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ANATOMICAL STUDY FOR THE ADAXIAL EPIDERMIS FROM LEAFLET OF SOME SPECIES OF GENUS *TRIFOLIUM* L. IN IRAQ.

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Absract

The aim of the study was to investigate the detail adaxial epidermal characteristics of the leaflets of 12 species of the genus *Trifolium* L. in Iraq. The qualitative parameters such as straight or sinuous of anticlinal cell walls ,types of stomata apparatus were studied by using light microscope (LM). Also quantitative traits such as length, width of epidermal cells was described in details. The results show high covariance in leaflets attributes which can be of great taxonomic significance between species in this study.

Key words : *Trifolium*, Adaxial Epidermis, Stomatal Types.

Introduction

Trifolium is one the largest genera of the paplionoideae of Leguminosae, comprising more than 250 species belonging to the tribe Trifolieae [Abberton (2007)]. In Leguminosae family anatomical similarity and differences between many genera of this family have pointed out by Metcalfe and Chalk (1950).

Taia (2004) studied the micromorphological characters including trichomes ,stomata and epidermal cells of leaflets in thirty one species distributed over four genera belonging to tribe Trifolieae . In a similar study Yagueddu *et al.* (2009) identified six species from papilionaceae by leaflets epidermal characteristics. Hassoun (2011) conducted a comparative anatomical study for leaf epidermis and cross section of stem, petiole and blade of six genera with in such family papilionoideae. Toma (1969) gave data about the type, number and size of stomata of *Trifolium* species from Romania. Akin and Robinson (1982) analyzed the anatomy of leaf, petiole and stem of *T. vesiculosum* and *T. incarnatum*. Krstic *et al.* (2008) carried out a microscopic analysis of leaflets, petiole and stem of twenty six populations out of seventeen *Trifolium* species. The study of Zoric *et al.* (2009) showed that epidermal characters, such as the shape of epidermal cells and their cell walls, wax deposition ,papillae, stomata and trichomes are useful in taxonomy. In recent study on twenty *Trifolium* species by the same researcher [Zoric *et al.* (2012)] indicated to the taxonomic significant to comparative analysis of qualitative anatomical characters of their vegetative organs . Leaf epidermal cell provided an identification key for determination of more species [Sivaranjani *et al.* (2013)]. Our work aims to elucidate the taxonomic potential by using leaflets epidermal features like stomata, epidermal cells and trichomes to delimit the species in the genus *Trifolium*.

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Materials and Methods

Fresh materials from different parts of Iraq were used in this study in addition to Iraqi herbarium samples .Adaxial surface from the leaflet epidermis were prepared according to Mubeen *et al.* (2014), with a fine needle and mounted on glass slides in glycerin and safranin (1 :1), covered with slips and edges of cover- slip sealed with nail varnish to prevent dehydration . Both qualitative and quantitative micromorphlogical foliar characteristics were observed using light microscope. Next they were photographed using a digital microscope camera.

Results and Discussion

Epidermal cells

In all examined species, leaflet epidermis cells are varied and some species are characterized by specific dimensions while others were overlapping. For the

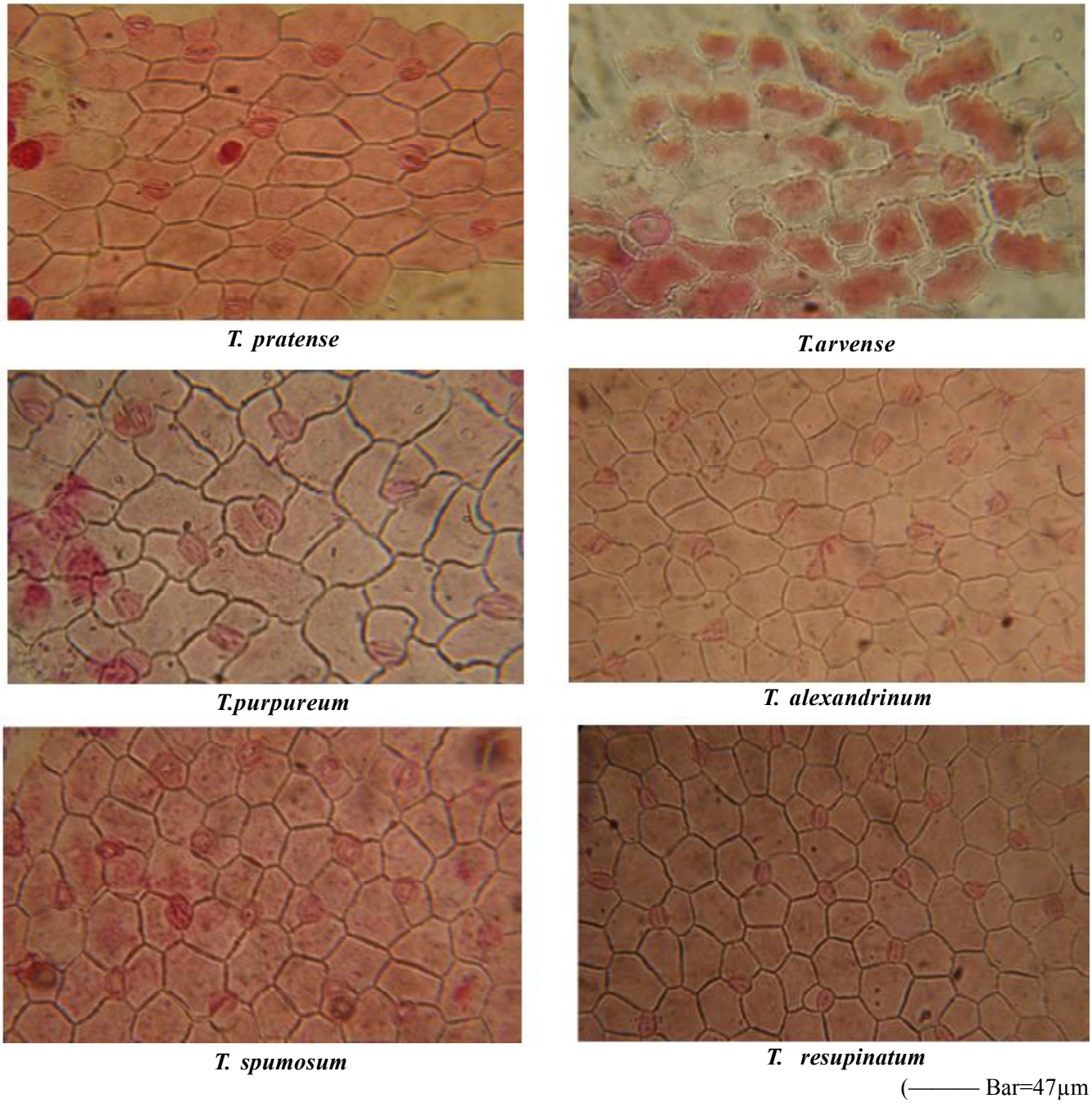
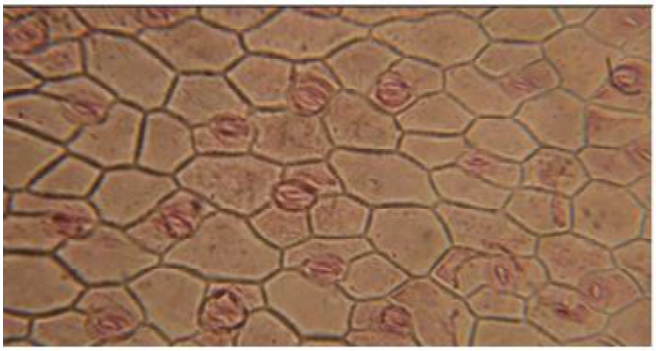
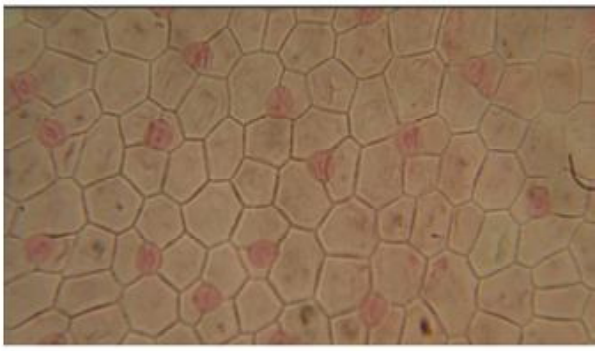


Plate 1: Upper epidermis of leaflet in *Trifolium* species

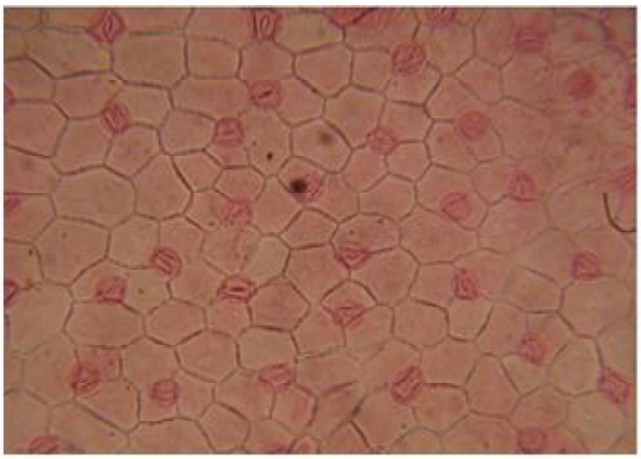
dimensions of upper (adaxial) epidermis, plates 1-4 and table 1, *T. campestre* recorded largest rate in length recorded 63.7 µm compared to *T. tomentosum* which recorded to 26.7µm and confined between 17.5- 42.5µm. The variation also included the width of these cells when *T. purpureum* showed the highest mean 43.2µm and the lowest mean 18.7µm for species *T. tomentosum*. Anticlinal cell walls were diagnosed in adaixal of epidermis in plates 1-4. Most species showed straight or straight-curved pattern with the exception of the two species *T. arvense* and *T. grandiflorum* which were undulate. The species *T. campestre* are semi undulate, while the species *T. pretense, T. arvense, T. campestre* and *T. grandiflorum* are appeared undulate, while the rest most species owned the straight curved and undulate pattern. Two other species possessed semiundulate pattern included *T. campestre, T. subterraneum* in adaxial epidermis. The pattern of epidermal cells wall for upper epidermis of leaflets which demonstrated in our study were similar to other study in *T. alexandrinum*



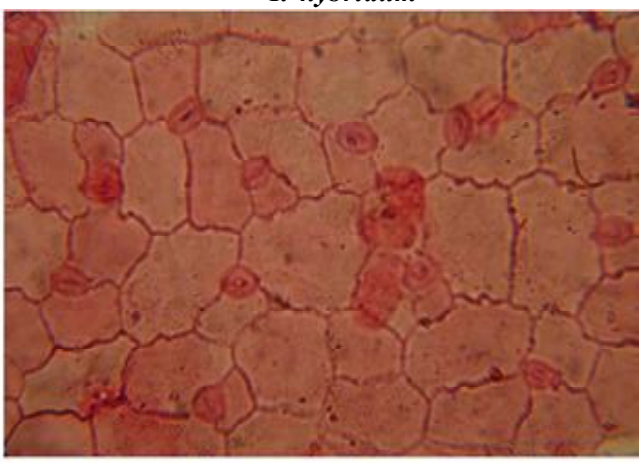
T. fragiferum



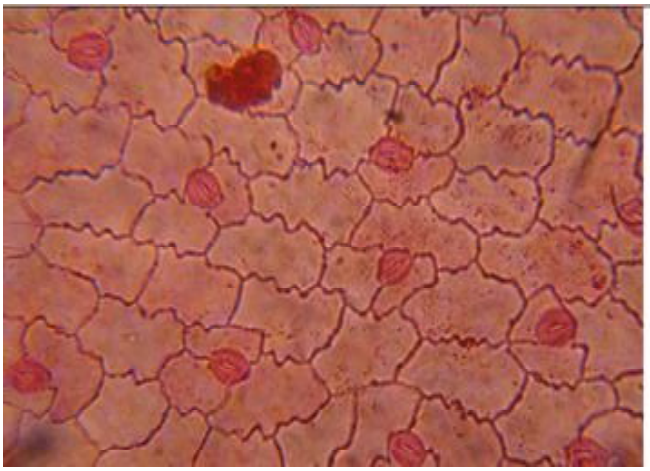
T. hybridum



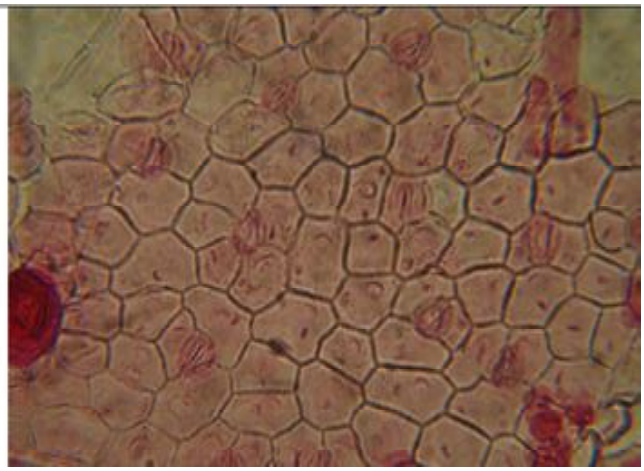
T. repens



T. campestre



T. grandiflorum



T. ochroleucon

(——— Bar=47µm

Plate 2: Upper epidermis of leaflet in *Trifolium* species

[Hassoun (2011)] and in *T. pratense, T. repens* [Yagueddu *et al.*(2009)].

Stomata

All species of *Trifolium* were amphistomatic and had four types of stomata which are illustrated in, plate 3. On the basis of stomata type in adaxial epidermis, plates 1-4 and table 1, species are divided into four groups as follow: First group included two species *T. resupinatum* and *T.frogiferum* (plate 3) was characterized by the presence of four types of stomata anomocytic, anisocytic, paracytic and hemiparacytic. Second group a species that contained three types of stomata and taken three forms, the first when contained anomocytic, anisocytic and paracytic included *T.alexandrinum, T. tomentosum, T. subterraneum and T.repens*, the second possessed the type anomocytic, anisocytic and hemiparacytic that are

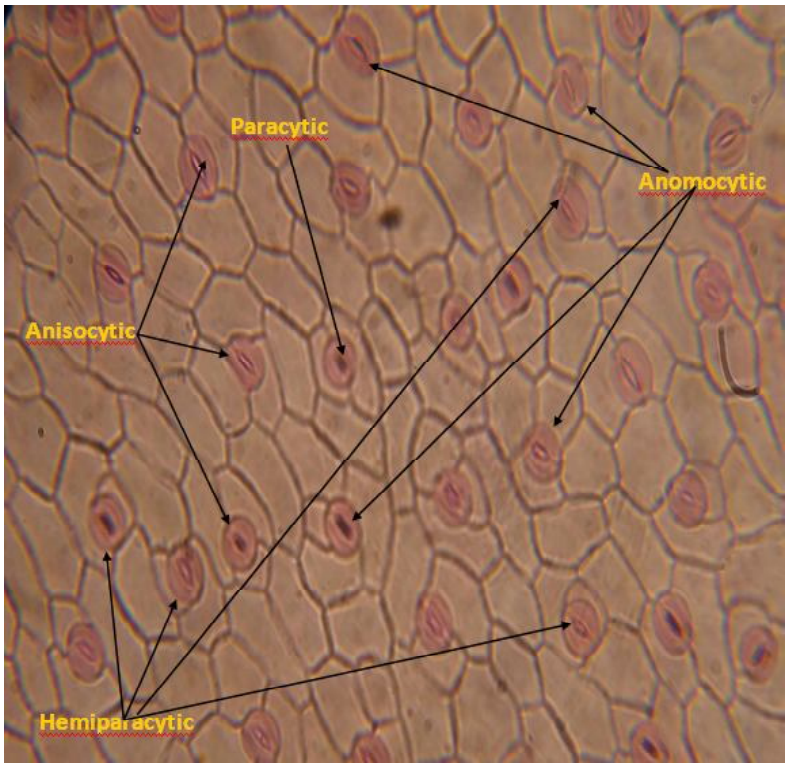


Plate 3: Four stomata types in leaflet lower epidermis of *T. resupinatum.*

Table 1: Epidermal cells and stomata in leaflet of *Trifolium* species

	Species	Upper epidermis (adaxial epidermis)					Stomata type
		Epidermal cells			Stomata		
		Length	Width	cell-wall pattern	Length	Width	
1	*T. pretense*	41.5 (25-50)	23.7 (12.5-30)	Straight	20 (17.5-22.5)	18.5 (17.5-22.5)	Anomocytic, Anisocytic
2	*T. arvense*	46.2 (22.5-62.5)	33 (12.5-50)	Undulate	21 (20-22.5)	17 (15-17.5)	Anomocytic, Anisocytic
3	*T. purpureum*	52.5 (30-75)	43.2 (20-62.5)	Straight-curved	24.7 (22.5-27.5)	17.5 (15-20)	Anisocytic, Paracytic, Hemiparacytic
4	*T. alexandrinum*	37.7 (27.5-50)	30 (25-37.5)	Straight-curved	15.2 (15-16.2)	15 (12.5-17.5)	Anomocytic, Anisocytic, Paracytic,
5	*T. subterraneum*	33.7 (25-42.5)	25.5 (17.5-35)	Semiundulate	19.7 (17.5-22.5)	13.5 (10-17.5)	Anomocytic, Anisocytic, Paracytic
6	*T. resupinatum*	34.5 (20-50)	29 (15-40)	Straight	17 (15-18.7)	12.5 (10-15)	Anomocytic, Anisocytic, Paracytic Hemiparacytic
7	*T. fragiferum*	33.5 (22.5-42.5)	31.7 (20-42.5)	Straight	21 (18.7-25)	17.5 (15-20)	Anomocytic, Anisocytic, Paracytic Hemiparacytic
8	*T. scabrum*	41.2 (25-52.5)	28.2 (12.5-45)	Straight-curved	17.5 (15-20)	16.5 (15-17.5)	Anomocytic
9	*T. repens*	37.5 (25-50)	28 (15-37.5)	Straight	19.5 (17.5-22.5)	16 (12.5-20)	Anomocytic, Anisocytic, Paracytic
10	*T. campestre*	63.7 (32.5-87.5)	39.2 (17.5-55)	Semiundulate	20.2 (20-22.5)	16 (12.5-20)	Anomocytic, Anisocytic, Hemiparacytic
11	*T. grandiflorum*	49.2 (25-72.5)	37.5 (17.5-50)	Undulate	21.2 (20-22.5)	16 (12.5-17.5)	Anomocytic, Anisocytic
12	*T. tomentosum*	26.7 (17.5-42.5)	18.7 (15-25)	Straight	14.7 (12.5-16.2)	14 (12.5-17.5)	Anomocytic, Anisocytic, Paracytic

Mean values, and in brackets minimum and maximum values, measured in µm.

found in species *T. campestre*, the third represented a single unique species T. purpureum to be type in the form of anisocytic, paracytic and hemiparacytic. Third group contained one type of stomata anomocytic and represented by species T. *subterraneum*. Last group is characterized by the existence of two types of stomata ,both anisocytic and anomocytic in a unique species *T. pratens, T.arvense and T. grandiflorum*.The dimensions

of stomata showed variation between species to give overlapping values in the upper epidermis, plates 1-4 and table 1, to record the highest rate value in each of length 24.7µm and value ranged 22.5 -27.5 µm in *T. purpureum* and width 18.5 µm in *T. pratense* to reach 17.5 -22.5 µm, while the rate of lowest values in length 14.7 µm in species *T.tomentosum* and width 12.5µm in species *T. resupinatum*. In our study recorded large stomata in lower epidermis compared to upper in terms of length and width, this agree with Zoric *et al.* (2009).

Conclusion

The species of the genus *Trifolium* could be separated on the basis of epidermal characters, such as anticlinal cell walls undulating, cells dimensions, stomatal type and dimensions.

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