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Leaf Characters Within Tribe *Trifolieae* (Family Leguminosae)

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Abstract: Leaf macro- and micro-morphological characters in 31 species distributed over four genera belonging to tibe *trifolieae* have been studied by both light and scanning microscope. Six groups have been distinguished according to type of leaves, trichome presence and type of hairs. All the studied species are amphistomatic with either paracytic, diacytic or anomocytic stomata. Brachyparacytic has been recorded in *Ononis pubescens* only. The epidermal cells are isodiametric or elongated with straight, undulated or wavy anticlinal walls. The data obtained support the separation of the genera *Ononis* and *Trifolium* each in a group of its own. *Trigonella* species are all glabrous and become very easily recognized by their leaf characters.

Key words: Macro characters, micro characters, trichomes-stomata, classification, plant taxonomy

INTRODUCTION

Leaf morphological characters considered one of the primary diagnostic features in segregating the major groups and put the leaf characters as the second to those of flowers and fruits in taxonomic studies^[11] while Tomlinson^[2] claimed the neglect of vegetative morphology in most of the recent taxonomic works. Stace^[3] has pointed to the necessity of using many tools in solving any taxonomic problem. Within the Paplionoideae the morphology of the leaf considered one of the first steps in distinguishing the taxa^[4-7]. Leaf macromorphology; shape, type, margin, apex and venation; alone cannot give a precise decision in taxonomic conclusions. For that it is necessary to search about the micro characters of the leaves to help in diagnosis. Stace^[1] has stated the characters that must be

studied in the leaf for the sake of clarity, especially if we use modern microscopes in this study. Taia^[8] made a survey of the leaf characters within the Egyptian papilionaceous species using both the light and scanning electron microscopes. Tribe *trifolieae* characterizes by having trifoliate leaves, genus *Ononis* has simple leaves, with serrate margins and acute apices. Micro characters of some species belonging to this tribe have been studied by Taia^[8]. This study is done during the period of 2000 and 2002 as complementary to the previous work and concentrate on species of tribe *trifolieae* to facilitate their separation and for better understanding their relation.

MATERIALS AND METHODS

Details of 31 species from the Egyptian flora distributed over four genera are listed in Table 1. Pieces of

Table 1: Taxa investigated	
Name of the taxon	Source/Locality/Collector and date
Ononis natrix L.	Alex. Univ./Rafah, near the sea/Boulos, 31-3-1977.
O. vaginalis Vahl.	Alex. Univ./Matruh-Siwa Rd./Ayyad et al., 21-3-1989.
O. reclinata L.	Alex. Univ./Burg El-Arab/El- Ghazaly et al., 2-4-1982.
O. sicula Guss.	Alex. Univ./Matruh, wadi El-Ramla/Tadros, 14-4-1948.
O. pubescens L.	Alex. Univ./Rafah/Ayyad et al., 14-4-1981.
O. serrata Forssk.	Alex. Univ./Rosetta/Rizk et al., 28-3-1992.
Trigonella arabica Del.	Alex. Univ./El-Arish/Boulos, 18-3-1955.
T. anguina Del.	Alex. Univ./Burg El-Arab/Ayyad et al., 27-2-1988.
T. occulta Del.ex DC.	CAI/Helwan desert/Ghabour and Moustafa, 13-3-1959.
T. monspeliaca L.	Alex. Univ./King Mariut/Zidan, 15-3-1994.
T. stellata Forssk.	CAI/Sinai, El-Hasnaa/Shamso, 3-4-1988.
T. laciniata L.	Alex. Univ./Burg El-Arab/Rizk et al., 24-3-1981.
T. maritime Del.	Alex. Univ./Siwa oasis/Ayyad et al., 4-4-1978.
T. hamosa L.	Alex. Univ./El-Arish/Boulos, 18-3-1955.
T. media Del.	Alex. Univ./AlexCairo Rd./Ayyad et al., 22-3-1980
Melilotus alba Medic	CAI/Ras El-Hekma/Tackholm et al., 30-4-1955.
M. sulcata Desf.	CAI/El-Sharkiya/Amer, 8-2-1982.
M. siculus (Turra) Jacks.	Alex. Univ./El-Mansoura/Boulos, 5-2-1981.
M. indica (L.)All.	Alex. Univ./El-Bousily/Rizk et al., 21-3-1989.
Trifolium fragiferumL.	Alex. Univ./Burg El-Arab/El-Ghazaly et al.,7-2-1981.

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Table 1: Continue	
Name of the taxon	Source/Locality/Collector and date
T. resupinatum L.	Alex. Univ./El-Bousily/Rizk et al., 21-3-1989.
T. tomentosum L.	Alex. Univ./AlexMatrouh Rd./Rashad, 20-3-1975.
T. procumbens L.	Alex. Univ./El-Omayed/Zedan, 14-3-1988.
T. philistaeum Gruenb. Et Zoh.	CAI/Sinai, El-Hasnaa/Shamso, 3-4-1988.
T. nervulosum Boiss. and Heldr.	Alex. Univ./El-Omayed,sand dune/Zedan 14-3-1988.
T. repens L.	Alex. Univ./AlexMatrouh Rd./Rashad, 20-3-1975.
T. alexandrenum L.	Alex. Univ./Samouha/Abdel-Aziz, 6-1-1948.
T. scabrum L.	Alex. Univ./Matrouh-Siwa Rd./Ayyad et al., 21-3-1989.
T. stellatum L.	Alex. Univ./Burg el-Arab/Fakhry, 23-3-1992.
T. purpureum Loisel	Alex. Univ./Saint Katren, Sinai/Ayyad et al., 15-4-1982.
T. desvauxii Boiss.et Bl.	Alex. Univ./Matrouh-Siwa Rd./Ayyad et al., 12-4-1987.

Abbreviations used: Alex. Univ.=Alexandria university herbarium, CAI= Cairo university herbarium, Rd.= road

the leaf lamina were peeled by boiling in 10% nitric acid for light microscope investigation. Portions of the laminae were mounted onto cleaned stubs with silver paint, then coated with gold and scanned at 15 kv. for the study of the micro characters. The terminologies used here are that of Dilcher^[9] for stomata, Strace^[1] for epidermal cells and Al-Shammary and Gornall^[10] for trichomes`.

RESULTS

From Table 2 and 3 it was observed that the most effective characters in delimiting the taxa within tribe trifolieae are leaflets shape, margin, apex beside trichome presence and type. From which it was found that the first three characters (leaflets shape, margin and apex) are useful tools in identifying some species, but cannot be used in delimiting the genera (Fig. 1-31). Trichomes presence and type of hairs have more weight in delimiting the genera (Table 3). Stomata in the studied taxa are mostly paracytic or diacytic. Anomocytic type founded in few species; Trigonella anguina, Melilotus indica and Trifolium procumbens (Fig. 32-40). Brachyparacytic stomata are found in Ononis pubescens only (Fig. 35). The epidermal cells are either isobilateral or elongated with wavy, straight or undulate walls (Fig. 41-74). Cuticle are either smooth (Fig. 45 and 46), striate (Fig. 47, 48, 49, 56 and 57) or granulate (Fig. 51, 52, 54 and 58) with, or without, wax secretions (Table 3). Accordingly, we can group the studied taxa in response to the type of leaf, presence or absence of trichomes and the type of hairs into six groups:

Group 1: *Ononis pubescens* **type:** This group has simple lanceolate leaves with smooth margin and acute apices, covered by multicellular glandular hairs. Stomata brachyparacytic with isobilateral, straight wall epidermal cells. Cuticle smooth with slight wax depositions. This group include one species; *Ononis pubesecens*.

Group 2: Ononis sp. type: Taxa with trifoliate leaves, leaflets having different shapes; lanceolate, ovate, trilete

or obovate; with smooth or dentate margins and emarginated, acute or apiculate apices. Leaflets enriched with multicellular, smooth walled glandular hairs. Stomata either paracytic or diacytic with isobilateral or elongated straight or wavy wall epidermal cells. Cuticle smooth ornamented with wax depositions. This group includes *Ononis natrix, O. vaginalis, O. reclinata, O. sicula and O. serrata.*

Group 3: *Trigonella* **sp. type:** Species with trifoliate leaves, with glabrous cordate, broadly ovate, trilete, rotund or even lanceolate leaflets, dentate margins and rounded, emarginated or apiculate apices. Stomata paracytic or diacytic rarely anomocytic surrounded by either isobilateral or elongated epidermal cells with straight, rarely undulate, walls. Cuticle smooth, striate or even granulate with or without wax secretions. This group gathers all the *Trigonella* species studies.

Group 4: *Melilotus* **sp. type:** Species with trifoliate leaves, leaflets lanceolate, narrow ovate, ovate or sometimes obovate with dentate margins and emarginated apices. Leaflets either glabrous or covered by unicellular or multicellular hairs. Stomata paracytic or anomocytic surrounded by isobilateral or elongated straight or undulated walls epidermal cells. Cuticle striate or granular, sometimes smooth, without wax secretions. This group includes *Melilotus* species studied.

Group 5: *Trifolium fragiferum* type: Taxa with glabrous trifoliate leaves. Leaflets lanceolate, clavate, ovate or triradiate shapes with dentate or serrate margins and rounded apices. Stomata paracytic or diacytic, occasionally onomocytic, surrounded by isobilateral or elongated, straight or undulated walls epidermal cells. Cuticle striate or granulate, rarely smooth with or without wax secretions. This group includes the following *trifolium* species: *T. fragiferum, T. resupinatum, T. tomentosum, T. procumbens, T. nervulosum* and *T. repens.*

Table 2: Macromorphological characters on leaflets of the studied taxa

Taxa/Char.	Туре	Length	Width	L./W.	Shape	Margin	Apex
Ononis natrix	trifoliate	5-7(6.2)	2-2.5	2.0	Lanc.	Dentate	Emarg.
O. vaginalis	trifoliate	3-4(3.8)	1.5-2	2.0	Obov.	Dentate	Acute
O. reclinata	trifoliate	5-6(5.2)	2-3	1.5-2	Trilete	Smooth	Apiculate
O. sicula	Trifoliate	12-15(13.8)	4-7	2.3-3	Lanc.	Dentate	Acute
O. pubescens	simple	10-12(11.3)	2-3	4.0	Lanc.	Smooth	Acute
O. serrata	Trifoliate	6-8(7.2)	2-3	2-3	Ovate	Dentate	Emarg.
Trigonella arabica	Trifoliate	7-10(9.0)	8-9	0.9-1.2	Cordate	Sl.dent	Notched
T. anguina	Trifoliate	13-15 (13.5)	7-9	1.6-1.8	Brov.	Dentate	Emarg.
T. occulta	Trifoliate	8-10(9.2)	3-4	2.5-2.7	Lanc.	Dentate	obtuse
T. monspeliaca	trifoliate	4-5(4.8)	3-4	1.25	Brobov.	Dentate	Rounded
T. stellata	trifoliate	5-7(5.8)	5-7	1.0	Rotund	Dentate	Rounded
T. laciniata	trifoliate	7-10(8.2)	2-3	3-3.5	Lanc.	Dentate	Apiculate
T. maritima	Trifoliate	8-10(9.2)	6-8	1.3	Brobov.	Dentate	Rounded
T. hamosa	Trifoliate	8-10(8.8)	4-5	2.0	Trilete	Dentate	Rounded
T. media	trifoliate	5-6(5.5)	4-5	1.2	Trilete	Dentate	Rounded
Melilotus alba	trifoliate	13-15(13.8)	3-4	3.7-4.3	Lanc.	Dentate	Emarg.
M. sulcata	trifoliate	10-14(12.8)	4-6	2.3-2.5	Narov.	Dentate	Emarg.
M. siculus	Trifoliate	15-17(16.2)	7-10	1.7-2.2	Obov.	Dentate	Emarg.
M. indica	Trifoliate	10-15(13.5)	4-6	2.5	Ovate	Dentate	Emarg.
Trifolium fragiferum	trifoliate	6-9(7.8)	3-5	1.8-2	Ovate	Sl.serr.	Rounded
T. resupinatum	trifoliate	12-15(13.2)	5-7	2-2.4	Clav.	Sl.dent.	Rounded
T. tomentosum	trifoliate	4-6(5.2)	2-3	2.0	Clav.	Dentate	Rounded
T. procumbens	trifoliate	10-13(12.2)	3-4	3.3	Lanc.	Dentate	Rounded
T. philistaeum	trifoliate	10-12(11.2)	3-4	3-3.5	Lanc.	smooth	Acute
T. nervulosum	trifoliate	9-11(10.2)	2-2.5	2.5-4	Trirad.	Dentate	Rounded
T. repens	Trifoliate	25-32(28.5)	15-19	1.5-1.8	Ovate	Serrate	Rounded
T. alexandrenum	trifoliate	30-33(32.8)	7-9	3.5-4	Lanc.	Sl.serr.	Acute
T. scabrum	trifoliate	6-9(7.4)	3-5	1.8-2	Obov.	Sl.serr.	Acute
T. stellatum	trifoliate	5-7(6.2)	3-4	1.7-2	Cord.	Sl.dent.	Emarg.
T. purpureum	trifoliate	27-32(30.5)	3-5	6-9	Lanc.	smooth	Acute
T. desvauxii	trifoliate	24-29(26.2)	3-4	7-8	Lanc.	smooth	Acute

The measurements by mm. of at least ten middle leaflets, minimum-maximum (mean) Abbreviations used: Lanc.=lanceolate Obov.=obovate Brov.=broadly ovate Narov.=narrowly ovate Clav.=clavate Trirad.=triradiatus; Sl.dent.=slightly dentate Sl.serr.=slightly serrate; Emarg.=emarginated

Table 3: Micromorphological characters on leaflets of the studied taxa

	Trichomes			Stomata	Epidermal cells			
Charac. Taxa	1	2	3	4	5	6	7	8
Ononis natrix	+++	Mg	Sm	diacytic	W	Iso.	Sm.	-
O. vaginalis	+++	Mg	Sm	diacytic	St.	Iso.	Sm.	+
O. reclinata	+++	Mg	Sm	paracytic	W	Iso.	Sm.	+
O. sicula	+	Mg	Sm	diacytic	W	El.p.	Sm.	+
O. pubescens	+	Mg	Sm	Brachypara.	St	Iso.	Sm.	+
O. serrata	++	Mg	Sm	paracytic	St	El.p.	Sm	+
Trigonella arabica				paracytic	St	El.	Str.	-
T. anguina				anomocytic	St	El.	Str.	-
T. occulta				paracytic	St	El.	Str.	-
T. monspeliaca				paracytic	St	El.	Sm.	+
T. stellata				paracytic	St.	El.	Sm.	+
T. laciniata				paracytic	St.	El.	Str.	-
T. maritima				diacytic	St.	Iso.	Gr.	-
T. hamosa				diacytic	Un.	Iso.	Gr.	-
T. media				paracytic	St.	El.	Gr.	-
Melilotus alba				Paracytic	Un.	El.	Str.	-
M. sulcata	+++	Mg.	Gra.	paracytic	St.	Iso.	Sm.	-
M. siculus				paracytic	Un.	El.	Str.	-
M. indica				anomocytic	St.	Iso.	Gr.	-
Trifolium fragiferum				diacytic	St.	El.	Str.	-
T. resupinatum				paracytic	St.	Iso.	Gr.	-
T. tomentosum				paracytic	Un.	Iso.	Str.	+
T. procumbens				anomocytic	Un.	Iso.	Sm.	-
T. philistaeum	++	Mp.	Gra.	paracytic	St.	El.	Sm.	-
T. nervulosum				diacytic	St.	El.	Str.	-
T. repens				paracytic	St.	El.	Str.	+
T. alexandrenum	+++	Mp.	Gra.	paracytic	St.	Iso.	Sm.	+
T. scabrum	+	Mp.	Gra.	paracytic	St.	El.	Str.	-
T. stellatum	++	М́р.	Str.	diacytic	St.	El.	Str.	-
T. purpureum	+++	М́р.	Sm.	diacytic	St.	Iso.	Sm.	-
T. desvauxii	++	Mp.	Sm.	paracytic	St.	El.	Gr.	-

Characters: 1=density 2=type 3=wall 5=anticlinal wall 6=shape 7=cuticle 8=wax secretions

- = absent + = present ++ = dense +++ = woolly Abbreviations used : Mg.=multicellular glandular, Ug.=unicellular glandular, Mp.=multicellular pointed; Sm.=smooth, Gra.=granulate, Str.=striate; Brachypara.=brachyparacytic; W=wavy, St.=straight, Un.=undulate; Iso.=isobilateral, El.p.=elongated pentagonal, El.=elongated; Gr.=granular



Fig. 1-31: Drawings of the leaflets of the 31 species, showing shape, margin and apex



Fig. 32-40: Drawings of some stomata, epidermal cells and hairs
32 and 33 illustration of the change in the five characters; 1 = leaflet shape, 2 = leaflet margin, 3 = leaflet apex, 4 = trichome presency, 5 = hair type, throughout the studied taxa, 34 = Ononis reclinata, 35 = Ononis pubescens, 36 = Trigonella monspeliaca, 37 = Trigonella maritime, 38 = Melilotus sulcata, 39 = Trifolium procumbens, 40 = Trifolium stellatum



Scanning electron micrographs for the leaflet surfaces of the studied taxa showing trichomes, epidermal cells, cuticle and wax secretions: Fig. 41 to 74: rix, laciniata. 4 4

1 = Ononis vaginalis,	42 = Ononis reclinata,	43 = Ononis sicula,	44 = Ononis natr
5=Ononis pubescens,	46 = Ononis serrata,	47 = Trigonella arabica,	48 = Trigonella la



Fig. 41 to 74: Scanning electron micrographs for the leaflet surfaces of the studied taxa showing trichomes, epidermal cells, cuticle and wax secretions:

49 =Trigonella occulta,50 =Trigonella anguina,53 =Trigonella stellata,54 =Trigonella media,

51 =Trigonella glabra, 52 =Trigonella maritime, 55 =Trigonella monspeliaca, 56 =Melilotus alba,



Fig. 41 to 74: Scanning electron micrographs for the leaflet surfaces of the studied taxa showing trichomes, epidermal cells, cuticle and wax secretions: 59 = Melilotus sulcata,

- 57 = Melilotus siculus,
 60 = Trifolium alexandrenum,
 63 = Trifolium philistaeum,

- 58 = Melilotus indica, 51 = Trifolium purpureum, 64 = Trifolium scabrum
- 62 = Trifolium fragiferum,





Fig. 41 to 74: Scanning electron micrographs for the leaflet surfaces of the studied taxa showing trichomes, epidermal cells, cuticle and wax secretions:

- 65 = Trifolium philistaeum,
- 68 = Trifolium procumbens,
- 71 = Trifolium tomentosum,
- 66 = Trifolium tomentosum, 69 = Trifoliun resupinatum, 72 = Trifolium stellatum

67 = Trifolium repens, 70 = Trifolium nervulosum,



Fig. 41 to 74: Scanning electron micrographs for the leaflet surfaces of the studied taxa showing trichomes, epidermal cells, cuticle and wax secretions: 73=Trifolium stellatum, 74= Trifolium desvauxii

Group 6: *Trifolium alexandrenum* type: Taxa with hairy, trifoliate leaves. Leaflets lanceolate, obovate or cordate enriched with multicellular pointed hairs with either smooth, granulated or even striated walls. Leaflets margins smooth, slightly serrate or slightly dentate and the apices are either acute or emarginated. Stomata are either paracytic or diacytic surrounded by isobilateral or elongated straight walls epidermal cells. Cuticle smooth, striate or granulate with or without wax secretions. This group includes the following species: *Trifolium philistaeum, T. alexandrenum, T. scabrum, T. purpureum, T. desvauxii.*

DISCUSSION

From this investigation, we can observe that leaf characters are, taxonomically, important in delimiting some taxa. Ononis pubesens has unique leaf type in the group, simple leaf, with unique stomatal type, brachyparacytic, which make it very easy to be distinguished. Leaflets shape, margin and apex are variable within the studied taxa, but the presence of hairs and their types can be of taxonomic use in delimiting some genera such as Trigonella species, which have glabrous leaves, as well as species of Trifolium, which have either glabrous or multicellular pointed hairs. Species of both Ononis and Melilotus have either glabrous or glandular hairs. Hair walls can be as well another good taxonomic character in identifying certain species such as the granular walls of the multicellular glandular ones in Melilotus sulcata and of the multicellular pointed ones in Trifolium philistaeum, T. alexandrenum and T. scabrum. Striate hair wall present in Trifolium stellatum only. Leaves are amphistomatic in which the stomata scattered randomly over the two surfaces of either the paracytic or diacytic types. Anomocytic stomata characterizes few species such as Trigonella anguina, Melilotus indica and Trifolium

procumbens. Brachyparacytic, a term first proposed by Dilcher^[9] and recorded by Subra *et al.*^[11] in some species of Tephrosia, is present in Ononis pubescens only. Taia^[8] has recorded this type in few species of Vicia and Psoralia plicata. Shape of epidermal cells as well as their anticlinal walls have no weight in the taxonomy of the group, this has been decided by Taia^[8,12] in the Papillionaceous leaves. Cuticle type which identified by Stace^[1,13] as the outer surface of the epidermal cells contains cutin in addition to cellulose and other cell wall polysaccharides, can help in the identification of some species (Table 3) beside the other leaf characters. Wax secretion, which considered by Barthlott^[14] as tertiary sculpturing of the leaf surface, plays a limited part in the taxonomy of the group as most of the species has either irregular wax particles or they have nowax at all. From the data obtained we can support the taxonomic opinions of Small^[15], Small *et al.*^[16,17] and Lersten^[18] in separating the genus Ononis in a monogeneric tribe and the genus Trifolium in another monogeneric subtribe; trifoliinae; and the rest of the genera in subtribe trigonellinae. As those two genera have their own characteristic features which enable them to be in a group of their own^[19] Genus Trigonella is the only genus which has glabrous leaves in all the studied taxa can be easily recognized.

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