | + | - | + | - | + | - | - |
| Druses crystals in medullary ray +/absent - | - | - | - | - | + | - | - |
| Secretory canals in pith present +/ absent- | - | - | - | - | - | + | - |
| Number of multiple epidermal layers | 3 | 3 | 4 | 2 | 2 | 2 | 3 |
| Number of xylem clusters | 3 | 7 | 4 | 4 | 5 | 7 | 9 |

Table 2: Anatomical characteristics of leaves of 7 cultivars belonging to *Ficus carica* of Moraceae with 6 qualitative characters recorded comparatively.

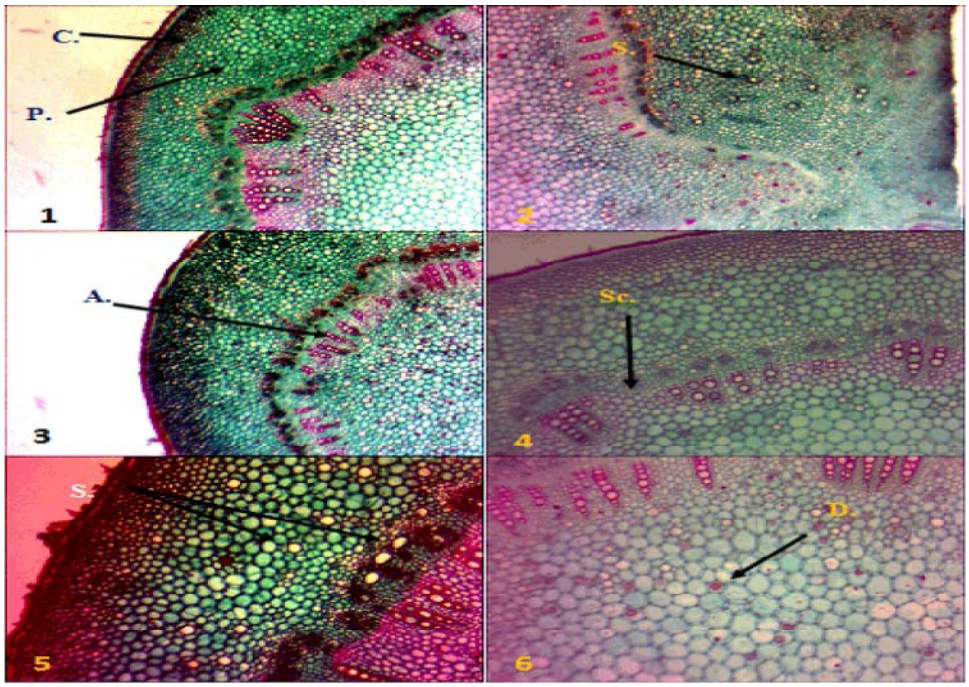
| Cultivars | | | | | | | |
|---|---|---|---|---|---|---|---|
| Characters | Aboudi | Black mission | Conadria | Gizy | Katoda | Sultany | White adcy |
| Palisade in one row +/ two rows | + | + | + | + | - | - | + |
| Druses crystal in mesophyll present +/ absent | - | - | - | - | - | + | - |
| Vascular bundle single +/ double - | - | + | + | - | - | - | + |
| Vascular bundle in crescent shape +/ circular- | - | + | + | - | - | - | + |
| Secretory canals in midrib region present +/ absent | - | - | - | - | + | - | - |
| Druses crystal in midrib region present +/ absent | + | - | - | - | + | + | - |

Table 3: Clusters analysis produces a phylogeny tree of cultivars based on similarity matrix

| Items | Aboudi | Black mission | Conadria | Gizy | Katoda | Sultany | White adcy |
|---|---|---|---|---|---|---|---|
| Aboudi | 1 | | | | | | |
| Black mission | 0.364 | 1 | | | | | |
| Conadria | 0.333 | 0.462 | 1 | | | | |
| Gizy | 0.2 | 0.364 | 0.333 | 1 | | | |
| Katoda | 0.364 | 0 | 0.154 | 0.182 | 1 | | |
| Sultany | 0.154 | 0.286 | 0 | 0.462 | 0.286 | 1 | |
| White adcy | 0.4 | 0.909 | 0.5 | 0.4 | 0 | 0.154 | 1 |

cultivar only (Fig. 5). Therefore, it could be concluded that, Sultany cultivar investigated by secretory canals in medullary ray, phloem tissue and pith. Druses crystal in pith was detected in Aboudi, Katoda and Conadria cultivars (Fig. 6). Whereas, druses crystal in medullary ray was observed only in Katoda cultivar. These results are in agreement with those obtained by Metcalfe and Chalk [16], who reported that, the epidermal layers consists of 1 to several layers of cells, which was not confused with true hypoderm and occurs in certain genera of Moraceae. Secretory canals which may be laticiferous or filled with gum-resin or mucilage are widely distributed in categories of secretory elements and present in the primary cortex and sometimes in the pith and in primary and secondary phloem. They also added that crystals are secreted mostly in the form of clusters or solitary types.

**Leaf Anatomical Structure:** Data presented in Table 2 showed the differences in leaf anatomical structure of the 7 fig cultivars. As illustrated in Figs. 7-12, the palisade tissue was consists of one raw of palisade cells as recorded in White adcy, Aboudi, Black mission Conadria and Gizy cultivars (Fig. 7). On the other side, palisade tissue consists of two layers of palisade cells (Fig. 8) were recorded in Katoda and Sultany cultivars. Collenchymatous cells in midrib region were recorded in all examined cultivars. One main leaf vascular bundle (single bundle), with crescent shape was detected in White adcy, Black mission and Conadria cultivars (Fig. 9), whereas, one main circular shape vascular bundle larger in size, with associated small size vascular bundle were detected in Aboudi, Katoda, Gizy and Sultany cultivars (double bundles) (Fig. 10). Secretary canals were only recorded in katoda cultivar (Fig. 11). Druses crystals were detected in both mesophyll and midrib zone of sultany cultivar (Fig.12), but it was observed only in midrib zone of Katoda cultivar. The present results are compatible with the findings of Metcalfe and Chalk [16], who reported that leaves of Moraceae usually dorsiventral, the epidermis is consisting of a single layer of cells.




Figs. 1-6: Cross sections in stems of *Ficus carica* cultivars

| | |
|---|---|
| 1-White adcy x 50 | 4- Black mission x 100 |
| 2-Black mission x 50 | 5- Sultany x 100 |
| 3-Conadria x 50 | 6- Katoda x 100 |

**Abbreviation:**

| | |
|---|---|
| C.= Collenchymatous cells | A.= arms of xylem |
| P.= Parenchymatous cells | Sc.= Sclerenchymatous cells |
| S.= Secretory canals | D.= druses crystals |

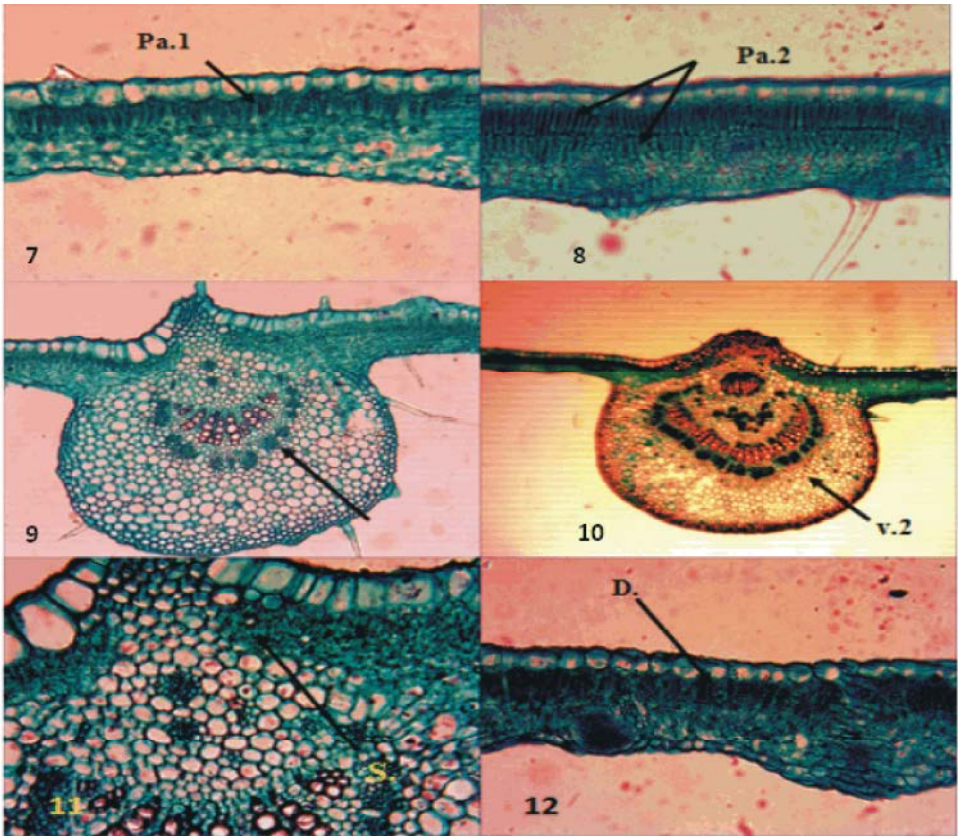
Veins very diverse in structure, may be a simple crescent of individually bundle as in *Morus alba* or closed cylinder of xylem which accompanied externally by numerous phloem groups. Wu and Huang [17] studied nine species belonging to eight genera of Moraceae and noticed druses crystals as preponderant types in these species. Sonibare *et al.* [18] examined the anatomical structure of leaves of twenty five species of *Ficus* from Nigeria, which characterized by palisade cells in 1-2 layers in certain species. Ravichandra and Paarakh [19] found that, collenchyma cells in the midrib below the upper and above the lower epidermis in the leaves of *Ficus microcarpa*. Kaur [20] recorded that, the leaf of *Ficus pumila* is bifacial, epidermis consists of a single layer and mesophyll region is distinctly differentiated into palisade and spongy tissues.

The present results also indicated possibility of selection four anatomical structures such as association of both small and large vascular bundles as well as two layers of palaside cells in mesophyll tissue of the leaves. Furthermore, number of multiple epidermis and number of xylem vessels cluster of stem cross- sections. These traits would be useful as indirect selection criteria for environmental stress.

Based on the observation anatomical features indented key [21] has been constructed to allow distinguishing the 7 cultivars of *Ficus carica*:

**Secretory Canals in Cortex Present:**

- No Druses Crystals in Medullary Ray
- Secretory canals in medullary ray and pith with druses crystals in mesophyll..... Sultany.
- No secretory canals in medullary ray but in phloem parenchyma with 2 layers of multiple epidermis..... Gizy.
- Multiple epidermis with 3 layers without secretory canals in midrib region.




Figs. 7-12: Transverse sections of leaves of

7- Aboudi x 250
8- Katoda x 250
9- White adcy x 100
10-Gizy x 40
11- Katoda x 400
12- Sultany x 250

**Abbreviation:**

Pa1= Palisade tissue consists of one layer

Pa2= Palisade tissue consists of two layers

V.1 = Single vascular bundle, With crescent shape

V.2= Large one main vascular bundle large in size, with associated small size vascular bundle, with circular shape

S.= Secretory canals

D.= Druses crystals

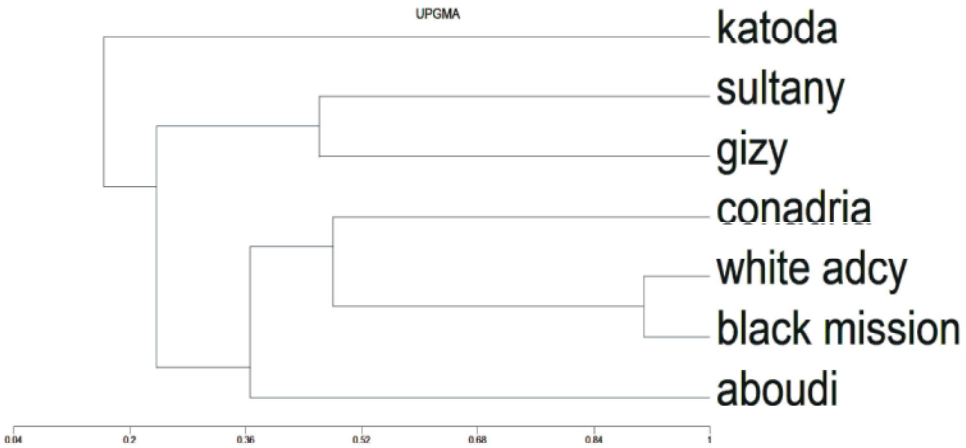



Fig. 13: Dendrogram is constructed on the basis of 16 anatomical features

C.1. Xylem with 9 clusters in stem .....White adcy.
C.2. Xylem with 7 clusters in stem .....Black mission
2. Druses Crystals in Medullary Ray and Secretory Canals in midrib region ..... Katoda.

**No Secretory Canals in Cortex:**

- Xylem formed from arms and undifferentiating clusters with 4 multiple epidermal layers and 4 xylem clusters and single vascular bundle …… Conadria.
- Xylem formed from arms and well differentiation clusters with 3 multiple epidermal layers and 3 xylem clusters with double vascular bundles ..... Aboudi.

**Cluster Analysis:** Cluster analysis produces a phylogeny tree of cultivar based on the similarity matrix (Table 3). As presented in Fig. 13 dendrogram is constructed on the basis of 16 anatomical features (Tables 1&2) which showed highly similarity between Black mission and White adcy as shown also in indented key, medium similarity between Sultany and Gizy, Conadria and Black mission, Aboudi and White adcy, White adcy and Conadria, Katoda and Aboudi, while showed a similarity at all between Sultany and Conadria and Katoda with Black mission. Other cultivars characterized by low similarity between some other. Generally, in order to cluster analysis 7 cultivars can divided into 3 groups, group 1 which included Katoda, Sultany and Gizy. Group 2 which included Conadria, Black mission and White adcy. Group 3 which included Aboudi only.

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