RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
55 (4) 1284-1293, 2023
http://doi.org/10.54910/sabrao2023.55.4.21
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

MORPHOLOGICAL AND ANATOMICAL CHARACTERIZATION OF THE SPECIES *TRIFOLIUM INCARNATUM* L. CULTIVATED IN IRAQ

R.H. ALOUSH

Department of Biology, College of Science, University of Tikrit, Tikrit, Iraq
*Corresponding author's emails: rana_aloush_plant@tu.edu.iq, ranaha85@gmail.com

SUMMARY

Trifolium incarnatum L. is a new species grown in America, Europe, and Iraq. The novel study described the plant parts based on morphological characteristics like root, stem, leaflet, and flower. The annual plant is erect, 20–70 cm tall, unbranched from the base, with stipules membranous oblong-lanceolate, leaflet cuneate, obovate, and broadly retuse, otherwise rounded or truncate, and the leaflet-shaped ovate-cordate, with hairy margins, leaflet petiolate, and pedunculate (5 cm). Its inflorescences are mostly terminal oblong, 1.8 cm in diameter and 4 to 7.5 cm in length, and flower peduncles. The pollen grain's analysis through the scanning electron microscope (SEM) revealed monad, symmetrical, isopolar, zono-colporate, and tri-porate. The leaflet anatomy displayed many features and recorded differences between upper (adaxial) and lower (abaxial) epidermis in shape, size, and stomatal complex. The species was amphistomatic and had many types of stomatal complex, i.e., Anomocytic, Anisocytic, Paracytic, and Actinocytic. The number of stomata within the microscopic field was 56–65 and 32–38 on the upper and lower surfaces, respectively, with occurring cross-sections in the leaflet and stem. The results showed the frond in a cross-section unifacial, the palisade tissue at two-three layers with a thickness of 82–100 µm, spongy tissue (62–70 µm), and the vascular bundle almost present in the central vein. The calcium oxalate crystals, especially prismatic crystals, lined along the veins, and the stem cross-section was a sub-triangle-circle–ovate, with three ovate closed vascular bundle sheaths distributed into three directions, with two facing each other.

Keywords: *Trifolium incarnatum* L., anatomy, morphological traits, root, stem, SEM pollen grains

Key findings: The unique study identified the morphological and anatomical traits and micromorphological characteristics of the pollen grains for *Trifolium incarnatus* L., cultivated as a new species in Iraq.

Communicating Editor: Prof. Zahoor Ahmed Soomro

Manuscript received: April 18, 2023; Accepted: June 8, 2023.

Citation: Aloush RH (2023). Morphological and anatomical characterization of the species *Trifolium incarnatum* l. cultivated in Iraq. *SABRAO J. Breed. Genet.* 55(4): 1284-1293. http://doi.org/10.54910/sabrao2023.55.4.21.

INTRODUCTION

Fabaceae is a large and economically valuable family, also known as the pea, faba, legume, and bean family, and considered the third-largest flowering plant, containing 730 genera and over 19,400 species after the Orchidaceae and Asteraceae (Aloush, 2014). *Trifolium* is one of the significant genera belonging to the Fabaceae family.

In the climate of the Northern and Southern Hemispheres, these species have broad edaphic tolerance and grow on soils ranging from markedly acid to highly calcareous, having a symbiotic relationship with nitrogen-fixing bacteria, with the species widely cultivated and used as forage and analgesics, antiseptics, expectorants, and rheumatic pain relief (Sabuda *et al.,* 2008). Evaluating the altitude, the effect of habitat change, and the influence of all environmental factors on plants can indicate successful demonstration of some plant taxa that have distribution abroad, such as, the *Trifolium* genus (Ball and Lacefield*,* 2000; Rizani *et al.,* 2021).

Trifolium incarnatum L. (Italian clover) commonly served as a cover crop in winter and summer and as green manure, forage, silage, and hay. Reports stated it is the most widely planted annual forage legume in the United States since 1940 (Bal and Lacefield, 2001), showing good growth under cool temperatures and the ability to produce a substantial seed crop (Clark, 2007), and a wide variety of native bees (UC SAREP, 2012). *Trifolium incarnatum* L., an essential component of cool-season forage, is a major seed crop in the United States (Ball and Lacefield, 2000), distributed as a native plant in Europe, Southwestern Asia, and introduced to the United States as a cultivated crop in the 1800s (Vincent and Isely, 2012).

Taxonomy, chorology, ecology, and biogeography enable a more suitable positioning of each biodiversity unit in the scientific system (Azka *et al*., 2021; Herman *et al*., 2023). In Turkey, about 103 species are grown as wild plants (Davis, 1970), and in Iraq, 33 species are mentioned in the flora of Iraq as wild plants (Townsend and Guest, 1974). *Trifolium* is a name in Pliny of a plant with 3-foliate leaves, tri-three, and folium leaf, and another label is like Nafal or Nifil (also NEFEL), and Winja (Kurdish name) or YUNJA, a term more usually applied to *Medicago sativa* and other medics. English names often refer to flowers, i.e., red, white, and yellow trefoils (Townsend and Guest, 1974). Classic taxonomy depends on morphology and anatomy characteristics, with 33 species recorded by Al-Rawi (1964) and 38 species counted by Ridda and Daood (1982). A taxonomical study has distinguished 11 species in the Erbil district (Darwesh, 2017).

Welham *et al.* (2002) studied the morphological plasticity of white clover (*Trifolium repens* L.) in response to spatial and temporal resource heterogeneity. Morphological traits, micromorphology, and chemical properties were studied in five clover species (*Trifolium* spp.) for their characterization (Ates, 2011) (Pedro *et al*., 2023). Recently, a pollen grain study by SEM, which deal with pollen grain characteristic, also helped classify and determine the species.

Plant anatomy is a foremost science for all botanists, and anatomical features may play a significant role in plants' classification (Metcalfe and Chalk, 1950). Fabaceae family comprising the genus *Trifolium* gained its name from Metcalfe and Chalk (1957). In the Leguminosae family, Toma (1969) has pointed out the anatomical similarity and differences among various genera of this family. Taia (2004) studied micromorphology characteristics, such as trichomes, epidermal, and stomata of leaflets for many species belonging to *Trifolium* genera. Akin and Robinson (1982) described the anatomy of the leaf petiole and stem of *T. vesiculosum* Savi. The study of Zoric *et al.* (2011) showed epidermal characteristics like papillae, stomata, trichomes, and cell walls, indicating its taxonomic significance to comparative analysis of qualitative anatomical features of their vegetative organs. Motar (2018) and AL-Dabbagh and Saeed (2020) determined the petioles' anatomical characteristics for some taxa of the genus *Trifolium* features. The attributes of the leaf epidermis play an indicative role in plant taxonomy, and various

studies dealing with the morphology and anatomy of epidermis, stomata, and indumenta (Hay *et al*., 1986; Navasero and Ramaswamy, 1991; Ditsch *et al.,* 1995).

The presented study aimed to identify the morphological and anatomical features of the newly cultivated species *Trifolium incarnatum*, as very little research engaged on micromorphological and anatomical characteristics of *T. incarnatum*, with this study may be the first in Iraq.

MATERIALS AND METHODS

The distinct study transpired at the College of Science, University of Tikrit, Tikrit, Iraq. Individual plants of *Trifolium incarnatum* L. gained collection from a field at Tikrit University, Iraq. The field sampling started during the flowering period (March–April 2022), with morphological characteristics (roots, stem, leaflet, and flowers) studied in fresh samples. For anatomical study, submerging some cut parts proceeded in FAA (formalin, acetic acid, and alcohol), peeling the epidermis (adaxial and abaxial) with a needle, and preparing the stem epidermis, cross-section of stem, and leaflet according to Mubeen *et al*. (2014).

The samples observed under a light microscope (LM) used a magnification of 40×, studying the pollen grains and determining the vital measurements of equatorial view, polar view maximum width, and length of the apertures, with all the samples measured using a 40× ocular micrometer, then prepared for viewing (SEM). Measurements ran on acetalized grains SEM-imaged. The acetolysis method comprised the prepared pollen grains, then suspended in 90% ethanol (Aldobaissi *et al.,* 2016), with the grains mounted on stubs and sputter coated with gold cover (Erdtman, 1969).

RESULTS

Morphology study

Trifolium incarnatum L. species was erect and ascending annually at 20–70 cm tall. The root is a tap, and its length ranges from 10 to 25 cm containing the root nodules. The plant is unbranched from base, stipules membranous, oblong-lanceolate, striate-nerved herbaceous, broadly acute 15 mm × 20 mm, and hairy. The leaves compound, leaflet cuneate-obovate broadly retuse, otherwise rounded or truncate, and generally leaflet-shaped ovate-cordate 27 mm × 10 mm to 30 mm × 15 mm, with cordate apex and hairy margins leaflet petiolate, and pedunculate (5 cm). The head Inflorescences are mostly terminal oblong, 1.8 cm in diameter and 4–7.5 cm long, and peduncles (4–7.2 cm). The florets open in succession from the bottom to the top (Figure 1), the calyx is 8 mm, veined, corolla deep red, standard lingulate, 14 mm × 10 mm truncate at the apex, wings at 13 mm and keel 11 mm. The upper teeth of the calyx are equal to the tube or slightly longer, pod-ovate, and seed-ovate – rounded green to light brown (3 mm) (Figure 1).

HABIT: Cultivated, flowering, and fruiting April – May.

Distribution: Europe, America in the United States, and records as a new species (wild plant) in Iran (Tabad *et al*., 2013).

Morphology study for pollen grains by SEM

The pollen grains are monad, symmetrical, isopolar, porate shaped, zono-colporate, and tri-porate, with an equatorial axis at 46–48 µm. In central view: widely-elliptical, polar axis at 40–42 µm; polar sight: subplate, P/E ratio at 0.8 µm, Apoculpium is 15 µm, Mesoculpium at 22 µm, colpus length is 28, and colpus end shape: rounded and reticulate ornamentation (Figure 2, Table 1).

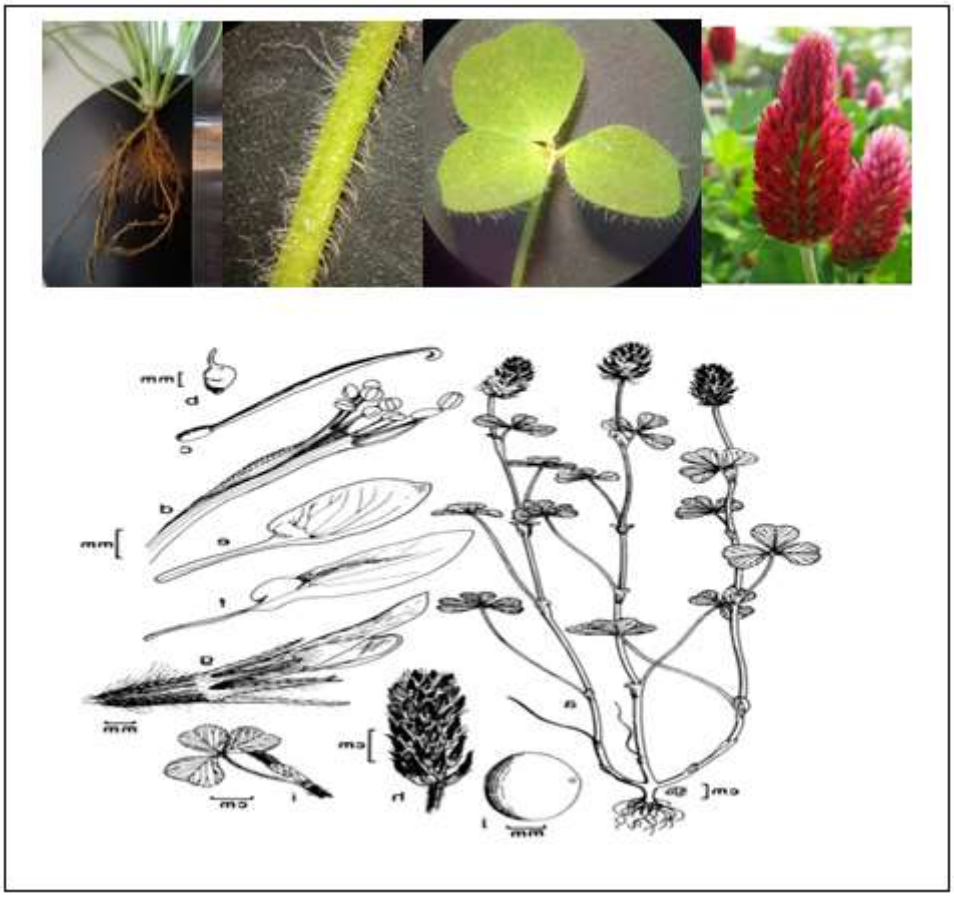

Figure 1. *Trifolium incarnatum* L. parts (roots, stem, leaflet, flowers, diagram of plant parts) - (a-habit, b-pod, c-pistil, d-staminal column and free stamen, e-keel, f-wing, g-flower, h-inflorescence, i-leaf, j-seed).

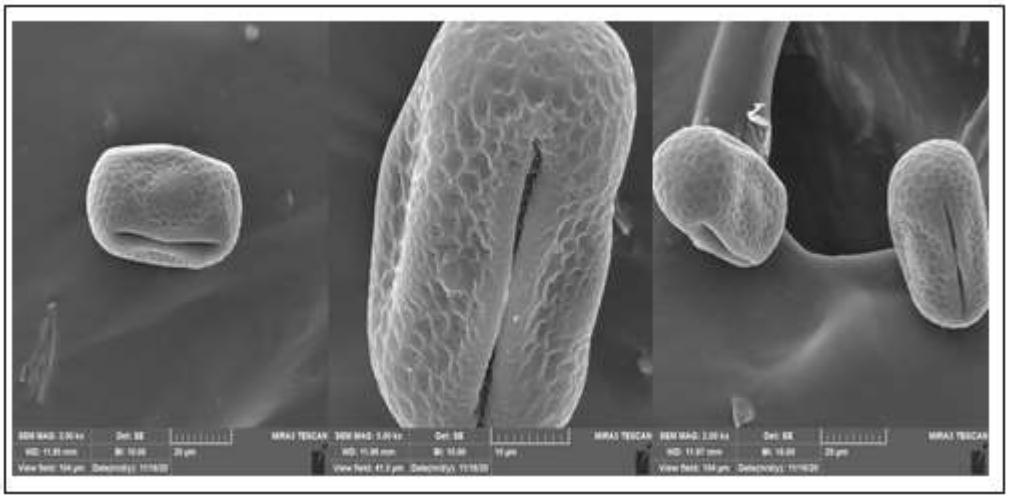

Figure 2. Pollen grains *Trifolium incarnatum* L. by SEM.

Table 1. Epidermis quantity and quantity characteristics of *Trifolium incarnatum* L.

Epidermis	Shape	Dimensions (µm)	Dimension of stomata(µm)	Number of stomata	Stomata Frequency
Upper	Curved	41.5x27.5	13.75x14	56-65	0.23
Lower	Zigzag	43.5x32.5	21.25x16	32-38	0.14

Anatomy study

Epidermis cells

The examined epidermis of the leaflet characteristics appears in Figure 3 - 1-3, showing the recorded differences between the upper (adaxial) and lower (abaxial) epidermis in shape, size, and stomatal complex. In the adaxial, the anticlinal cell wall was a straight-curved pattern, while in the abaxial, the cell wall was zigzag (Figure 3, Table 1). The dimensions of the upper epidermis cells were 41.5 µm × 27.5 µm, and for the lower, it was 43.5 µm × 32.5 µm. Papillae spread on both surfaces, and also trichome, which was two types, non-glandular (length between 70–90 µm), width (12.5–15 µm), wall thickness (2.5 µm), and the base (25–30 µm), with a rough trichome surface. The epidermis in the stem was oblong, the anticlinal cell was straight-oblique, and the spread of the trichome glandular trichome capitate divides into multiple cells (7-8) in the head (2-4), and the rest in the stalk, having a length of 40–50 µm, and the width was 15–20 µm.

Stomata

The species was amphistomatic and had many types of stomatal complex, i.e., anomocytic, anisocytic, paracytic, and actinocytic. The stomata exhibited a kidney shape, and the dimensions of the stomata in upper and lower surfaces were 13.75 µm × 14 µm and 21.25 µm × 16 µm, respectively (Table 1, Figure 3). The number of stomata within the microscopic field was 56–65 and 32–38 in the upper and lower surfaces, respectively. However, the stomata frequencies were 0.23 on adaxial and 0.14 on abaxial surfaces.

Cross section (leaflet anatomy)

The result showed that the cuticle thickness was 5 µm, an upper epidermis compound from one layer (15 µm), and palisade tissue was from two-three layers, with a thickness of 80–100 µm. The palisade cell was cylindrical, below the palisade tissue, which is spongy (62–70 µm), differing in sizes, thick collateral vascular bundles (182–200 µm), and arranged in one row surrounded by parenchymatous sheath (Figure 3). The sclerenchyma tissue was in adaxial and abaxial from leaflet, vascular bundle almost present in the central vein, and sclerenchyma developing in the phloem part of the bundle. Additionally, calcium oxalate crystals, especially prismatic crystals, lined along the veins, also containing sandy crystals (Figure 3 [3-12], Table 2).

Cross section of stem

In the stem, the shape of a microscopic cross-section was sub-triangle-ovate, with a thickness of 1000 µm, and the epidermis was one layer with a mean of 18–20 µm. The stomata and non-glandular trichomes emerged, with unicellular trichomes observed, two-three layers of lamellate collenchyma and angular collenchyma, and upper vascular bundle sheaths. The cortex of two-three layers had supplementation from parenchymatous tissue (chlorenchyma) with a thickness of 300 µm, three-ovate closed vascular bundle sheaths distributed into three directions, with two facing each other, a thick vascular bundle (180–200 µm), and the fibers surrounded the vascular bundles, parenchyma tissue inward to the pith, the tricked of it was 400 µm (Table 1, Figure 3 [14-16]).

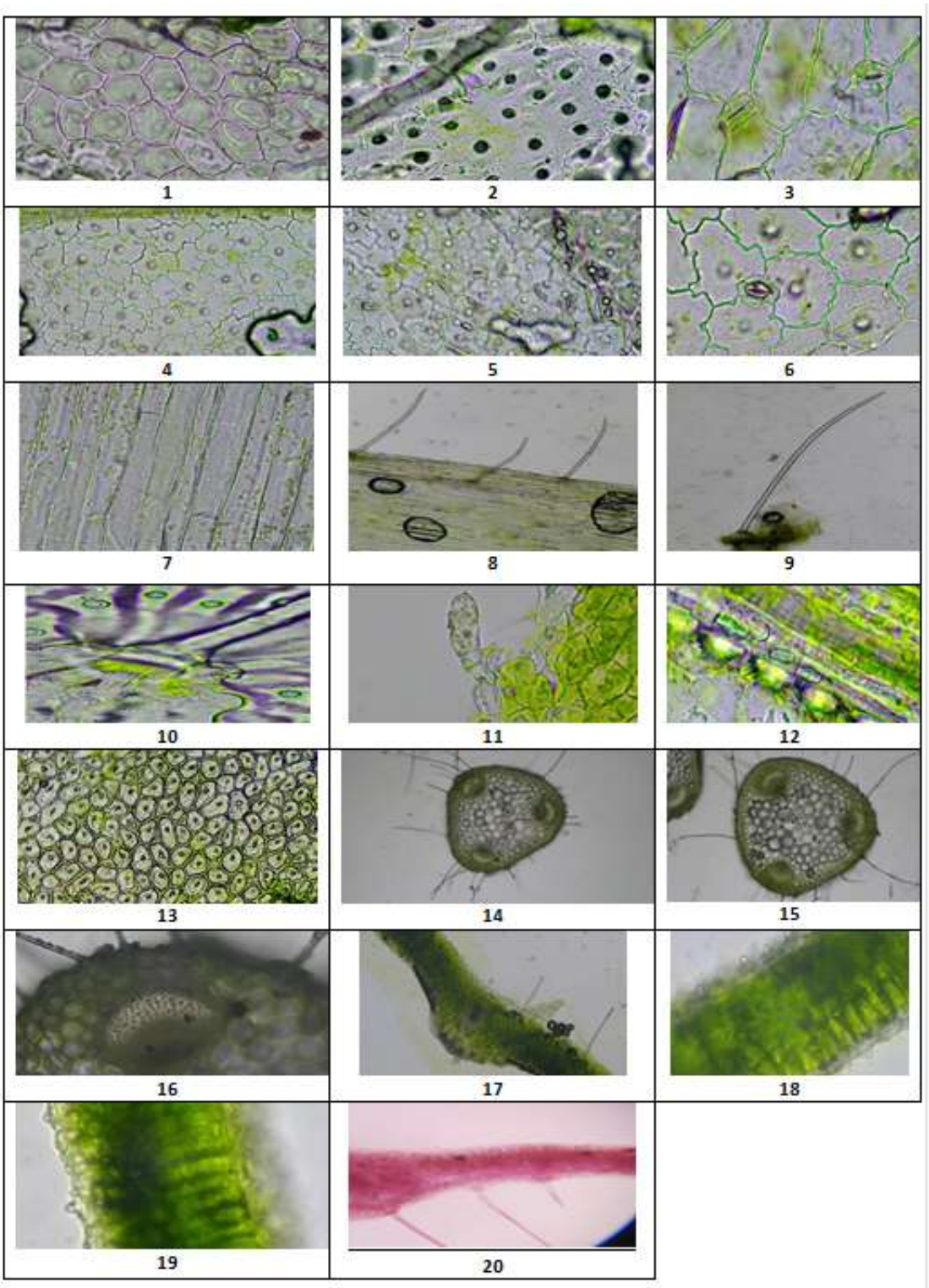

Figure 3. Anatomy characteristics for *Trifolium incarnatum* L. :(1-3) 10× ,40× upper epidermis (Adaxial), (4-6) 10×, 40× lower epidermis (Abaxial), (7) stem epidermis, (8-9) Non-glandular trichome, (10) base of trichome, (11) glandular trichome, (12) crystal, (13) papillae on the leaflet surface, (14-16) cross-section in stem 10×, 40×, (15) bundle sheath in stem 40×, (17-20) cross-section in leaflet 10×,40×.

Table 2. Anatomy and pollen grain quantity characteristics of *Trifolium incarnatum* L.

Stem (µm)		Leaflet (µm)		Pollen grains (µm)	
Epidermis thick	18-20	Cuticle thick	5	Equatorial axis	46-48
Cortex	300	Upper Epidermis	15	Polar axis	40-42
Pith	400	Palsied	80-100	P/E	0.8
Vascular bundles thick	180-200	Spongy tissue	62-70	Apocolpium	15
		Lower Epidermis	12	Mesocolpium	22
		Leaflet thick	182-200	Colpus Length	28

DISCUSSION

Trifolium incarnatum L. is a new species cultivated in Iraq, which grows as a wild and cultivated plant in Europe and the USA. It suggests that the red clover adaptation was successful with the change of ecological factors, and the plant has phenotypic plasticity, which helped to grow under different environmental conditions, allowing faster adjustment to a changing environment (Hoffman and Sgro, 2011). It may refer to the correlation among the genotypes, which express phenotypic differences under varied habitats and according to the mechanism for individual adaption to environmental heterogeneity (Franel and Steindor, 2013).

The phenotypic characteristics reckon vital in the classical taxonomy despite their instability and change according to the environment, especially the plant organs' dimensions. In this study, the root was a tap containing root nodules, and it refers to the plant's ability for nitrogen-fixing as natural in the Fabaceae species, already used in intercalated crops due to their ability to increase the supply of nitrogen to the ecosystem (Howald, 2000). The root growth inside the soil reduces the taxonomic importance. The presence of trichomes in the stem, compound leaf, and the shape of leaflets, which was ovate-cordate, helped determine the species flower's papilionoid, which compounded from the standard (14 mm × 10 mm), wings (13 mm), keel (11 mm), and the florets opening in succession from the bottom to the top.

Pollen analysis, as a part of palynology, deals with the morphological characteristics to determine the species; scanning electron microscopy examinations of the surface structures offer the possibility of taking high-resolution images of this structure, and this technology serves better in classifying the pollen taxa (Guggenheim, 1975). The results of SEM showed some pollen features of taxonomic significance, such as shape, which was porate, and the pollen grains were monad, isopolar, zono-colporate ornamentation reticulate, with these results in analogy with the past findings, which dealt with some species of *Trifolium* also identified as favorite plants of honey bees in Istanbul (Kocyigit *et al*., 2013).

Using microscopic methods and anatomical characteristics for taxonomic purposes has proven helpful for identifying plant taxa and herbarium specimens (Metcalfe and Chalk, 1957). Various attributes change with varying environments and, therefore, require more precision in selecting the qualities useful in taxonomy, where taxonomy decreases with the increasing plasticity of the plant. The micro-morphological trait is crucial for the taxonomic analysis, since the leaf base shape benefitted taxonomy of *Alnus* sp. (Sabeti, 1965).

The taxonomist studies the anatomy of plant organs, such as, the leaf, which reacts with environmental changes so that the internal structure develops in different conditions—the epidermis, stomata, indumentum, and influential characteristics for taxonomy. In this study, the epidermis consisted of one layer and both surfaces, non-glandular trichomes, and papillae spread. The leaf epidermis parameters proved beneficial in the taxonomy of *Trifolium* species (Zoric *et al*., 2008).

The anatomical characteristics of the leaf and stem are vital in determining species taxonomy (Scatena *et al*., 2005). The results

showed that the leaf was hypostomatic, with the stomata numbered 56–65 and 32–38 on the upper and lower surfaces. These results conform with the findings of Kofidis and Bosabalidis (2008) as they studied some species of *Trifolium*. The environmental factors (air, light, humidity, and CO_2 concentration) could influence the stomata density (Woodward and Kelly, 1995).

The latest study pointed out that the leaflet internal structure compound from the thick cuticle, uniseriate epidermis, two-three layers of palsied tissue, the leaflet unifacial, two-three and below the palsied were spongy tissue, collateral vascular bundles ovate shape, and in the middle of the midrib, sclerenchyma was in adaxial and abaxial from a leaflet. Akin and Robinson (1982) examined the anatomy of leaflet for *T. vesiculosum* and *T. incarnatum* and observed similar results. EL-Faiki and Kady (1990) examined the *T. alexandrium and T. polystachyum* and added the calcium oxalate crystals (prismatic crystals) lined along the veins. Aloush (2014) studied *Lathyrus* species in Iraq, while Aloush and AL-Khesraji (2016) studied some of the *Astragalus* species and referred that the species of Fabaceae contained prismatic crystal which lined along the veins.

The stem anatomy results pointed to the cross-section shape, which was sub-triangle-ovate and thick (1000 µm) and uniseriate epidermis with some trichomes. EL-Faiki and Kady (1990) studied some species of *Trifolium*, and their findings revealed that the epidermal cell wall was thick without lignified elements. The concerned study showed that two-three lamellate collenchyma and angular collenchyma appeared in upper vascular bundles sheaths, with the sclerenchyma tissue giving additional strength to a plant and supplying mechanical support and protection mechanism against biotic and abiotic factors. The legume fiber emerges in the stem, particularly in the xylem, sclerenchyma, and sclerenchymatous parenchyma (Zoric *et al*., 2008). Metcalfe and Chalk (1957) indicated the presence of sclerenchyma parenchyma in the center of a stem in some species of *Trifolium*, and this study corresponds with EL-Faiki and Kady (1990) for some species of *Trifolium*; however, their work did not include *T. incarnatum* L.

CONCLUSIONS

The Fabaceae family species are widely spread globally and can adapt to different circumstances. The species have various morphological characteristics, such as the specialized shape of stem and inflorescence, and the pollen grains were zono-colporate, a unique quality for the Fabaceae family. The anatomy of epidermis proved to determine the species, spreading the crystal along the veins, which could serve as a distinct trait of *Trifolium* species and of Fabaceae, in general.

ACKNOWLEDGMENTS

The author thanks the College of Sciences, University of Tikrit, Tikrit, Iraq, for providing the facilities required for the presented investigations, and professor Dr. Abdulkareem Eraibi Sabba Alkurtany, for providing the seeds from the University of Kentucky, USA.

REFERENCES

Akin DE, Robinson EL (1982). Structure of leaves and stems of arrowleaf and crimson clovers as related to in vitro digestibility. *Crop Sci*. 22(1): 24-29.

Al-Dabbagh ST, Saeed JF (2020). Morphological and anatomical variations of fruits in some taxa of Valerianaceae Batsch family. *Iraqi J. Agric. Sci*. 51 (Special Issue): 101-115.

Aldobaissi I, AL-Shammary KH, AL-Mosawi AHA (2016). Palynological study of some species of Asteracea and Papilionaceae grown in Erbil-Iraq. *Iraqi J. Sci*. Special vol. (A): 26-37.

Aloush RA, AL-Khesraji TO (2016). Comparative morphological study of leaflet and stems of some species of the genus *Astragalus* L. in relation to photosynthetic pathways. *Tik. J. Pure. Sci.* 21(4): 6-11.

Aloush RH (2014). Biosystematic study of species genera *Lathyrus* L. (Papilinaceae) in Northern and Middle Iraq. A Thesis, College of Pure Sciences, Tikrit University, Iraq (in Arabic).

Al-Rawi A (1964). Wild plants of Iraq with their distribution. Ministry of Agriculture and Irrigation, State Board for Agricultural and Water Resources Research, National Herbarium of Iraq, Baghdad, pp. 81-84.

Ates E (2011). Some chemical and morphological properties of five clover species (*Trifolium* sp.) at different aspect of pasture in Belovets Village (Razgard) Bulgarai. *Int. J. Plant Prod.* 5(3): 255-262.

Azka NA, Taryono, Wulandari RA (2021). Assessment of tea plant (*Camellia sinensis* L.) accessions for pollen sources in natural crossing by using microsatellites. *SABRAO J. Breed. Genet.* 53(4): 673-684. https://doi.org/ 10.54910/sabrao2021.53.4.10

Ball DM, Lacefield GD (2000). Crimson clover. Circular 00-1. Oregon Clover Commission, Salem. http://www.oregonclover.org/ downloads/files/crimso nclover.

Clark A (2007). Managing cover crops profitably. *3rd Ed*. National SARE Outreach Handbook Series, Book 9. National Agric. Laboratory.

Darwesh TD (2017). Plant biodiversity in the vicinity of Choman in terms of non-wood forest products, Erbil-Iraq. M.Sc. Thesis, Graduate School of Natural and Applied Science, Kahramanmaraş Sütçü İmam University, Turkey.

Davis PH (1970). Flora of Turkey and the East Aegean Islands, *Vol. 3. (3rd Ed.)*. Univ. Press, Edinburgh. pp: 384-448.

Ditsch F, Patha H, Barthott W (1995). Micromorphology of epicuticular waxes in *Fabales* L. and it is systematic significance. *Biol. PFI* 68: 297-310.

EL-Faiki MA, Kady KA (1990). Comparative morphological and anatomical studies on some *Phaseolea* and *Trifolieae*. Zagazig *J. Agric Res*. 17(2): 313-324.

Erdtman G (1969). Handbook of Palynology (Morphology-Taxonomy-Ecology). Hafner Publishing Co. New York, pp. 486.

Franel L, Steindor K (2013). Preliminary studies on the morphological variability among Arabidopsis halleri populations from contrasting habitats. *Ann. Bot. Fennici* 50: 142-148.

Guggenheim R, Rasterelektronenmikroskopische UND (1975). Morphometrische Untersuchungen an Tilia-Pollen: Ein Beitrag zur Artunterscheidung von Tilia platyphyllos SCOP. und Tilia cordata Mill. in der Palynologie. (Scanning Electron Microscopic and morphometric studies of Tilia pollen: A contribution to species differentiation of *Tilia platyphyllos* SCOP. and *Tilia cordata* Mill in palynology). *Flora* 164: 287-338.

Hay MJM, Dunlop J, Hopcropft HD (1986). Phosphate uptake and anatomy of unthickened and secondarily thickened adventitious roots of field-grown white clover (*Trifolium repens* L.). *New Phytol*. 103: 659-668.

Herman, Al-Khairi H, Wansyah AR, Asmania, Utami RI, Anzaelina DN, Oktaviano Z, Lestari W, Adiwirman, Roslim DI (2023). The ability of matK and trnL-trnL-trnF intergenic spacer to discern certain species accessions of the families Solanaceae and Fabaceae. *SABRAO J. Breed. Genet*. 55(1): 97-106. http://doi.org/10.54910/sabrao2023.55.1.9.

Hoffman AA, Sgro CM (2011). Climate change and evolutionary adaptation. *Nature* 470: 479-485.

Howald AM (2000). A Floral of Valentine Eastern Sierra Reserve, *part I*. *2nd Edition.* Museum of Systematics and Ecology. University of California, Santa Barbara, Publication 1.

Kocyigit M, Keskin M, Dastan T (2013). Pollen morphology of some *Trifolium* species which are favorite plants of honey bees in Istanbul. Istanbul Ecz. Fak. Derg. *J. Fac. Pharm. Istanbul* 43(2): 2013 s. 85-8.

Kofidis G, Bosabalidis AM (2008). Effects of altitude and season on glandular hairs and leaf structural traits of *Nepeta nuda* L. *Bot. Studies* 49(4): 363-372.

Metcalfe CR, Chalk L (1957). Anatomy of the Dicotyledons, Leaves, Stem, and Wood in Relation to Taxonomy with Notes on Economic Uses. *Vol. 1*. Oxford, Great Britain: Oxford Univ. Press. pp: 502.

Metcalfe CR, Chalk L (1950). Anatomy of the Dicotyledons. *Vol. I.* London, UK: Oxford Univ. Press. pp: 15-19.

Motar AA (2018). Anatomical study for the adaxial epidermis from leaflet of some species of genus *Trifolium* L. in Iraq. *Plant Arch*. 18(2): 2580-2584**.**

Mubeen M, Periyanayagam MK, Basha SS (2014). Anatomical investigation on the leaves of *Piper betle* L. var. sirugamani1(SGMI) links an ethnomedical important medicinal plant and its pharmacognostic relevance. *Int. J. Pharm. Technol. Res*. 6(1): 244-251.

Navasero RC, Ramaswamy BS (1991). Morphology of leaf surface trichomes and its influence on egglaying by *Heliothis virescens*. *Crop Sci*. 31: 342-353.

Pedro WLJ, Nyncke JH, Janrinze VS, Nick VE (2023). White clover (*Trifolium repens*) population dynamics partly dependent on timing of seminal taproot death. *Grass Forage Sci.* 78(1): 1-11.

Ridda TJ, Daood WH (1982). Geographical Distribution of Wild Vascular Plants of Iraq.

Baghdad, Iraq: National Herbarium of Iraq. pp. 13-14.

Rizani KL, Šoljan D, Berisha N (2021). Morphological variability of *Trifolium repens* L. (Fabaceae). Univ. Prishtina, https://orcid.org/0000-0002-7443-4368 K.

Sabeti H (1965). Trees and Shrubs Native to Iran. Tehran Univ. Press. pp. 430.

Sabuda K, Temine N, Guler N (2008). Review article *Trifolium* L. – A review on its phytochemical and pharmacological profil. *Phytotherapy Res*. 22(4): 544-549.

Scatena VL, Giulietti AM, Borba EL, Van-der-Berg C (2005). Anatomy of Brasilian Eriocaulaceae: Correlation with taxonomy and habitat using multivariate analyses. *Plant Syst. Evol.* 253: 1-22.

Tabad MA, Nemati M, Jalilian N (2013). *Trifolium Incarantum* L. (Papilionaceae). A new record for the flora of Iran. *Iran J. Bot.* 21(2): 2015.

Taia WK (2004). Leaf characters within tribe Trifolieae (Family Leguminosae). *Pak. J. Biol. Sci.* 7(8): 1463-1472.

Toma C (1969). Studiul epidermei frunzelor la genul *Trifolium* L. din flora Romaniei. *An. St. Univ. Lasi., Sect Iia,* XV(1): 41-47.

Townsend CC, Guest E (1974). Flora of Iraq. *Leguminales Vol. 3.* Ministry of Agriculture, Republic of Iraq, Baghdad, Iraq. pp. 150-196.

UC SAREP (2012). Program, Univ. of California, Crimson clover. In: SAREP cover crops database. *Sustainable. Agric. Res. Edu. Prog.,* http://www.sarep.ucdavis.edu/database/covercrops.

Vincent MA, Isely D (2012). Flora, *Trifolium incarnatum* L., Jepson Flora (v. 1.0). Jepson Project. http://ucjeps.berkeley.edu/IJM.html.

Welham CVJ, Turkington R, Sayre C (2002). Morphological plasticity of white clover (*Trifolium repens* L.) in response to spatial and temporal resource heterogeneity. *Oecologia* 130(2): 231-238.

Woodward FI, Kelly CK (1995). The influence of Co_2 concentration on stomatal density. *New Phytol*. 131: 311-327.

Zoric L, Merkulov L, Lukovic J, Boza P, Polic D (2008). Leaf epidermal characteristics of *Trifolium* L. species from Serbia and Montenegro. *Flora* 204: 198-209.

Zoric L, Merkulov L, Lukovic J, Bozo P (2011). Comparative analysis of qualitative anatomical characters of *Trifolium* L. (Fabaceae) and their taxonomic implications: Preliminary results, Plant systematics and Evolution, pp. 205-219.