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MICROMORPHOLOGICAL AND ANATOMICAL STUDIES OF ANTHERS IN CITRUS TAXA FROM IRAQ

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SUMMARY

The innovative study comprised a comparative evaluation of phenotypic and anatomic characters of anthers in 14 citrus taxa grown in Iraq. The results revealed the classification of citrus anthers into three groups based on the apex shape—round in five taxa, round-flattened apex in three taxa, and acute apex in six taxa. The epidermis surface patterns also exhibited four groups—reticulated surface, pitted, smooth, and slightly disassembled. The tapetum was evident in three forms, viz., densely folded, folded orbicules, and almost unfolded. The anatomical study disclosed that the cross-section shape of the anther was like a butterfly in all taxa. The anther wall of the studied citrus taxa consisted of four distinct cell layers. The endothecium cells of the studied citrus taxa had the characteristic of semi-circular outline thickenings with needle-shaped crystals appearing in the anther tissue in some taxa. The stamen filament was irregular in the citrus taxa, comprising an epidermis with cuticle, followed by the cortex with raphides crystals, with the vascular bundle located in the center. The results confirmed that the citrus anther micromorphology and anatomy represent effective tools in supporting taxonomy and breeding by facilitating the differentiation of taxa and improving the understanding of relationships within *Citrus*.

Keywords: Citrus taxa, citrus anther, Iraqi citrus, anther anatomy, SEM

Key findings: Classification tools vary to differentiate taxonomic taxa down to the smallest structure; therefore, the anther's adoption morphologically and anatomically served as one of the distinguishing marks between citrus taxa, and with hybridization, their characterization resulted in a high degree of convergence and similarity.

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INTRODUCTION

Citrus belongs to the family Rutaceae, characterized by remarkable levels of polymorphism and genetic diversity (Rymbai, 2024). This led to an ambiguous number of taxa, with estimates ranging from a few accepted species to more than 150 (Ollitraulta *et al.*, 2020). In Iraq, Townsend (1980) listed nine taxa native to the country. Many citrus taxa have nutritional and health values, serving as fresh fruits and juices, as well as inclusion in some food recipes. Moreover, citrus plants have become a source of vitamin C with traditional medicinal values (Okeke and Mbagwu, 2001).

Citrus has a wide distribution throughout the world, and its diversity primarily depends on apomixis, the highest frequency of bud mutations, hybridization, sexual compatibility between *Citrus* and related genera, and the long history of its cultivation (Nicolosi *et al.,* 2000). This phenomenon resulted in an extensive cultivar list of mostly hard-to-identify diverse and interesting morphological characteristics (Jin *et al.,* 2022). The presence of numerous uncertain taxa renders citrus taxonomy confusing, controversial, and complicated. However, this perplexity has prompted taxonomists to delve into the depths of characters, especially phenotypic traits (Rymbai, 2024).

Phenotypic characters serve as primary tools for botanists for isolating and determining a taxon, with most of these characters evolving dramatically after the discovery of the two types of electron microscope. This advanced technology helped much in solving taxonomic identifications, since it describes the smallest details of plant parts at the microscopic level and the study of external and internal structures of plants (Germanà, 2007).

The variations in shape are evident in the plant's reproductive parts, considered important taxonomically due to fixed structures and mostly unaffected by the environment over generations (Begcy *et al.,* 2024). Qualitative and quantitative key characters are effective in the identification and description amidst the large number of taxonomic structures. The anthers are the neglected morphological and anatomical features, where studies relied on pollen grains. The external and internal structure of the anther affects the process of pollen production and has a viable role in male sterility (Chaban *et al.,* 2020). Therefore, the anther has a remarkable role in plant breeding (Das *et al.,* 2018) and has reached a wide range of studies in plant species (Aboshama, 2011).

The well-differentiated anther wall comprises four layers (Begcy *et al.,* 2024). The tapetum, or the inner surface of the anther, plays a vital role in transporting nutrients to the developing pollen grains, with the primary function of forming the outer wall. In *Citrus,* the tapetum is typically the parietal tapetum (Saffari *et al.,* 2021). Most previous studies relied on the fruits. Given the lack of any comprehensive study on morphological and anatomical anthers of this genus in Iraq, the presented study sought to clarify the ambiguity and investigate the taxonomic status to better understand the relationship within citrus taxa (Alanbari *et al*., 2025).

MATERIALS AND METHODS

Identification of plant specimens

Given the lack of integration of the Iraqi plant encyclopedia, which includes the genus *Citrus*, this study used the taxonomic keys published in many other floras. These are the Flora of British India (Hooker, 1885), the Flora of Syria, Palestine, and Sinai (Post, 1933), the Flora of Iran (Parsa, 1943), the Flora of Japan (Ohwi, 1965), the Flora of Java (Backer, 1965), and the Flora of Turkey (Davis, 1975).

Samples collection

Samples collected of the genus *Citrus* taxa comprising different species came from different Iraqi governorates, such as Anbar, Babel, Baghdad, Basrah, Diyala, Erbil, Karbala, Salahaldeen, and Suleimanyiah, during different growth and development stages (Table 1). The 30 field trips extended from October 2024 to May 2025, approximately visiting each site more than twice. These field

Table 1. Characteristics of morphological features of anther of *Citrus* taxa in Iraq.

No.	Taxa	Length of anther (mm)	Diameter of anther (mm)	Shape of apex anther	Shape of outer surface	Shape of inner surface
1	*C. aurantifolia* var. *acidica*	0.4-2.1 (1.0)	0.4-1.2 (1.0)	round	smooth	densely flooded
2	*C. aurantium*	1.1-3.1 (2.0)	1-3 (2.0)	round	clearly disassembled	flooded
3	*C. deliciosa.*	0.2-1.4 (1.0)	0.6-2 (1.0)	round flattened	slightly disassembled	densely flooded
4	*C. grandis*	3.7-8.1 (5.0)	1-3 (1.5)	acute	clearly disassembled	densely flooded
5	*C. japonica*	1-2 (1.2)	0.5-1.4 (0.9)	round flattened	pitted	densely flooded
6	*C. latifolia*	2-3.7 (3.0)	0.8-2.5 (1.0)	round	*slightly disassembled*	densely flooded
7	*C. limetta*	1.5-4.5 (3.0)	0.9-1.5 (1.0)	acute	*smooth*	flooded
8	*C. medica*	2 -4 (2.3)	0.4-1.2 (1.0)	acute	*clearly disassembled*	flooded
9	*C. paradisi*	2.2-5.7 (3.0)	1-1.2 (1.0)	round	*clearly disassembled*	not flooded
10	*C. reshni*	1.1-3 (2.0)	0.7-1.6 (1.0)	round flattened	*clearly disassembled*	densely flooded
11	*C. reticulata* var. *clementine*	1.5-3 (2.0)	0.6-2.1 (1.0)	acute	reticulated	densely flooded
12	*C. sinensis*	0.4-5 (2.0)	1-3.1 (1.5)	round	*clearly disassembled*	densely flooded
13	*C. aurantium x C. trifoliata*	1.5-3.5 (2.0)	0.5-4.1 (1.5)	acute	pitted	flooded
14	*C. sinensis* var. *moro*	2 -4 (3.0)	0.9-4.2 (2.0)	acute	*clearly disassembled*	densely flooded

trips succeeded in their organization in all seasons of the year, at different stages of growth, including the floral buds stage, the vegetative growth stage, and the stage of flowers and fruits. The tripaxon of the genus *Citrus* entailed distribution in province centers and some parts of Kurdistan and Southern Iraq. These areas, orchards, and home gardens have reached earlier identification for sample collection in the existing environment.

Morphological study

Primarily for accurate diagnosis, all the specimens underwent detailed studies using dissecting and compound microscopes. The samples collected from the flowering buds were at the stage just before their initiation and stored in 70% ethanol. The sample preparation preceded the investigation using both light microscopy (LM) and electron microscopy (SEM). Morphological and anatomical studies proceeded in the laboratories of the Biology Department at the College of Education for Pure Sciences, University of Baghdad, Iraq. Depositing the specimens continued at the herbarium at the College of Education for Pure Sciences, University of Baghdad, Iraq. The samples for LM sustained mounting in silicone oil before sealing with paraffin. Then, the samples incurred observation, followed by photography with an Olympus BH2 light microscope (Germany). For SEM studies, the samples attained suspension in 100% alcohol before putting on a stub and coating with gold. The samples proceeded to assessment under an LEO 1450VP electron microscope (England), taking the measurements from 25 samples for each citrus taxon.

Anatomical study

For studying the different parts of anthers, the research followed the methodology of Thammathaworn (1996).

RESULTS AND DISCUSSION

All taxa of the genus contained an androecium with numerous fertile stamens, ranging from 10 to 40, comprising arrangements in 3–20 bundles (polydelphous) and having protandrous phenomena. A stamen comprised a filament and an anther, with these characters shown in Figure 1A and Table 1. Yellow, smooth, and filiform filaments were notable in all taxa; their length varied from the lowest length (5.6 mm) in the species *C. deliciosa* to the highest length (17.9 mm) in the species *C. sinensis.* These results were greatly analogous to past findings, which also reported varying sizes in different species when compared (Illoh,

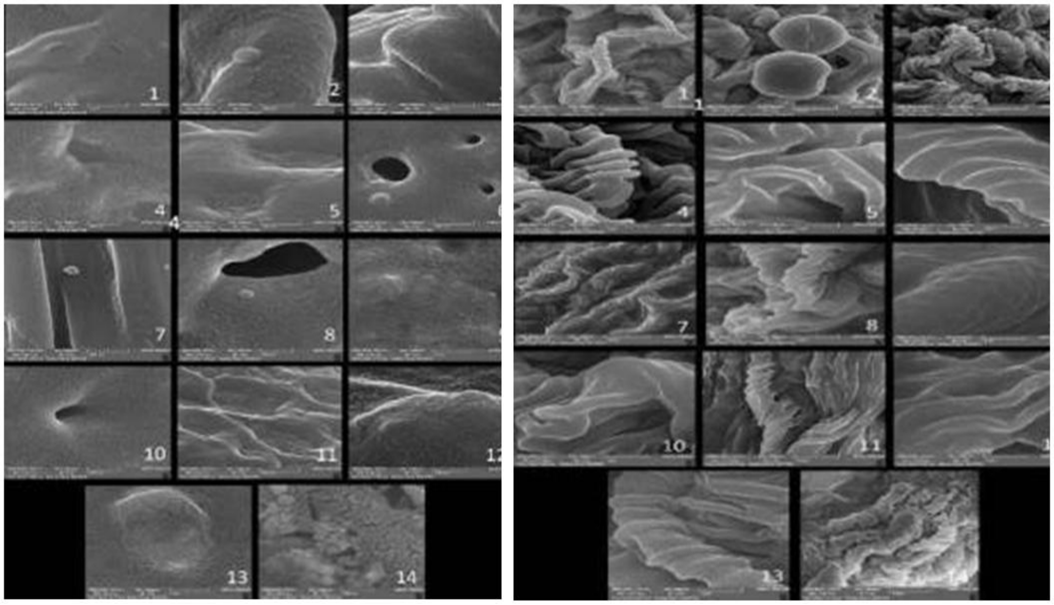

Figure 1. Anther of *Citrus* taxa. A: Outer surface, B: Inner surface, 1. *C. aurantifolia* var. *acidica,* 2. *C. aurantium,* 3. *C. deliciosa,* 4. *C. grandis,* 5. *C. japonica,* 6. *C. latifolia,* 7. *C. limetta,* 8. *C. medica,* 9. *C. paradisi,* 10. *C. reshni,* 11. *C. reticulata* var*. clementine,* 12. *C. sinensis,* 13. *C. aurantium x C. trifoliata,* and 14. *C. sinensis* var. *moro*.

1995; Adedeji and Jewoola, 2008; Saheed and Illoh, 2010).

The anther length was the minimum (1 mm) in the species *C. aurantifolia* var. *acidica* and *C. deliciosa*. However, the species *C. grandis* had the longest anther length (5 mm). Ogundare and Saheed (2012) also mentioned the epidermal cell could be too isolated in closely related taxa of *Citrus*. In all other taxa under study, the anthers were between above two ranges. The anther diameter was greatest (2 mm) in the species *C. aurantium* and *C. sinensis* var. *moro*, while the smallest anther diameter (0.9 mm) resulted in the species *C. japonica*. Lattar *et al*. (2012) also reported varied values for anther length and diameter in various families. The light microscopy (LM) study showed the anther composed of two lobes in all taxa had an oblong shape and longitudinal dehiscence. The surface was glabrous and yellow and has a property of being spiral after opening, with the same shape of anthers reported in the species *C. limetta* (Cao *et al.,* 2025).

The SEM study of the apex and the top of anthers reached three classifications of groups, such as round apex in the species *C. aurantifolia* var. *acidica*, *C. aurantium*, *C. latifolia*, *C. paradisi*, and *C. sinensis*. The other group is round-flattened apex in the species *C. deliciosa*, *C. japonica*, and *C. reshni*. The third group, the acute top, was noteworthy in the species *C. grandis*, *C. limetta*, *C. medica*, *C. reticulata* var. *clementine*, *C. aurantium x C. trifoliata*, and C*. sinensis* var. *moro*. Cai *et al*. (2021) suggested that returns to degradation of tapetum impair pollen maturation, which also supported these present results (Li *et al*., 2011; Yu *et al*., 2016).

The shape of the outer surface of the anther or epidermis reached a classification into four groups. The species *C. reticulata* var. *clementine* was visible with a reticulated surface (Figure 1A); the pitted surface appeared in the species *C. japonica* and *C. aurantium x C. trifoliata;* a smooth surface was apparent in the species *C. aurantifolia* var. *acidica* and *C. limetta*. However, the slightly disassembled surface emerged in the species *C. deliciosa* and *C. latifolia*, while the outer ones with clearly disassembled surfaces were in *C. aurantium*, *C. grandis*, *C. medica*, *C.*

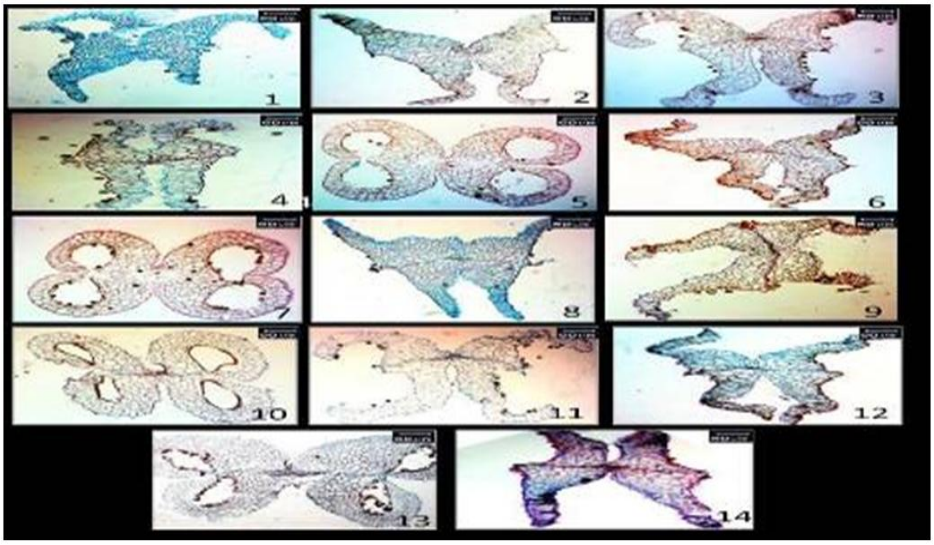

Figure 2. The cross-section shape of *Citrus* taxa. 1. *C. aurantifolia* var. *acidica,* 2*. C. aurantium,* 3. *C. deliciosa,* 4. *C. grandis,* 5. *C. japonica,* 6. *C. latifolia,* 7. *C. limetta,* 8. *C. medica,* 9. *C. paradisi,* 10. *C. reshni,* 11. *C. reticulata* var. *clementine,* 12. *C. sinensis,* 13*. C. aurantium x C. trifoliata,* and 14. *C. sinensis* var. *moro*.

paradisi, *C. reshni*, *C. sinensis*, and *C. sinensis* var. *moro*. The presented results were in contrast with past findings of Çetinbaş and Ünal (2015), who described that epidermal cells were considerably stretched and flattened. The SEM may provide the new results for the anther structure, whereas the variations in the epidermis anther can be beneficial as a taxonomic tool to differentiate the taxa. The latest results also gained support from previous studies in some other genera (Illoh, 1995; Adedeji and Illoh, 2004; Saheed and Illoh, 2010).

The inner surface of the anther contained bicule sorubisch bodies reaching classification into three groups (Figure 1B). The first one was densely flooded bicules (less than 2 micrometers flooded) observed in the species *C. aurantifolia* var. *acidica*, *C. deliciosa*, *C. grandis*, *C. japonica*, *C. latifolia*, *C. reshni*, *C. reticulata* var. *clementine*, *C. sinensis*, and *C. sinensis* var. *moro.* The second was flooded orbicules (2–5 micrometer flooded) in the species *C. aurantium*, *C. limetta*, *C. medica*, and *C. aurantium x C. trifoliata*. However, the unflooded showed in the species *C. paradisi*. Therefore, one can conclude that the orbicules were obviously noticeable in plants with the smaller fruits (Cai *et al*., 2021; Rymbai, 2024).

Anatomical study

The outer shape of the anther cross-section was like a butterfly in all the *Citrus* species (Figure 2). The cross-section of the anthers in all the taxa showed that the epidermis was thicker, covered by a cuticle layer (Figure 3A). Previous studies have reported that the anther epidermis constitutes a protective layer against harm and mechanical stress (Rezanejad 2008; Skrzydeł *et al.,* 2021). Similarly, it further protects the genetic materials from the abiotic and biotic stress effects (Somaratne *et al.,* 2017) and indirectly saves the developing pollen grains (Yang *et al.,* 2014). All the *Citrus* taxa had the composition of four cell layers—one row of the epidermis, four rows of the endothecium, and two rows of the tapetum (Figure 3A). The similar structure of the anther wall bore a description in the flowers of some species of the family Rosaceae (Saffari *et al.,* 2021).

The location of endothecium cells occurred under the epidermis, found to be

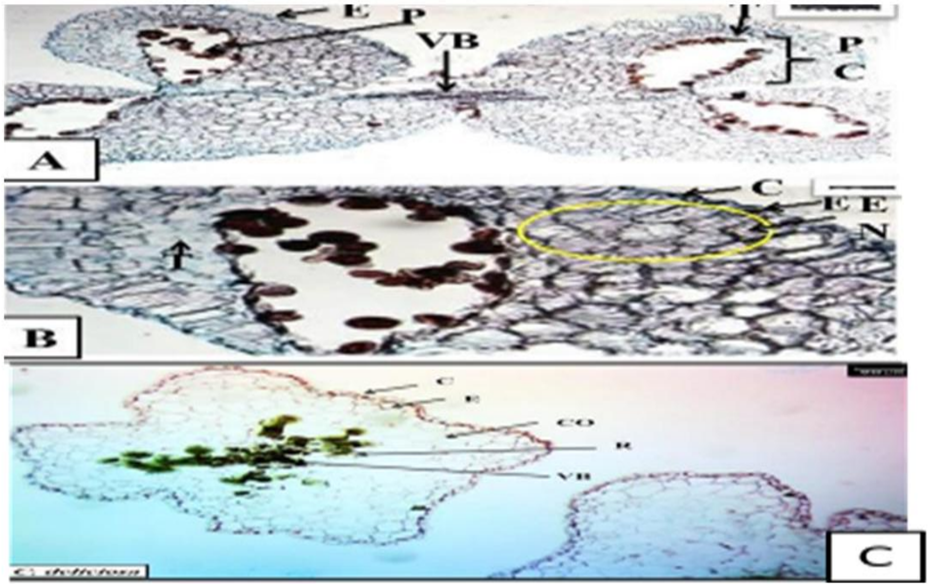

Figure 3. A: The cross-section shape of *C. aurantium x C. trifoliata*. E = epidermis, P = pollen grains, VB = vascular bundle, T = tapetum, PC = pollen sac, B = enlarged part of the anther, C = cuticle, EN: thickening of endothecium, filament of stamen showed, C = cuticle, CO = cortex, and R = raphides crystal.

larger than the epidermal cells, and exhibited the ledge-shaped thickenings of parallel ledges, regularly arranged semicircular (Figure 3B). Previous studies revealed the endothecium cells in other species comprised of one or several layers (Bhanwra *et al.,* 2006; Xue *et al.,* 2021). The endothecium cells of the studied citrus taxa had characteristics of thickening with a semicircular outline, with the needle-shaped crystals or raphides crystals observed in anther tissue sections in some *Citrus* species, such as *C. aurantium*, *C. deliciosa*, *C. japonica*, *C. sinensis* var. *moro*, and *C. reshni* (Figure 3C). These needle-shaped crystals consisted of calcium oxalate. These endothecium cell thickenings form the tetrad stage, and the cell wall has the fibrous thickenings that enlarged during anther development (Reinoso *et al.,* 2002; Çetinbaş and Unal*,* 2014).

The study results further revealed that in the *Citrus* taxa, the stamen filament was irregular in form and included one layer of cuticle epidermis. The cortex fills the inner part, comprising parenchyma tissues, and the vascular bundle sits in the center of it, consisting of many raphides crystals around it (Figure 3C). The central part of the filament cross-section contains a collateral vascular bundle surrounded by a multilayer of parenchyma tissue and a single epidermal layer (De-Carvalho *et al.,* 2016).

CONCLUSIONS

Although the stamen is a small structure, it can be effective as a supporting classification tool to separate taxa from one another, especially within the *Citrus* genus, due to the close similarity and likeness between its taxa. Identifying micro-morphological and anatomical features serves as a vital method for separating the taxa. The difference between the outer and inner surfaces of the stamens and the fold ratio is a dividing factor for taxa. Moreover, the anatomy of the structure, its external shape, the presence of crystals, and epidermal thickness have demonstrated differences among taxa.

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