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Embryo and seedling morphology of some *Trifolium* L. species (Fabaceae)

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Abstract

The present study aims to describe and characterize the morphology of the embryo and the seedling and their importance in the taxonomy of the studied *Trifolium* species. Macro and micromorphological studies were carried on 15 *Trifolium* species using light microscopy. Qualitative and quantitative characteristics of the embryo, seedling with cotyledonary leaves, seedling with first unifoliate and first trifoliate leaves indicated that cotyledon characters such as cotyledon shape; L/W ratio, apex, colour, radicle position; shape, apex; cotyledonary leaves such as hypocotyl colour, blade shape, L/W ratio; first and second leaves (shape, apex base, margin and texture) proved to be useful for identification of different taxa. Numerical analysis is carried out and phenogram illustrating the relationship between the studied taxa was constructed by calculating the average taxonomic distance. Results show a taxonomic significance in the identification and differentiation between the studied taxa. An identification key was prepared based on embryo and seedling morphology.

***Keywords*:** embryo, Fabaceae, first & second foliage morphology, seedling, *Trifolium*.

Introduction

The genus *Trifolium* L. (Fabaceae) includes approximately 250–300 species, mostly annual, biennial and short-lived perennial, distributed throughout the temperate and subtropical regions (Allen & Allen, 1981; Zohary & Heller, 1984 and Ellison *et al*., 2006). The genus is cosmopolitan with species that occur mostly in Northern hemisphere; it includes a large number of food crops and forage plants.

The genus has been divided into eight sections: *Chronosemium*, *Involucrarium*, *Lotoidea*, *Mystillus*, *Paramesus*, *Tricocephalum*, *Trifolium* and *Vesicaria*, where *Lotoidea* is the largest and has served as a source taxon for the evolution of other sections (Zohary & Heller, 1984).

The seedling stage is arguably the busiest phase in a plant's lifetime. Once germination has occurred, the seedling depends on its own morphological and physiological characteristics to cope with the various factors threatening its survival (Farnsworth, 2008).

Seedling morphological characters are as important, reliable, and conservative characters as that of floral ones, and should be used in the delimitation of different taxonomic groups (Mundhra *et al*., 2012).

Different workers have also carried out investigations on seedling morphology at the level of genus with reference to their taxonomic importance (Scott & Smith, 1998).

Morphological studies were carried on seedling giving on different taxonomic groups: Leguminosae (Compton, 1912), Juglandaceae (Conde & Stone, 1970), Sapotaceae (Bokdam, 1977), Iridaceae (Tillich, 2003) and Malpighicaceae (Barbosa *et al*., 2014). Seedling morphology has also been utilized in the preparation of seedling flora (Lubbock, 1892, Burger, 1972, Muller, 1978). Seedlings of Dicotyledons are some of the noteworthy contributions in this regard.

Vogel (1980) classified the dicotyledons based on seedling morphology and germination pattern, embryo and seed size. Paria *et al*. (1991) described seedling

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morphology of 14 species under 13 genera related to four families of Malvales and utilized the data in the construction of artificial keys to the identification of the taxa showing affinities within them. Das (2001) clarified the taxonomic significance of mangrove seedling morphology, depending upon special morphological character like, seedling type, cotyledons differentiation and hypocotyls elongation. Characters of seedlings have taxonomic implications on *Bauhinia* L. (Bandyopadhyay, 2002). Khalik & Van der Maesen (2002) used radicle/cotyledons position as a significant character to separate different tribes of Brassicaceae.

Seedlings in Fabaceae were previously studied and provided taxonomic characters that was useful in delimiting different levels of taxonomic groups (Baudet, 1974; Duke & Polhill, 1981; Ye, 1983; Nozzolillo, 1985; Lima, 1989; Oliveira, 2001 and Rodrigues & de Azeve do Tozzi, 2007), it was found useful in the tribe Trifoliae in segregating particular species like *Medicago* (Buendia Lazaro *et al*., 1966). Sanyall & Paria (2015) used seedling morphological characters of twenty-five taxa under eighteen genera to determine inter-relationships among these taxa and to construct artificial key for identification purpose. Turki *et al.* (2016) studied 29 *Medicago* species, used morphological characters of cotyledons, seedlings and first foliage leaves in differentiation between the studied taxa.

Karaismailoglu (2015) treated embryo size as useful taxonomic character of the genus *Romulea* (Iridaceae) in Turkey. Characters of cotyledons in Fabaceae correlated with taxonomic grouping at several levels within the family (Smith 1983). Based on cotyledons characters; Scott & Smith (1998) recognized the *Acacia* subgenera. According to the number of leaf primordial, Nemoto & Ohashi (1993) recognized two distinct types of plumule in genus *Lespedeza*. Gavadi and Yamaguchi (2004) used plumule features as a parameter for species identification and resolution of species relationship of genus *Cicer*.

Numerical analyses as a useful statistical tool play an important role in clarifying the relationships between different taxonomic levels (Van de Wouw *et al.,* 2001; Khalik *et al*., 2002; Turki *et al*., 2013 and Kendir *et al*., 2015).

According to the literature, there is no previous work about embryo and seedling description of the *Trifolium* species. The present study aims to study the detailed description of the embryo and seedling structure of *Trifolium* species to evaluate the systematic value of these characters as an aid in recognition of the studied species.

Materials & Methods

Seeds of 15 species belonging to *Trifolium* obtained from the Institute of Plant Genetics and Crop Plant Research (IPK) - Germany (Table 1) and stored at 5 C° at refrigerator until their use. Seed soaked (5-10 seeds) in tap water for 2-3 hours, remove seed coat gently, examine by using Stereomicroscope using different magnification.

Seeds planted (7-10 seeds) at about 1cm in plastic pots (10 cm diameter) filled with soil (sand and peat moss 2:1 respectively), irrigation carried out by tap water.

Seedlings measurements were taken at the first growth stage (cotyledonary leaves stage) and the second growth stage (first unifoliate and trifoliate foliage leaf stage). Description of hypocotyls, petioles and blades followed the terminology used by Hickey (1973), Vogel (1980), Ahammed & Paria (1996) and Khalik & Van der Maesen (2002).

For minor details in morphology, the seedlings were examined using stereomicroscope then photographed using Zeiss research microscope using different magnification.

For statistical analysis, characters were encoded according to the multistate method; they were coded as (0, 1) for different

character states; 31 characters included 152 character states were recorded for each taxon. The codes were analyzed with NTsys version 2.1 (Rohlf, 2000) which is specialized in the numerical analysis data. Phenogram illustrating the relationship between the studied taxa were constructed by calculating the average taxonomic distance.

Table 1: List of the studied species of *Trifolium* L. and the sources of seeds.

	Species	*Synonyms*	Source of seeds (IPK)
1	*Trifolium argutum* Banks & Sol.	*Trifolium xrocphalum* Fenzl, *T. moriferum* Boiss.	Portugal (PRT)
2	*Trifolium bocconei* Savi.	-	Portugal (PRT)
3	*Trifolium campestre* Schreb.	*Trifolium agrarium* L., *T. procumbens* L., *T. lagrangei* Boiss.	Portugal (PRT
4	*Trifolium dubium* Sibth.	*Trifolium minus* Sm.	Portugal (PRT
5	*Trifolium fragiferum* L.	*Trifolium congestum* Link, *T. neglectum* C. Mey.	Australia (AUS)
6	*Trifolium glomeratum* L.	*Amoria glomerata* (L.) Sojak	Portugal (PRT)
7	*Trifolium lappaceum* L.	*Trifolium carteiense* Coincy	Portugal (PRT)
8	*Trifolium ligusticum* Balb. ex Loisel.	-	Frankreich (FRA)
9	*Trifolium michelianum* Savi.	*Trifolium macropodum* Guss.	Bulgaria (BGR)
10	*Trifolium nigrescens* Viv.	*Amoria nigrescence* (Viv.) Fourr.	Portugal (PRT
11	*Trifolium physodes* M. Bieb.	*Amoria physcodes* (M. Bieb.) Roskov	Portugal (PRT
12	*Trifolium purpureum* Loisel.	*Trifolium angustifolium* L. subsp. *purpureum* (Loisel.) Ponert	Lebanon (LBN)
13	*Trifolium repens* L.	*Amoria repens* (L.) C. Presl	Portugal (PRT)
14	*Trifolium squarrosum* L.	*Trifolium dipsaceum* Thuill., *T. panormitanum* C. Presl	Italy (IAT)
15	*Trifolium tomentosum* L.	*Amoria tomentosa* (L.) Roskov	Portugal (PRT)

Results

The embryo characters, description of the seedling in the cotyledonary leaves; description of seedling with the first unifoliate leaf and, of the first trifoliate foliage leaf of the fifteen investigated taxa are summarized in tables 1-4 and fig. 1-9.

Description of embryo (Fig. 1-4, Table 2)

Cotyledons shape were obovate in *T. glomeratum, T. michelianum, T. nigrescens, T. physodes*, and *T. repens,* elliptic in *T. campestre, T. ligusticum* and *T. tomentosum* and oblong in the remaining seven studied species. Cotyledons length × width were 2.8-3 × 1.2-1.3mm in *T. squarrosum* and 0.8-2 × 0.3-1 mm in the remaining 14 studied species. Cotyledons were pale brown in *T. argutum, T. ligusticum, T. michelianum* and *T. physodes* and pale yellow in the remaining 11 species. Radicles were accumbent (i.e. cotyledons having their edges folded against the hypocotyl) in *T. physodes* and incumbent in the remaining 14 studied species. Radicle flattened in *T. argutum,* semicylindrical in *T. purpureum* and cylindrical in the remaining 13 studied species. Radicle apex were acuminate in *T. argutum* and *T. squarrosum*, obtuse in *T. ligusticum, T. nigrescens* and *T. physodes*, rounded in *T. tomentosum,* acute-subacute in *T. fragiferum* and acute in the remaining eight studied species. Radicles length × width were 0.3-0.5 × 0.2 mm in *T. bocconei*, 0.6-1.2 × 0.2 mm in *T. campestre, T. dubium, T. glomeratum, T. lappaceum, T. ligusticum, T. nigrescens,* and *T. purpureum,* 1.3-1.7 × 0.2 mm in *T. fragiferum, T. michelianum, T. physodes, T. repens* and *T. tomentosum,* 1.9-2 × 0.2 mm in *T. argutum* and up to 2.3-2.5 × 0.2 mm in *T. squarrosum.* Mucilage attached to embryo in *T. bocconei, T. fragiferum,T. lappaceum, T. nigrescens, T. physodes, T. purpureum, T. repens, T. squarrosum* and *T. tomentosum* and not attached in the remaining six studied species**.**

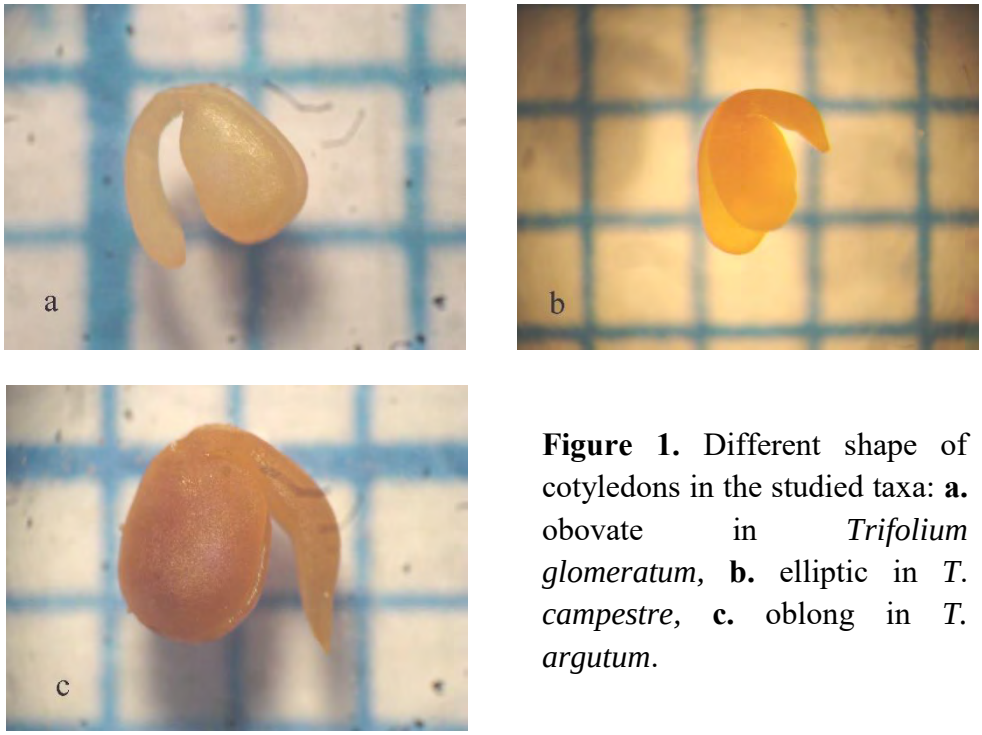

Figure 1. Different shape of cotyledons in the studied taxa: **a.** obovate in *Trifolium glomeratum,* **b.** elliptic in *T. campestre,* **c.** oblong in *T. argutum*.

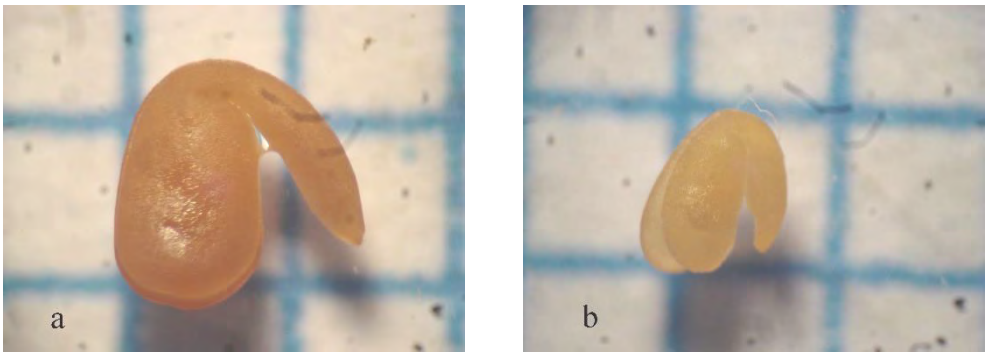

Figure 2. Cotyledons colour: **a.** pale brown in *Trifolium argutum,* **b.** pale yellow in *T. bocconei*

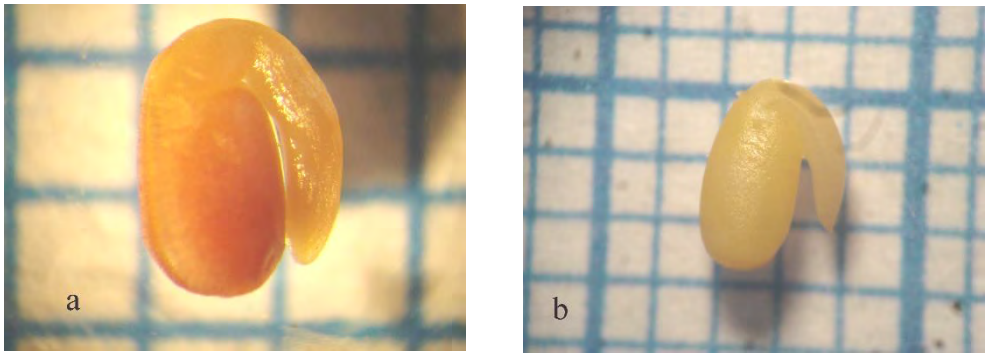

Figure 3. a. accumbent radicle in *Trifolium physodes*, **b.** incumbent radicle in *T. argutum*.

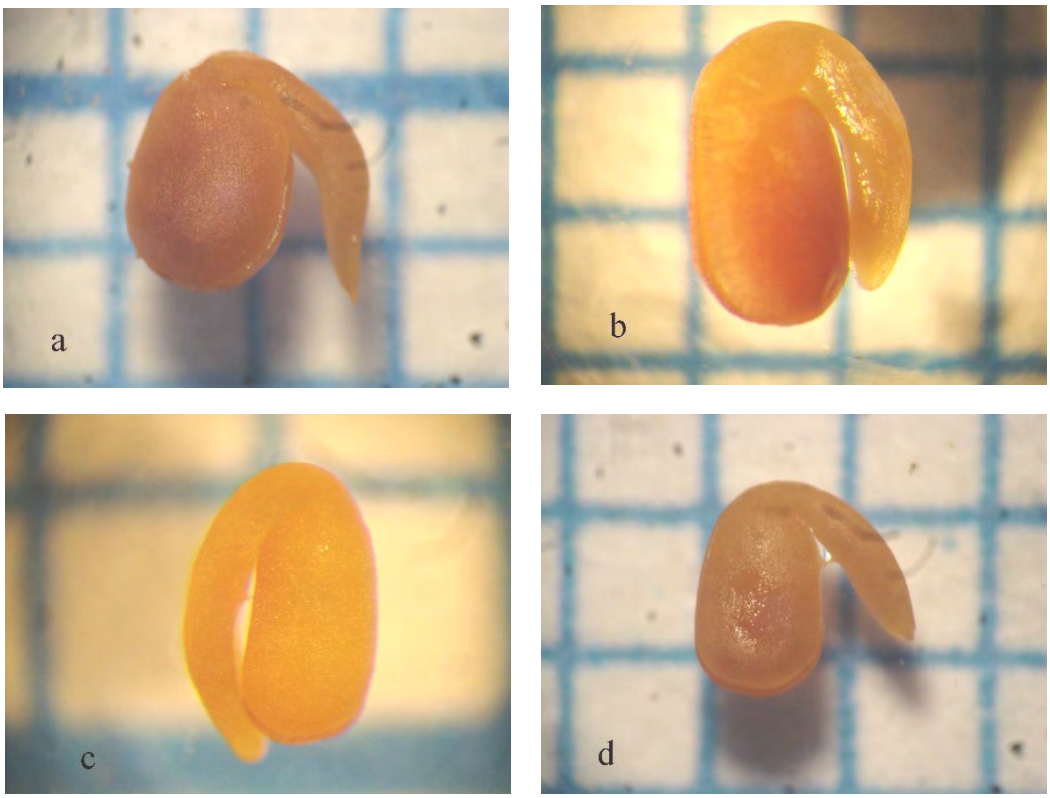

Figure 4. Radicle apex: **a.** acuminate in *Trifolium argutum,* **b.** obtuse in *T. ligusticum,* **c.** rounded in *T. tomentosum*, **d.** acute in *T. bocconei.*

Description of the seedling in the cotyledonary leaves stage (Fig. 5, Table 3)

Hypocotyl colour yellowish green in *T. repens,* green in *T. fragiferum, T. physodes* and *T. squarrosum,* reddish green in *T. lappaceum,* whitish green in *T. argutum* and pale green in the remaining nine studied species. Hypocotyl length up to 27-28 mm in *T. purpureum,* 17-19 mm in *T. dubium* and *T. physodes,*14-15 mm in *T. argutum,*10-12 mm in *T. campestre* and *T. ligusticum,*7-10 mm in *T. fragiferum, T. lappaceum T. michelianum,* and *T. squarrosum,* and 1-5 mm in the remaining five species. Length of the cotyledonary leaf petiole were 4.5-7 mm in *T. argutum, T. fragiferum, T. michelianum*, and *T. physodes,* and were 2-3 mm in *T. glomeratum, T. lappaceum, T. purpureum, T. squarrosum* and *T. tomentosum* and up to 2 mm in the remaining 6 studied species. Blade shape obovate in *T. glomeratum* and *T. squarrosum,* oblong in the remaining 13 studied species. Blade apex obtuse in *T. ligusticum* and *T. repens* while rounded in the remaining 13 studied species. Blade base cuneate in *T. glomeratum* and *T. tomentosum* while rounded in the remaining 13 studied species. Blade length × width; 7-8 × 2-3mm in *T. squarrosum,* 4-6 × 2-3.5 mm in *T. argutum, T. lappaceum, T. ligusticum, T. michelianum,* and *T. physodes,*1.2-1.5 × 0.8-1 mm in *T. purpureum* and up to 4 × 1.3-2 mm in the remaining eight studied species.

Table 2. Characters of embryo structure of 15 studied *Trifolium* species

Species	Cotyledon				Radicle				Attachment of mucilage to embryo
	Shape	L×W (mm)	L/W ratio	Colour	Position	Shape	Apex	L×W (mm)	
T. argutum	oblong	1.6-1.8 × 0.9-1	1.7	pale brown	incumbent	flattened	acuminate	1.9-2 × 0.2	not attached
T. bocconei	oblong	0.8-1 × 0.3-0.4	2.5	pale yellow	incumbent	cylindrical	acute	0.3-0.5 × 0.2	attached
T. campestre	elliptic	1.3-1.5 × 0.5-0.6	2.5	pale yellow	incumbent	cylindrical	acute	0.8-0.9 × 0.2	not attached
T. dubium	oblong	1.2-1.5 × 0.5	2.7	pale yellow	incumbent	cylindrical	acute	0.8-0.9 × 0.2	not attached
T. fragiferum	oblong	1.5-2 × 0.8-1	1.9	pale yellow	incumbent	cylindrical	acute-subacute	1.3-1.5 × 0.2	attached
T. glomeratum	obovate	1.3-.5 × 0.6	2.3	pale yellow	incumbent	cylindrical	acute	0.7-0.9 × 0.2	not attached
T. lappaceum	oblong	1.8-2 × 0.8-1	2.1	pale yellow	incumbent	cylindrical	acute	1-1.2 × 0.2	attached
T. ligusticum	elliptic	1.2-1.4 × 0.8	1.6	pale brown	incumbent	cylindrical	obtuse	0.6-0.7 × 0.2	not attached
T. michelianum	obovate	1.7-1.9 × 0.4	4.5	pale brown	incumbent	cylindrical	acute	1.4-1.5 × 0.2	not attached
T. nigrescens	obovate	0.8-1 × 0.3-0.4	2.5	pale yellow	incumbent	cylindrical	obtuse	0.7-0.8 × 0.2	attached
T. physodes	obovate	1.8-2 × 1	1.9	pale brown	accumbent	cylindrical	obtuse	1.4-1.6 × 0.2	attached
T. purpureum	oblong	1.8-2 × 0.9-1	2	pale yellow	incumbent	semi-cylindrical	acute	1-1.2 × 0.2	attached
T. repens	obovate	1.2-1.4 × 0.8	1.6	pale yellow	incumbent	cylindrical	acute	1.4-1.6 × 0.2	attached
T. squarrosum	oblong	2.8-3 × 1.2-1.3	2.3	pale yellow	incumbent	cylindrical	acuminate	2.3-2.5 × 0.2	attached
T. tomentosum	elliptic	1.2-1.5 × 0.8	1.6	pale yellow	incumbent	cylindrical	rounded	1.5-1.7 × 0.2	attached

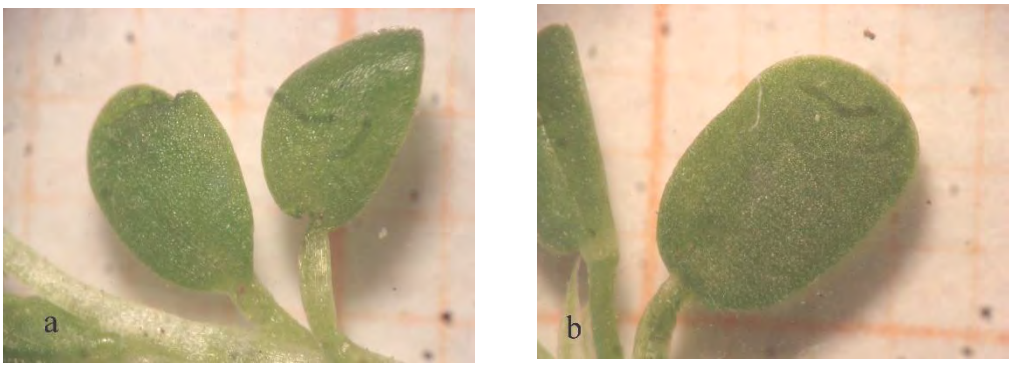

Figure 5. Different shapes of cotyledonary leaf: **a.** obovate in *Trifolium glomeratum*, **b.** oblong in *T. campestre*.

Table 3. Description of the seedling in the cotyledonary leaves stage.

Species	Hypocotyl		Petiole length (mm)	Blade				L/W ratio
	Colour	Length (mm)		Shape	Apex	Base	L×W (mm)	
T. argutum	whitish green	14-15	6-7	oblong	round	round	5-6 × 2.5-3	2
T. bocconei	pale green	4-5	1-1.5	oblong	round	round	2.5-3 × 1.5-2	1.5
T. campestre	pale green	11-12	1-1.5	oblong	round	round	2.8-3 × 1.8-2	1.5
T. dubium	pale green	17-18	1-1.5	oblong	round	round	2.8-3 ×1.3-1.5	2.1
T. fragiferum	green	9-10	5.5-6.5	oblong	round	round	3-4 × 2-2.5	1.5
T. glomeratum	pale green	2-3	2.5-2.8	obovate	round	cuneate	3.5-4 × 1.8-2	1.9
T. lappaceum	reddish green	9-10	2-2.5	oblong	round	round	4-5 × 2-3	1.6
T. ligusticum	pale green	11-12	1.5-2	oblong	obtuse	round	4-5 × 2-3	1.8
T. michelianum	pale green	7-8	6-6.5	oblong	round	round	4-5 × 2.5-3	1.6
T. nigrescens	pale green	4-5	1.5-2	oblong	round	round	2.3-2.5 ×1.5-2	1.3
T. physodes	green	18-19	4.8-5	oblong	round	round	5-6 × 3.2-3.5	1.6
T. purpureum	pale green	27-28	2-2.5	oblong	round	round	1.2-1.5 ×0.8-1	1.5
T. repens	yellowish green	1-1.2	1.3-1.5	oblong	obtuse	round	2.3-2.5 ×1-1.5	1.9
T. squarrosum	green	9-10	2-2.5	obovate	round	round	7-8 × 2-3	3
T. tomentosum	pale green	2-2.5	2-2.5	oblong	round	cuneate	3.5-4 × 1.5-2	2.1

Description of seedling with the first unifoliate leaf stage (Fig. 6-7, Table 4)

Petiole length up to 39-41 mm in *T. purpureum*, 28-31 mm in *T. argutum* and *T. physodes,* 23-27 mm in *T. michelianum* and *T. tomentosum,*17-20 mm in *T. fragiferum* and *T. ligusticum,* 12-15 mm in *T. glomeratum* and *T. nigrescens* and up to10 mm in the remaining six studied species.

Petiole surface pubescent in *T. fragiferum* and *T. ligusticum,* pilose in *T. bocconei*, *T. lappaceum, T. purpureum* and *T. squarrosum* and glabrous in the remaining nine studied species. Blade shape cordate in *T. repens,* obcordate in *T. michelianum,* oblong in *T. argutum*, oblate in *T. dubium, T. lappaceum* and *T. ligusticum,* elliptic in *T. purpureum,* square in *T. bocconei,* ovate in *T. physodes* and *T. tomentosum,* depressed ovate in *T. campestre* and broadly ovate in *T. fragiferum, T. glomeratum* and *T. nigrescens.* Blade length × width; 11-12 × 5-6 mm in *T. purpureum,* 6-7 × 5-6 mm in *T. dubium, T. fragiferum, T. physodes* and *T. squarrosum,* 4-5 × 4-6 mm in *T. campestre, T. ligusticum* and *T. tomentosum*, up to 2 × 2 mm in *T. repens,* 3-3.5 × 3.5-4 mm in *T. michelianum,* 4-4.5 × 3-3.5 in *T. argutum,* 2.5-3×2.8-3.5 mm in *T. lappaceum,* 3.5-4 × 3.5-4.5 mm in *T. bocconei* and *T. glomeratum,* and 2.5-3 × 3-3.5 mm in *T. nigrescens.* Blade apex acute in *T. argutum* and *T. tomentosum*, emarginate-apiculate in *T. glomeratum,* rounded in *T. lappaceum* and truncate in *T. michelianum* and emarginate in the remaining ten studied species. Blade base rounded in *T. argutum* and *T. lappaceum,* reniform in *T. nigrescens,* cuneate in *T. purpureum* and *T. squarrosum,* and truncate in the remaining ten studied species. Blade margin ciliate in *T. argutum,* dentate in *T. fragiferum, T. glomeratum, T. nigrescens, T. physodes, T. repens* and *T. tomentosum,* serrate in *T. michelianum* and entire in the remaining seven studied species. Blade surface pilose in *T. bocconei, T. lappaceum, T. purpureum* and *T. squarrosum,* pubescent in *T. ligusticum,* subglabrous in *T. fragiferum* and glabrous in the remaining nine studied species.

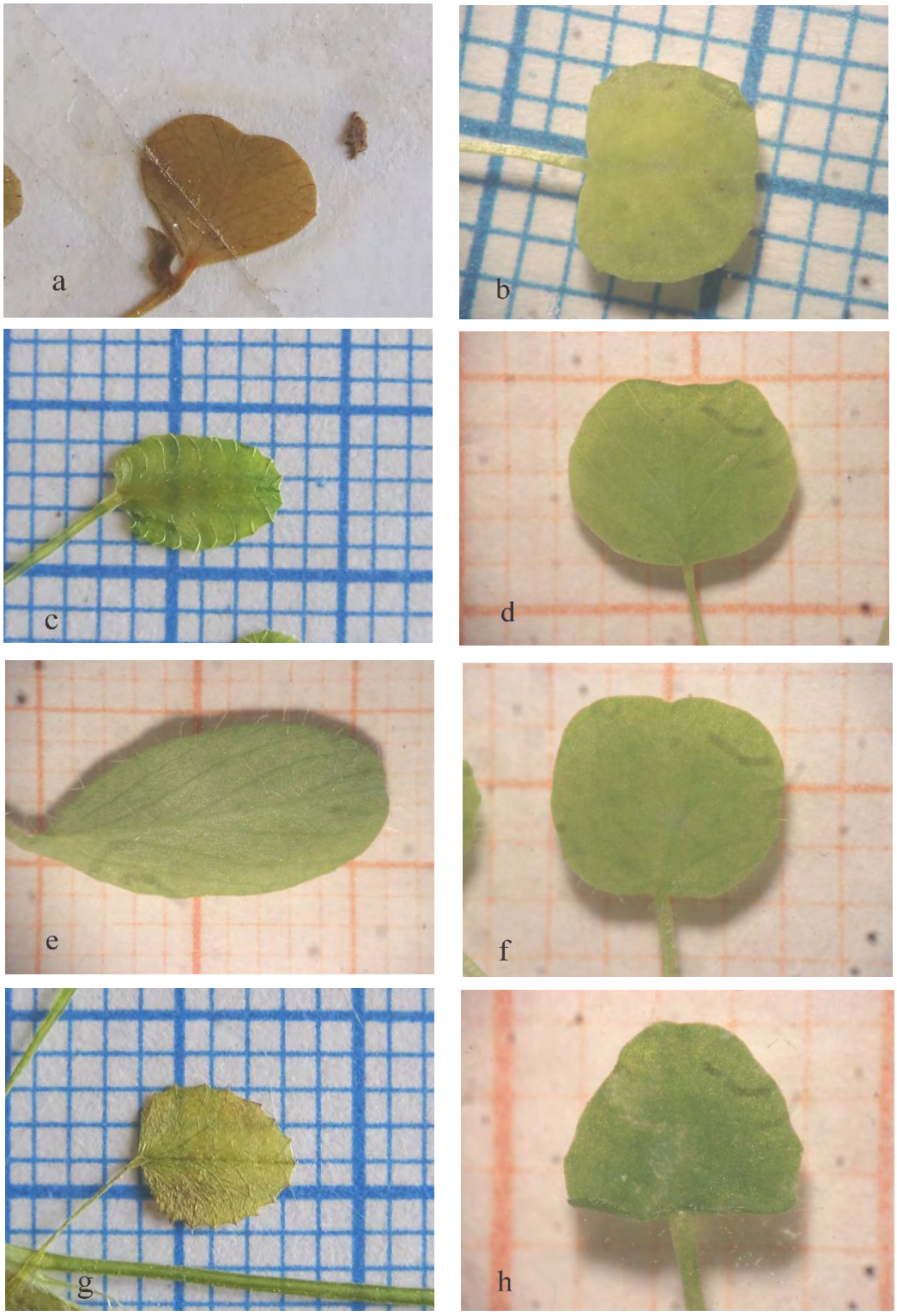

Figure 6. The first unifoliate leaf shape: **a.** obcordate in *Trifoium michelianum*, **b.** depressed ovate in *T. campestre*, **c.** oblong in *T. argutum*, **d.** oblate in *T. lappaceum*, **e.** elliptic in *T. purpureum*, **f.** square in *T. bocconei*, **g.** ovate in *T. physodes*, **h.** broadly ovate in *T. fragiferum*.

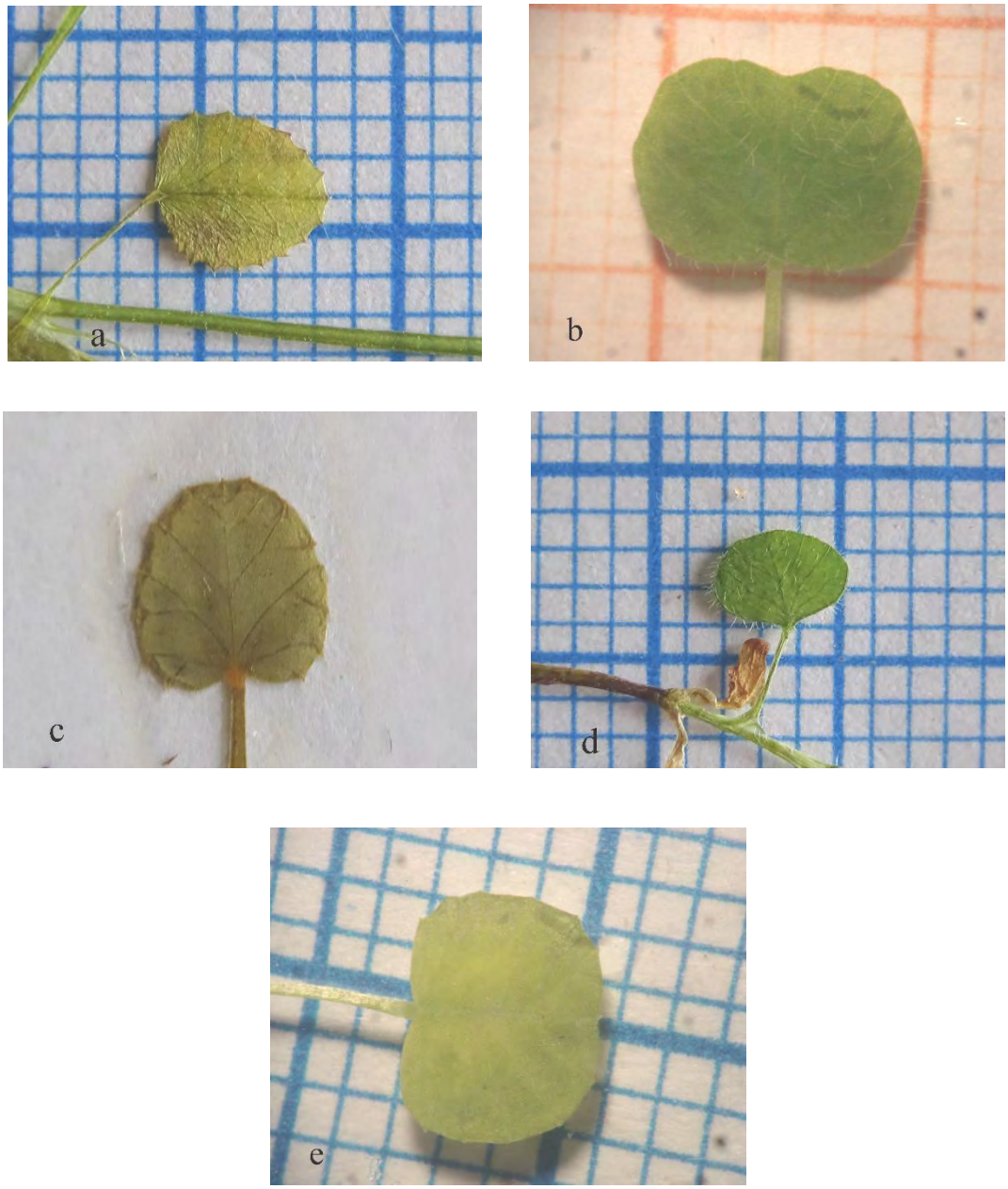

Figure 7. The first unifoliate leaf apex: **a.** acute in *Trifolium argutum*, **b.** emarginate in *T. bocconei*, **c.** emarginate-apiculate in *T. glomeratum*, **d.** rounded in *T. lappaceum*, **e.** truncate in *T. michelianum*.

Table 4. Description of seedling with the first unifoliate leaf stage

Species	Petiole Length (mm)	Blade						
		Shape	L × W (mm)	L/W ratio	Apex	Base	Margin	Surface
T. argutum	30-31	oblong	4-4.5 × 3-3.5	1.3	Acute	rounded	ciliate	glabrous
T. bocconei	7-8	square	3.5-4 × 3.5-4	1	emarginate	truncate	entire	pilose
T. campestre	5-6	depressed ovate	4-5 × 5-6	0.8	emarginate	truncate	entire	glabrous
T. dubium	9-10	oblate	4-4.5 × 5-5.5	0.8	emarginate	truncate	entire	glabrous
T. fragiferum	17-18	broadly ovate	4-4.5 × 4.3-4.8	0.9	emarginate	truncate	dentate	subglabrous
T. glomeratum	13-15	broadly ovate	3.5-4 × 4-4.5	1.1	emarginate apiculate	truncate	dentate	glabrous
T. lappaceum	3-4	oblate	2.5-3 × 2.8-3.5	0.8	rounded	rounded	entire	pilose
T. ligusticum	18-20	oblate	4-5 × 5-6	0.8	emarginate	truncate	entire	pubescent
T. michelianum	23-25	obcordate	3-3.5 × 3.5-4	0.8	truncate	truncate	serrate	glabrous
T. nigrescens	12-13	broadly ovate	2.5-3 × 3-3.5	0.8	emarginate	reniform	dentate	glabrous
T. physodes	28-29	ovate	6-7 × 5-6	1.1	emarginate	truncate	dentate (at upper third)	glabrous
T. purpureum	39-41	elliptic	11-12 × 5-6	2.1	emarginate	cuneate	entire	pilose
T. repens	7-8	cordate	1.8-2 × 1.8-2	1	emarginate	truncate	dentate (at upper half)	glabrous
T. squarrosum	5-6	obovate	6-7 × 5-6	1.1	emarginate	cuneate	entire	pilose
T. tomentosum	25-27	ovate	4-5 × 4-5	1	Acute	truncate	dentate	glabrous

Description of the first trifoliate foliage leaf stage (Fig. 8-9, Table 5)

Leaf petiole length show wide variation; 4-42 mm in the studied species. Petiole surface pilose in *T. bocconei, T. purpureum,* and *T. squarrosum,* pubescent in *T. ligusticum,* subglaborus in *T. lappaceum*, glabrous in the remaining ten studied species. Leaflet shape circular in *T. nigrescens*, elliptic in *T. fragiferum, T. physodes* and *T. tomentosum,* obovate in *T. purpureum,* Semicircular in *T. argutum,* oblong in *T. squarrosum,* obcordate in the remaining eight studied species. Blade length × width shows differentiation between the studied 15 species listed in the table. Blade apex acuminate in *T. tomentosum*, acute in *T. argutum,* obtuse in *T. purpureum,* emarginate in the remaining 12 studied species. Leaf base rounded in *T. michelianum, T. nigrescens* and *T. physodes,* truncated in *T. dubium,* cuneate in the remaining 11 studied species. Leaf margin ciliate in *T. argutum*, serrate in *T. michelianum* and *T. repens,* dentate in *T. fragiferum, T. glomeratum, T. physodes* and *T. tomentosum*, entire in the remaining eight studied species. Blade surface pubescent in *T. ligusticum,* pilose in *T. bocconei, T. lappaceum, T. purpureum* and *T. squarrosum*, and glabrous in the remaining ten studied species.

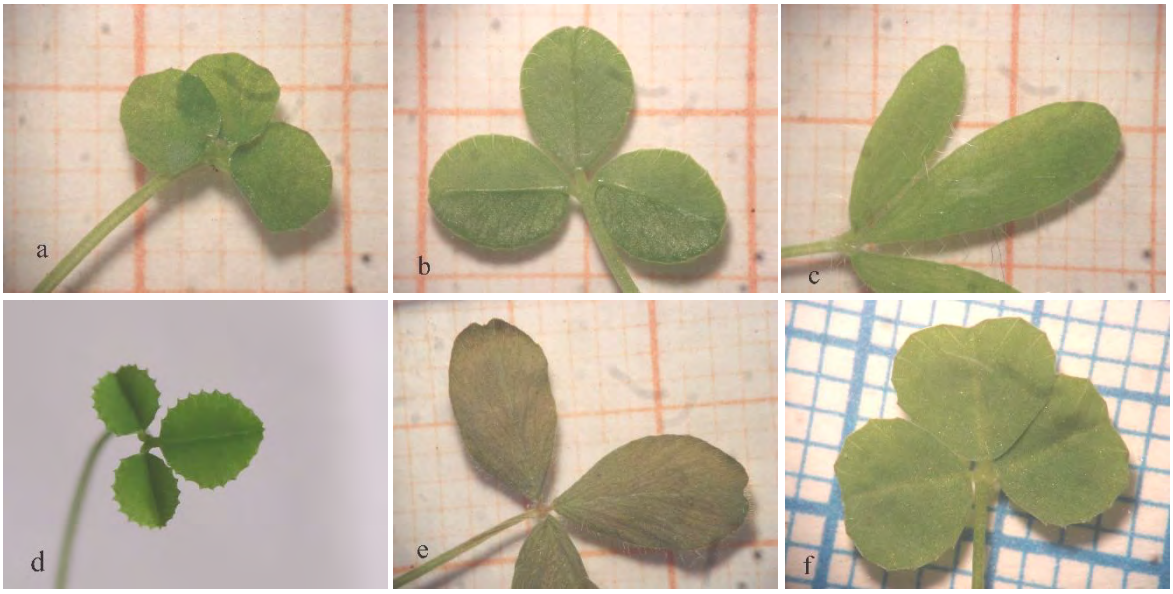

Figure 8. The first trifoliate leaflet shape: **a.** circular in *Trifolium nigrescens*, **b.** elliptic in *T. fragiferum,* **c.** obovate in *T. purpureum*, **d.** semicircular in *T. argutum*, **e.** oblong in *T. squarrosum*, **f.** obcordate in *T. bocconei*.

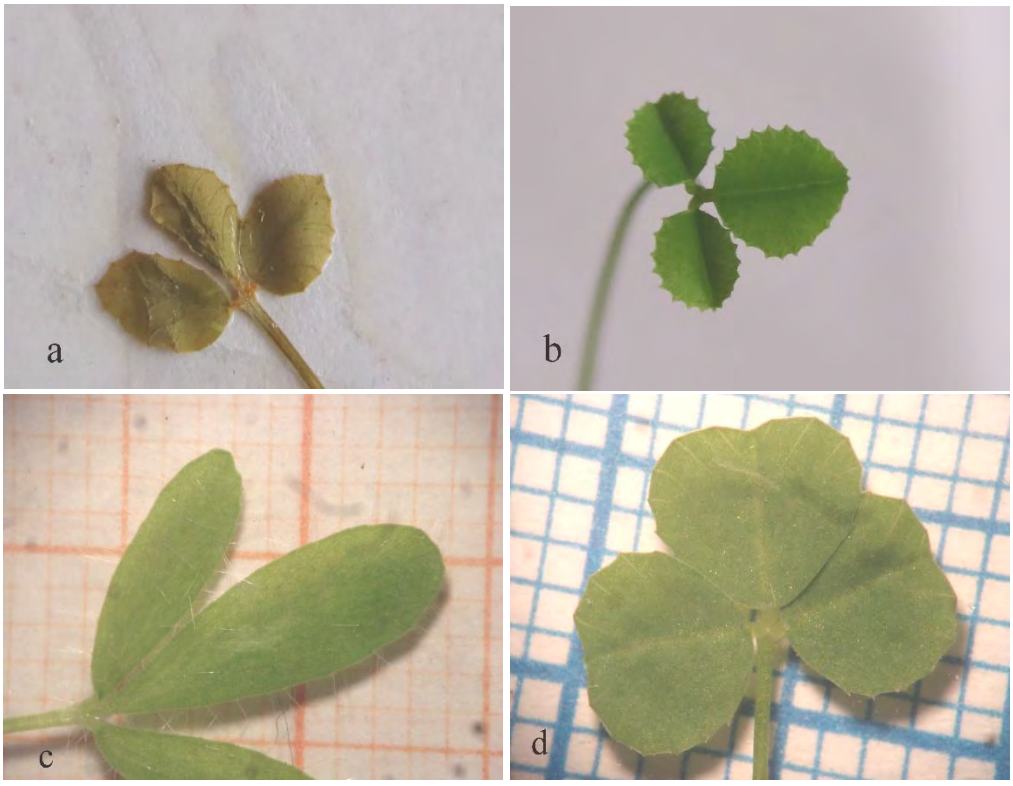

Figure 9. The first trifoliate leaflet apex: **a.** acuminate in *T. tomentosum*, **b.** acute in *T. argutum*, **c.** obtuse in *T. purpureum*, **d.** emarginate in *T. bocconei.*

Table 5. Description of seedlings in the first trifoliate foliage leaf stage

Species	Petiole Length mm	Surface	Blade Shape of leaflet	L×W mm	L/W ratio	Apex	Base	Margin	Surface
T. argutum	28-30	glabrous	semicircular	4-4.5 × 3-3.5	1.3	acute	cuneate	ciliate	glabrous
T. bocconei	11-12	pilose	obcordate	3-3.5 × 3-3.5	1	emarginate	cuneate	entire	pilose
T. campestre	5-6	glabrous	obcordate	3-3.5 × 3-3.5	1	emarginate	cuneate	entire	glabrous
T. dubium	11-12	glabrous	obcordate	3-3.5 × 2.5-3	1.2	emarginate	truncate	entire	glabrous
T. fragiferum	25-26	glabrous	elliptic	4-4.5 × 3.5-4	1.3	emarginate	cuneate	dentate	glabrous
T. glomeratum	20-21	glabrous	obcordate	3-3.5 × 3-3.5	1	emarginate	cuneate	dentate	glabrous
T. lappaceum	4-5	subglaborus	obcordate	2.8-3 × 2.8-3	1	emarginate	cuneate	entire	pilose
T. ligusticum	32-33	pubescent	obcordate	3.5-4 × 4.5-5	0.7	emarginate	cuneate	entire	pubescent
T. physodes	40-42	glabrous	obcordate	4-4.5 × 4-5	0.9	emarginate	round	serrate	glabrous
T. nigrescens	15-16	gabrous	circular	2-2.5 × 2-2.5	1	emarginate	round	entire	glabrous
T. michelianum	20-22	glabrous	elliptic	3-4 × 2-3	1.4	emarginate	round	dentate	glabrous
T. purpureum	40-41	pilose	obovate	10-11 × 3-3.5	3.2	obtuse	cuneate	entire	pilose
T. repens	11-12	glabrous	obcordate	5-5.5 × 4-4.5	1.2	emarginate	cuneate	serrate	glabrous
T. squarrosum	20-21	pilose	oblong	7-7.5 × 3.5-4	1.9	emarginate	cuneate	entire	pilose
T. tomentosum	28-30	glabrous	elliptic	3.5-4 × 2.5-3	1.3	acuminate	cuneate	dentate	glabrous

Numerical analysis

The dendrogram produced from using distance measure (Fig. 10) showed that on the bases of total characters (Appendix 1), *Trifolium* species was split off at distance level 1.67 into two series; the first series represented by *T. argutum* which characterized by flattened radicle shape, whitish green hypocotyl colour, oblong unifoliate leaf, with ciliate margin, second leaf with acute apex and ciliate margin.

The second series include the remaining 14 studied species and characterized by cylindrical, semi-cylindrical radicle shape, pale green, reddish green, yellowish green hypocotyl colour; unifoliate leaf shape square, depressed ovate, oblate, broadly ovate, obcordate, ovate, elliptic, cordate, obovate with entire, dentate, serrate margin, the second leaf with emarginated, obtuse, acuminate margin, and entire, serrate margin.

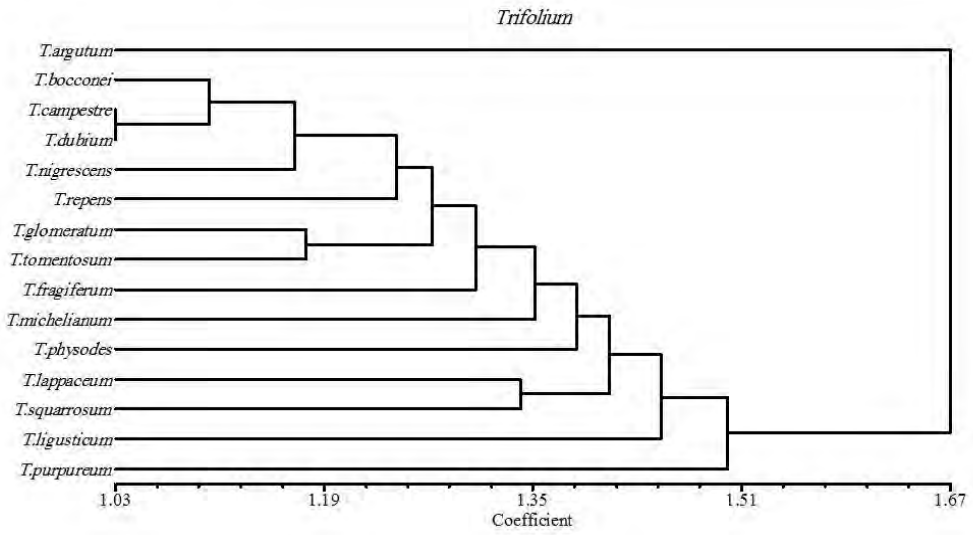

Figure 10. Dendrogram illustrating the relationships between the studied 15 *Trifolium* species on the bases of the morphological characters of the embryo and the seedlings cotyledonary leaves and the first unifoliate and trifoliate leaf stages.

The second series spilt off at distance level 1.49 into two subseries, the first represented by *T. purperum* and characterized by semi-cylindrical radicle shape, hypocotyl length 27-28 mm, petiole length of unifoliate leaf 39-41 mm, elliptic unifoliate leaf shape and L/W ratio 2.1, the second leaf L/W ratio 3.2, with obtuse apex.

The second subseries characterized by cylindrical radicle shape, hypocotyl length up to 19 mm, petiole length of unifoliate leaf up to 29 mm, square, depressed, ovate, oblate, broadly ovate obcordate, ovate, cordate, obovate unifoliate leaf shape and L/W ratio 1.1, second leaf L/W ratio up to 1.9, with emarginated, acuminate apex.

The second subseries split off at distance level 1.45 into two clusters, the first cluster represented by *T. ligusticum* and characterized by pubescent unifoliate leaf surface and petiole and surface of second leaf, L/W ratio of the second leaf up to 0.7, the second cluster represented by the remaining studied species and characterized by glabrous, subglaborus, pilose unifoliate leaf surface, glabrous, subglabrous, pilose petiole and surface of second leaf, L/W ratio of second leaf up to 0.9-1.9.

The second cluster split off at distance level 1.4 into two subclusters, the first subcluster split off into two groups at distance level 1.34, the first group represented by *T. squarrosum* and characterized by acuminate radicle apex, radicle length 2.3-2.5 mm, cotyledonary blade L/W ratio 3, obovate; first leaf obovate; the second leaf oblong-obcordate.

The second group represented by *T. lappaceum* and characterized by acute radicle apex, radicle length 1-1.2 mm, cotyledonary blade L/W ratio 1.6, first leaf oblate, the second leaf obcordate.

The second subcluster split off at distance level 1.38 into two groups, the first group represented by *T. physodes* and characterized by brown cotyledon colour, radicle position accumbent, petiole length of first leaf 28-29 mm, dentate (upper 1/3) margin, L/W ratio of second leaf 1.4.

The second group characterized by pale brown, pale yellow, radicle position incumbent, first leaf with petiole length up to 27 mm; entire, dentate, serrate margin, the second leaf L/W ratio up to 1.3.

The second group split off at 1.35 into two subgroups, the first subgroup represented by *T. michelianum* and characterized by cotyledonary ratio 4.5, unifoliate blade shape obcordate with truncated apex and serrate margin.

The second subgroup characterized by cotyledonary ratio up to 2.7, unifoliate blade shape square, depressed ovate, oblate, broadly ovate, ovate, cordate with acute, emarginate apex and entire, dentate margin.

The second subgroup split off at 1.3 into two classes, the first class represented by *T. fragiferum* and characterized by subacute radicle apex, hypocotyl green, first leaf with pubescent petiole surface; petiole length of second leaf 25-26 mm.

The second class characterized by acute-rounded radicle apex, hypocotyl pale green-yellowish green, first leaf with glabrous-pilose petiole surface and petiole length of second leaf up to 22 mm.

The second class split off at distance level 1.27 into two subclass, the first subclass split of at distance level 1.17 into two clades. The first clade represented by *T. tomentosum* and characterized by elliptic cotyledon shape, rounded radicle apex, mucilage attached to embryo, oblong cotyledon shape, petiole length of first leaf 23-27 mm; ovate with acute apex, petiole length of second leaf 28-30 mm; elliptic with acuminate apex.

The second clade represented by *T. glomeratum* and characterized by obovate cotyledon shape, acute radicle apex, mucilage non-attached to embryo, obovate cotyledon shape, petiole length of first leaf up to 15 mm; broadly ovate with emarginated-apiculate apex, petiole length of second leaf 20-22 mm; obcordate with emarginate apex.

The second subclass split off at distance level 1.24 into two clades, the first clade represented by *T. repens*, and characterized by cotyledon L/W ratio up to 1.6, yellowish green hypocotyl colour, obtuse cotyledon apex, cordate unifoliate leaf with dentate (upper 1/2) margin, the second leaf with serrate margin.

The second clade characterized by cotyledon L/W ratio 1.9-2.7, pale green hypocotyl colour, rounded cotyledon apex, square, depressed ovate, oblate, broadly ovate first leaf with entire margin, the second leaf with entire margin.

The second clade split off at distance 1.16 into two subclades, the first subclade represented by *T. nigrescens* and characterized by obovate cotyledon shape, obtuse radicle apex, first leaf broadly ovate with dentate margin and reniform base, second leaf circular with rounded base.

The second subclade represented by *T. bocconei, T. campestre, T. dubium* and characterized by oblong, elliptic cotyledon shape, acute radicle apex, square, depressed ovate, oblate first leaf with entire margin and truncated base, obcordate, cordate second leaf shape with cuneate, truncated base.

The second subclade split off at distance 1.1 which represented by *T. bocconei* characterized by radicle length up to 0.5 mm, mucilage attached to embryo, first leaf square, petiole and blade pilose; second leaf petiole and blade pilose.

T. campestre and *T. dubium* separated at distance 1.03, *T. campestre* characterized by elliptic cotyledon shape, hypocotyl length up to 12 mm, first leaf depressed ovate, petiole length up to 6 mm, second leaf cordate with cuneate base, petiole length up to 6 mm.

T. dubium characterized by oblong cotyledon shape, hypocotyl length 17-19 mm, first leaf oblate, petiole length 9-10 mm, second leaf obcordate with truncated base, petiole length 11-12 mm.

Discussion

The important of seedling morphological characters such as cotyledons shape and colour, radicle shape, colour and position and plumule colour, prophyl leaf shape and margin were variable and could be used to identify *Trigonella* species (Abozeid *et al.,* 2017) and *Vicia* species (Abozeid *et al.,* 2018).

In present study, *Trifolium* species possess many characters that play an important role in the differentiation between the studied species; cotyledon leaf shape; radicle apex, second leaf shape distinguished *T. tomentosum* from other species; Cotyledon colour, radicle apex, first leaf shape, second leaf hairness (indumentums) distinguished *T. campestre* from *T. ligusticum*; radicle apex, cotyledon L/W ratio, hypocotyl length, first and second leaf shape distinguished *T. physodes* from *T. michelium*. Radicle apex, first leaf base, second leaf shape distinguished *T. nigrescens* from other species.

Cotyledon L/W ratio, mucilage attachment to embryo, cotyledon leaf shape, first leaf shape, second leaf margin distinguished *T. glomeratum* from *T. repens*.

Radicle apex, hypocotyls colour, first and second leaf shape distinguished *T. argutum* from, *T. fragiferum*. Radicle apex, cotyledon leaf shape distinguished *T. squarosum* from other species. Mucilage attachment to embryo, cotyledon L/W ratio, first leaf hairness (indumentums), second leaf base distinguished *T. dubium* from other species.

Cotyledon L/W ratio, first leaf shape distinguished *T. bocconei* from other species. Radicle shape, hypocotyls colour, first and second leaf shape distinguished *T. lappaceum* from *T. purpureum*. Sinjushin and Akopian (2011) considered seedling features of *Vavilovia*, *Pisum* and *Lathyrus* can used to identify species of studied genera. Feitoza *et al*. (2014) considered variation in embryos, plumule characters of *Macrolobium* Schreb. species (Leguminosae, Caesalpinioideae) provided characters with taxonomic relevance that differing among the studied species and aid species identification.

In conclusion, it is clear that embryo, cotyledonary, seedling, and first foliage leaf features are the most useful characters in the identification of *Trifolium* species as shown in the following key:

1a. Cotyledon shape elliptic……...……..…….……2
1b. Cotyledon shape oblong, obovate……..……..…….…..4
2a. Radicle apex rounded, cotyledon leaf base cuneate, first leaf apex acute, second leaf elliptic…….……...…*T. tomentosum*
2b. Radicle apex acute, obtuse, cotyledon leaf base rounded, first leaf apex emarginate, second leaf obcordate…….…….……..……..…..….3
3a. Cotyledon pale yellow, radicle apex acute, first leaf depressed ovate, second leaf glabrous…….……..…….…….…….....*T. campestre*
3b. Cotyledon pale brown, radicle apex obtuse, first leaf oblate, second leaf pubescent…….……..…….*T. ligusticum*
4a. Cotyledons obovate……..…….……..……5
4b. Cotyledons oblong……..…….……..…….9
5a. Cotyledons pale brown, brown……..…….……..……...6
5b. Cotyledons pale yellow…….……..……7
6a. Radicle apex obtuse, cotyledon L/W ratio up to1.9; hypocotyl length 18-19 mm; first leaf ovate, second leaf elliptic…….……...……*T. physodes*
6b. Radicle apex acute, cotyledon L/W ratio 4.5; hypocotyl length up to 8 mm; first leaf obcordate, second leaf obcordate…..…….*T. michelianum*
7a. Radicle apex obtuse, second leaf circular ……..…… *T. nigrescens*
7b. Radicle apex acute, second leaf obcordate…….……....…….……..8
8a. Cotyledon L/W ratio 2.3; mucilage not attached to embryo; cotyledonary leaf obovate, first leaf broadly ovate, the second leaf with dentate margin ……..…….…….*T. glomeratum*
8b. Cotyledon L/W ratio 1.6; mucilage attached to embryo; cotyledonary leaf

oblong, first leaf cordate, the second leaf with serrate margin……..…....*T. repens*
9a. Cotyledon L/W ratio up to 1.9…….……..……10
9b. Cotyledon L/W ratio more than 2…….……..……..11
10a. Radicle apex acuminate, hypocotyl whitish green; first leaf oblong, second leaf semicircular-obovate.……...…….……*T. argutum*
10b. Radicle apex acute, hypocotyl green, first leaf broadly ovate, second leaf elliptic……..……...……*T. fragiferum*
11a. Radicle apex acuminate; cotyledonary leaf obovate……..…....*T.squarosum*
11b. Radicle apex acute, cotyledonary leaf oblong…….……..…..12
12a. First unifoliate leaf glabrous with truncated base……..….....*T. dubium*
12b. First unifoliate leaf pilose, with cuneate base …….……...13
13a. First unifoliate leaf square……..……...……...……*T. bocconei*
13b. First unifoliate leaf oblate, elliptic…….……..……14
14a. Radicle cylindrical; hypocotyl reddish green; first unifoliate leaf oblate, second leaf obcordate……..……..*T. lappaceum*
14b. Radicle semicylindrical; hypocotyl pale green, first unifoliate leaf elliptic, second leaf obovate……..……..*T. purpureum*

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix 1. Data matrix of (0, 1) codes of different characters states used in statistical analysis of *Trifolium* L. species (1) *T. argutum*, (2) *T. bocconei*, (3) *T. campestre*, (4) *T. dubium*, (5) *T. fragiferum*, (6) *T. glomeratum*, (7) *T. lappaceum*, (8) *T. ligusticum*, (9) *T. michelianum*, (10) *T. nigrescens*, (11) *T. physodes*, (12) *T. purpureum*, (13) *T. repens*, (14) *T. squarrosum*, (15) *T. tomentosums*

Characters			Species														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Embryo	Cotyledon shape	Oblong	1	1	0	1	1	0	1	0	0	0	0	1	0	1	0
		Elliptic	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1
		Obovate	0	0	0	0	0	1	0	0	1	1	1	0	1	0	0
	Cotyledon L/W ratio	1.6	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1
		1.7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		1.9	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
		2.1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
		2.3	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
		2.5	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0
		2.7	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
		4.5	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	Cotyledon colour	Pale brown	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
		Pale yellow	0	1	1	1	1	1	1	0	0	1	0	1	1	1	1
		Brown	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	Radicle position	Incumbent	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
		Accumbent	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	Radicle shape	Flattened	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Cylindrical	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1
		Semicylindrical	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	Radicle apex	Acuminate	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
		Acute	0	1	1	1	1	1	1	0	1	0	0	1	1	0	0
		Subacute	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
		Obtuse	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0
		Rounded	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Radicle length (mm)	0.3-0.5	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		0.7-0.9	0	0	1	1	0	1	0	1	0	1	0	0	0	0	0
		1-1.2	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
		1.3-1.6	0	0	0	0	1	0	0	0	1	0	1	0	1	0	1
		1.9-2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		2.3-2.5	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	Mucilage attachment to embryo	Non attached	1	0	1	1	0	1	0	1	1	0	0	0	0	0	0
		Attached	0	1	0	0	1	0	1	0	0	1	1	1	1	1	1
Hypocotyl	colour	Whitish green	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Pale green	0	1	1	1	0	1	0	1	1	1	0	1	0	0	1
		Green	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0
		Reddish green	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
		Yellowish green	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	length (mm)	1-1.5	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
		2-3	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
		4-5	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
		7-8	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
		9-10	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0
		11-12	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
		14-15	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		17-19	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
		27-28	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

Cotyledonary leaves	Blade shape	Oblong	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1
		Obovate	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
	Blade apex	Rounded	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1
		Obtuse	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
	Blade base	Rounded	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0
		Cuneate	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
	Blade L/W ratio	1.3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
		1.5	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0
		1.6	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0
		1.8	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
		1.9	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
		2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		2.1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
		3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	Petiole length (mm)	1-2	0	1	1	1	0	0	0	1	0	1	0	0	1	0	0
		2.1-3	0	0	0	0	0	1	1	0	0	0	0	1	0	1	1
		5-7	1	0	0	0	1	0	0	0	1	0	1	0	0	0	0
First unifoliate foliage leaf	Petiole length (mm)	3-4	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
		5-6	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
		7-8	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
		9-10	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
		12-15	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
		17-20	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
		23-27	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
		28-29	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
		30-31	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		39-41	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	Petiole surface	Glabrous	1	0	1	1	0	1	0	0	1	1	1	0	1	0	1
		Pilose	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0
		Pubescent	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
	Blade shape	Oblong	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Square	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		depressed ovate	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
		Oblate	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0
		broadly ovate	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0
		Obcordate	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
		Ovate	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
		Elliptic	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
		Cordate	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
		Obovate	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	Blade L/W ratio	0.8	0	0	1	1	0	0	1	1	1	1	0	0	0	0	0
		0.9	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
		1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1
		1.1	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0
		1.3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		2.1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	Blade apex	Acute	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
		Emarginate	0	1	1	1	1	0	0	1	0	1	1	1	1	1	0
		Emargainate apiculate	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
		Rounded	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
		Truncate	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	Blade base	Rounded	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
		Truncate	0	1	1	1	1	1	0	1	1	0	1	0	1	0	1
		Reniform	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
		Cuneate	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
	Blade margin	Ciliate	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Entire	0	1	1	1	0	0	1	1	0	0	0	1	0	1	0

	Blade margin	Dentate	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1
		Serrate	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
		Dentate ⅓ upper	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
		Dentate ½ upper	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Blade surface	Glabrous	1	0	1	1	0	1	0	0	1	1	1	0	1	0	1
		Pilose	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0
		Subglabrous	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
		Pubescent	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
First trifoliate foliage leaf	Petiole length (mm)	4-6	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
		11-12	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0
		15-16	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
		20-22	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0
		25-26	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
		28-30	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
		32-33	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
		40-42	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
	Petiole surface	Glabrous	1	0	1	1	1	1	0	0	1	1	1	0	1	0	1
		Pilose	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0
		Subglabrous	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
		Pubescent	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	Blade shape	Semicircular	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Obovate	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
		Obcordate	0	1	0	1	0	1	1	0	1	0	0	0	1	1	0
		Cordate	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
		Elliptic	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1
		Circular	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
		Oblong	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	Blade L/W ratio	0.7	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
		0.9	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
		1	0	1	1	0	0	1	1	0	0	1	0	0	0	0	0
		1.2	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
		1.3	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1
		1.4	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
		1.9	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
		3.2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	Blade apex	Acute	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Emarginate	0	1	1	1	1	1	1	1	1	1	1	0	1	1	0
		Obtuse	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
		Acuminate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Blade base	Cuneate	1	1	1	0	1	1	1	1	0	0	0	1	1	1	1
		Truncate	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
		Rounded	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0
	Blade margin	Ciliate	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Entire	0	1	1	1	0	0	1	1	0	1	0	1	0	1	0
		Dentate	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1
		Serrate	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
	Blade surface	Glabrous	1	0	1	1	1	1	0	0	1	1	1	0	1	0	1
		Pilose	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0
		Pubescent	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0