

Morphological characterization of *Medicago littoralis* (Fabaceae) in Egypt, with two new varieties.

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Abstract. This study aims at evaluating the macromorphological and seed characters of *Medicago littoralis* Rodhe ex Loisel in Egypt by light and scanning electron microscopy. The results have indicated that plant height, color, stipule margin, pod diameter, seed characters, hilum shape, outline of cells, and relief and thickness of cell boundary are taxonomically important in systematic differentiation between the *M. littoralis* varieties: var. *littoralis*, var. *dentata* and var. *aegyptiaca*. Var. *dentata* and var. *aegyptiaca* are new varieties. A key to identification of the varieties has been prepared.

Key words: *Medicago littoralis*, morphology, seed, SEM

Introduction

Medicago L. is a genus with approximately 87 species of herbs and shrubs, widespread from the Mediterranean to Central Asia (Small & Jomphe 1989; Lewis & al. 2005; Small 2010). Taxonomically, *Medicago*, along with *Melilotus* Mill. (Sweet Clovers) and *Trigonella* L., are included in the tribe *Trigonellinae*, first recognized by Schultz (1901).

Boissier (1872-1873) classified *Medicago* into three sections: *Falcao*, *Spirocarpos* and *Lupulina*. Small & Jomphe (1989), Small (1990a&b) and Gillespie & McComb (1991) stated that *M. littoralis* belonged to the section *Spirocarpos*, subsection *Pachyspirae*.

Heyn (1963) divided *M. littoralis* into two varieties: var. *littoralis* with spiny pods, and var. *inermis* Moris, without spines or with tubercles. He also recorded high variability in the size of spines and the number of coils. Negre (1956) mentioned a form with lobed leaves, f. *laciniatifolia*.

Husain & al. (1994) suggested a key based on the seed coat to identify the studied species of the genus of *Medicago*.

Gandhi & al. (2011) studied 17 legume species belonging to three genera of *Faboideae*, the results showed that the seed coat ornamentation/spermoderm pattern can be helpful in identification of the species.

Medicago littoralis from Israel, Greece, Italy, and Spain seemed to differ morphologically, the plants of each country being substantially discriminable; variations appeared continuous, overlapping, and not conducive to formal infraspecific recognition according to Small & Brookes (1990).

Based on pod characters, Täckholm (1974) reported two varieties of *M. littoralis* in Egypt – var. *littoralis* Rohde ex Loisel and var. *inermis* Boiss., while El Hadidi & Fayed (1994/95) and Boulos (1995, 1999, 2000, 2009) recorded the species without infraspecific taxa.

This work has been undertaken for a more accurate identification of the infraspecific taxa of *M. littoralis* in Egypt.

Material and methods

The present study is based on fresh material collected from natural habitats in Egypt and collections kept in

the Cairo University Herbarium (CAI) and in the Menoufia University Herbarium (MNF) (Table 1, Plate 1).

Seeds study. At least 20 seeds from 10 individuals within the same taxon were examined by light microscopy (LM) to assess the morphological characters and general features of the spermoderm. For SEM microscopy, seeds were mounted on brass stubs and coated with a thin layer of gold and examined with JEOL JSM 530P SEM at the Electron Microscopic Unit, Faculty of Science, Alexandria University. Terminology followed Lersten (1981), Brochmann (1992), Stearn (1992), Kirkbride & al. (2003).

Results

Key to *Medicago littoralis* varieties in Egypt:

- 1a**-Plants up to 20 cm in height, internodes up to 1.5 cm long, pods up to 2 mm in diameter, outer periclinial wall flat **var. littoralis**
1b-Plants over 20 cm in height, internodes longer than 1.5 cm, pods 4–5 × 5 mm in diameter, outer periclinial wall concave **2**
2a-Yellow green plants, stipules lacinate, pods clockwise, hilum elliptic **var. aegyptiaca**
2b-Grey green plants, stipules subulate-dentate, pods anticlockwise, hilum circular **var. dentata**

Medicago littoralis Rohde ex Loisel., Not. Fl. France 118(1810) **var. littoralis** (Plate 2, Table 2)

Syns: *Medicago arenaria* Ten. Cat. Pl. Hort. Neapol. App. 1: 66 (1815). *Medicago cylinderacea* DC., Cat. Pl. Hort. Monsp. 123(1813).

Annual herb, 10–20 cm high, stem erect to procumbent, hairy, branched from base, lower branches angular, upper branches grooved, internodes (0.5)1–1.5 cm long. Leaves trifoliolate (0.8)1–1.5 cm long, petiolate; petiole 0.3–0.5 cm long, lamina 4–5 × 2–4 mm, varies from obtriangular to obovate, both sides densely hairy, terminal leaflets larger than lateral ones, upper two-thirds of leaflet dentate on the margin, apex emarginate, seldom obtuse. Stipules 3–4 × 1–1.5 mm, ovate, lacinate at margin, acuminate at apex. Inflorescence simple raceme, as long as or longer than the bracts, 2–3(5)-flowered. Flowers subsessile; 4.5–5.0 mm long; calyx hairy, 2.5 mm long, teeth longer than tube, tube 0.8–1.0 mm long, teeth 1.2–1.5 mm long; corolla yellow, twice longer than calyx, petals clawed; standards and keels longer than wings, standards 4.5–5.0 mm long, elliptic, obtuse at apex, truncated and with wavy tip; wings 3.5–3.8 mm long, obtuse at apex, up to 1.8 mm long, claw 1.8–2.0 mm long; keels up to 4.2 mm long, obtuse to subacute at apex, claw up to 2.2 mm long. Androecium diadelphous, 4.5–5.0 mm long, tube 3.5–4.0 mm long, free filaments 0.3–0.5 mm long, anthers ovate, 0.3 × 0.2 mm. Gynoecium 4.5–5.0 mm long; ovary 3.0–3.5 mm long, oblong, smooth; style 1.5–2.0 mm long; stigma up to 0.2 mm long, granulated. Pods 1.0–1.5 cm long, as long as or longer than bracts, spiral, anticlockwise, truncated, with 10–12 curved ridges, pod diameter 1.5–2.0 mm. Pod 3–5-coiled, spiny, with spines horizontal to spread, spines 1–2 mm long, with straight apex, sparsely hairy. Seed 3–4 × 1.5 mm, reniform, smooth, yellow-brown, with lateral hilum.

Table 1. Plant material used in the present study.

Taxa	Localities	Geographical coordinates	Collector	Date of collections
<i>Medicago littoralis</i> var. <i>littoralis</i>	Wadi Om Rakham between Matrouh and Agiba (CAI).	–	Täckholm	3–1975
	El-Mathani Bahari, coastal road (MNF).	31°27.999'N 26°45.033'E	Turki & al.	4–2009
	El Hasana road 7 km before El Hasana (CAI).	–	I.El Garf	4–1988
	El Kharga Oasis (CAI).	–	M. Imam	2–1959
<i>Medicago littoralis</i> var. <i>aegyptiaca</i>	Matrouh- Ras El-Hekma road (examined fresh and preserved in Acronym MNF).	31°10.387'N 27°34.807'E	Turki & al.	4–2009
	20 km east of Burg El-Arab, King Mariout-Burg El-Arab road (examined fresh and preserved in Acronym MNF).	31°00.725'N 29°44.947'E	Turki & al.	4–2009
<i>Medicago littoralis</i> var. <i>dentata</i>	Matrouh- Ras El-Hekma road examined fresh and preserved in Acronym MNF).	31°10.387'N 27°34.807'E	Turki & al.	4–2009
	Burg El Arab – El Alamine (examined fresh and preserved in Acronym MNF)	30°56.972'N 29°31.337'E	Turki & al.	4–2010

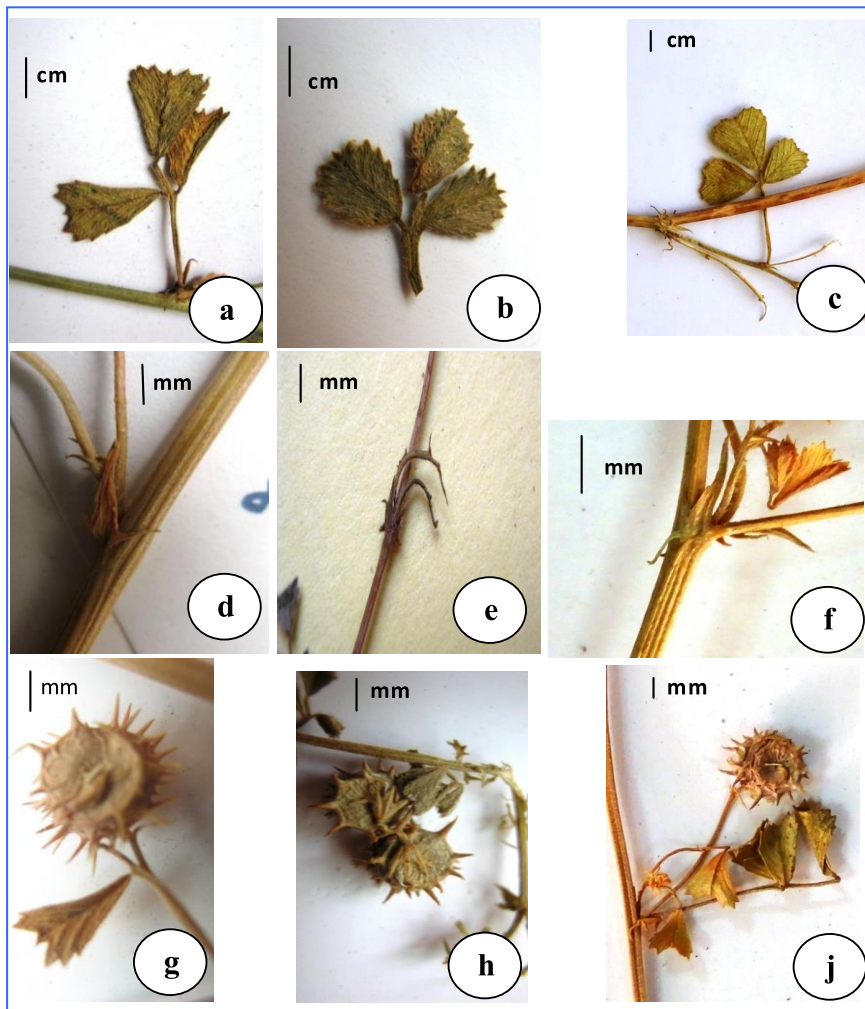


Plate 1. Macromorphological characters of *Medicago littoralis*; **a, d, g**; var. *littoralis*; **b, e, h**; var. *dentata*; **c, f, i**; var. *aegyptiaca*; **a, b, c**; Leaf, **d, e, f**; Stipule, **g, h, i**; Fruit.

Medicago littoralis var. *aegyptiaca* Turki, El Shayeb & Shehata, **var. nov.**

Typus: Egypt, Matrouh- Ras El-Hekma road, 31°10.387'N 27°34.807'E, 2009, Turki & al., MNF.

Plant grayish green, 30–40 cm in height, densely hairy, internodes up to 4 cm long (longer than in the type variety), leaves as long as in the type. Standard obovate, margins serrate, ovary papillate, pods 2–3-coiled, spines horizontal.

Medicago littoralis var. *dentata* Turki, El Shayeb & Shehata, **var. nov.**

Typus: Egypt, Matrouh- Ras El-Hekma road, 31°10.387'N 27°34.807'E, 2009–2010, Turki & al., MNF.

Plant 30–40 cm in height, hairy, internodes 2.5–4.0 cm long (longer than in the type variety), leaves longer than in the type variety, 1.5–2.5 mm long. Stipules subulate-dentate. Inflorescence 1–2-flowered, calyx

teeth longer than tube. Pods clockwise, bigger than in the type variety; 4–5 × 5 mm, seeds oblong.

Seed characters

Seeds reniform-oblong, yellow brown, 0.8–1.4 mm long, with rounded poles in all studied varieties. Seed length-width ratio 1.9–2.4. Hilum lateral in all studied varieties; elliptic in var. *littoralis*, transversely elliptic in var. *aegyptiaca*, circular in var. *dentata*. Seed coat papillate. Outline of cell tetra pentagonal in var. *littoralis*, isodiametric-elliptic in the other studied varieties. Anticlinal wall straight – slightly curved in var. *littoralis*, lobed in the other studied varieties. Relief of cell boundary slightly channeled – superficial in var. *littoralis*, channeled in the other studied varieties. Cell boundary thin in var. *littoralis*, thick in the other varieties. Curvature of outer periclinal wall flat in var. *littoralis*, concave in the other studied varieties (Plate 2).

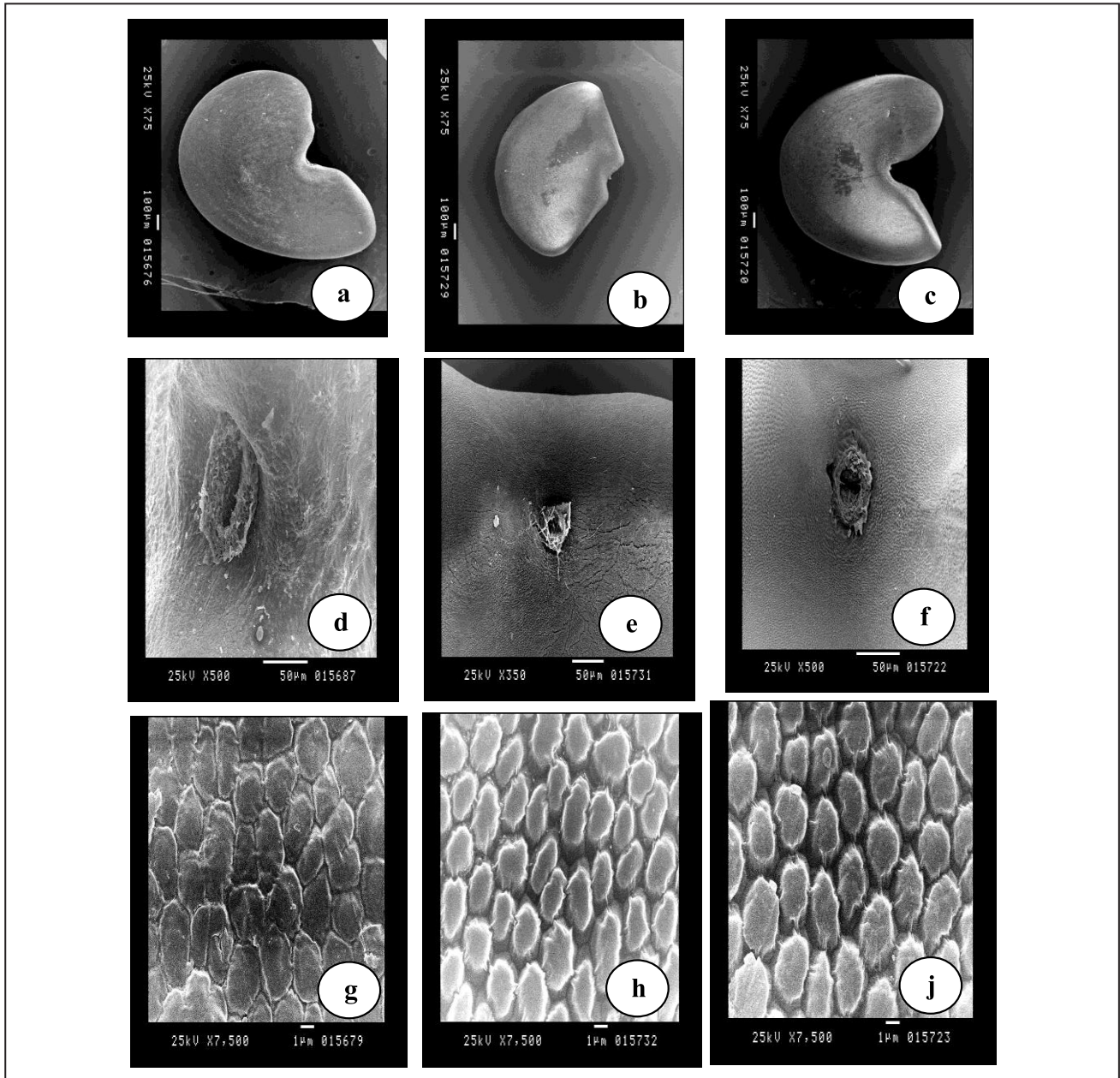


Plate 2. Seed morphology and spermoderm under SEM of *Medicago littoralis* varieties: **a, d, g;** var. *littoralis*; **b, e, h;** var. *dentata*; **I, f, j;** var. *aegyptiaca*; **a, d, g;** Seed **b, e, h;** Hilum position **c, h, m;** Hilum shape **I, f, j;** Seed spermoderm.

Discussion

In the present study, the plant height, hairs, internode length, stipule shape, flower characteristics (number, standard shape, ovary surface), and fruit characters (diameter, spine direction) are considered to be of systematic value in differentiating the studied taxa. Plant height varied between the different taxa: up to 20 cm in var. *littoralis* and more than 20 cm in the other varieties. Plants were densely hairy in var. *aegyptiaca*,

sparsely hairy in the other varieties. Internode length was up to 1.5 cm in var. *littoralis* and more than 1.5 cm in the other two varieties. Stipule shape was subulate-dentate in var. *dentata*, ovate in the other two varieties.

The number of flowers was 1–2 in var. *dentata*, 2–3(5) in the other varieties. Standard shape was obovate in var. *dentata*, elliptic in the other varieties. Ovary was papillate in var. *aegyptiaca*, smooth in the other varieties.

Table 2. Morphological characters of the studied *M. littoralis* varieties.

Taxa		<i>M. littoralis</i> var. <i>littoralis</i>	<i>M. littoralis</i> var. <i>aegyptiaca</i>	<i>M. littoralis</i> var. <i>dentata</i>
Plant	Habit	Erect to procumbent	Erect to procumbent	Erect to procumbent
	Height (cm)	10–20	30–40	30–40
	Colour	Green	Grayish green	Green
Stem	Surface	Hairy	Densely hairy	Hairy
	Internode (cm)	(0.5)1–1.5	(0.5)1–1.5	2.5–4
Stipule shape		Ovate–dentate	Laciniate	Subulate–dentate
Leaflet shape		Obtriangle–obovate	Obovate	Obtriangle–obovate
No of flowers		2–3(5)	2–3(5)	1–2
Ovary		Smooth	Papillate	Smooth
Pod		3–5 coils	2–3 coils	3–5 coils
Seed	Shape	Reniform	Reniform	Oblong
	Color	Deep yellow–pale brown	Deep yellow–pale brown	Deep yellow–pale brown
Seed size	LxW (mm)	1.2–1.4 × 0.6–0.8	1.1–1.3 × 0.8–0.9	1–1.2 × 0.8–0.9
	L/W ratio	1.9	1.4	1.7
Seed poles		Rounded	Truncated end & other rounded	Both ends truncated
Seed coat pattern		Shallow papillae	Striated papillae	Striated papillae
Hilum	Shape	Elliptic	Transversely elliptic	Circular
	Size (µm)	181 × 75	56 × 100	50 × 64
Outline of cells		Tetra pentagonal	Isodiametric–elliptic	Isodiametric–elliptic
Anticlinal wall		Straight–slightly curved	Lobed	Lobed
Relief of cell boundary		Slightly channeled– superficial	Channeled	Channeled
Thickness of cell boundary		Thin	Thick	Moderately thick
Curvature of outer periclinal wall		Flat	Slightly concave	Concave

Täckholm (1974) and Ball (1981) considered the fruit characters as major characters for identification. The present study has indicated that the fruit characters varied between the different taxa: pods up to 2 mm in diameter in var. *littoralis*, 4–5 × 5 mm in diameter in the other varieties. Spine direction was horizontal in var. *aegyptiaca* and horizontal to spreading in the other varieties.

Gandhi & al. (2011) studied 17 legume species belonging to three genera of Faboideae and the results showed that the seed coat ornamentation/spermoderm pattern could be helpful in identification of species.

In the present study, SEM spermoderm investigations have indicated the presence of differences between the studied taxa, represented in hilum shape, which was elliptic in var. *littoralis*, transversely elliptic in var. *aegyptiaca*, and circular in var. *dentata*. Curvature of the outer periclinal wall was flat in var. *littoralis*, and concave in the other varieties.

In the present study, three varieties were recognized within *M. littoralis* according to their macromorphological characters (stipule shape, hairs) and seed coat pattern (curvature of outer periclinal wall), which have

confirmed the morphological results to a great extent. This also revealed that such characteristics can play a decisive role in differentiating between the varieties and distinguishing them from one another.

Based on all earlier criteria, the varieties of *M. littoralis* in Egypt can be divided into var. *littoralis*, var. *dentata* and var. *aegyptiaca*. Both var. *dentata* and var. *aegyptiaca* are new varieties. The characters of all available and examined specimens (deposited in the herbaria and recently collected) do not match the characters of the variety *inermis* as reported by Täckholm (1974).

References

- Ball, P.W. 1981. *Scorpiurus* L. – In: Tutin T.G. & al. (eds), Flora Europaea. Vol. 2, p. 185. Cambridge Univ. Press, Cambridge.
- Boissier, P.E. 1872–1873. Flora Orientalis. Vol. 2, p. 77. H. Georg., Genevae et Basileae.
- Boulos, L. 1995. Flora of Egypt Checklist. Al Hadara Publishing, Cairo.
- Boulos, L. 1999. Flora of Egypt. Vol. 1, pp. 259–289. Al Hadara Publishing, Cairo.
- Boulos, L. 2000. Flora of Egypt. Vol. 1, Al-Hadara Publishing, Cairo.

- Boulos, L.** 2009. Flora of Egypt Checklist. Revised annotated ed. Al Hadara Publishing, Cairo.
- Brochmann, C.** 1992. Pollen and seed anatomy of Nordic *Draba* (*Brassicaceae*) phylogenetic and ecological implications. – *Nordic J. Bot.*, **12**(6): 657-673.
- El Hadidi, M.N. & Fayed, A.A.** 1994/95. Material for excursion flora of Egypt. – *Taekholmia*, **15**: 1-223.
- Gandhi, D., Albur, S. & Pandya, N.** 2011. Morphological and micromorphological characterization of some legume seeds from Gujarat, India. – *Environm. Exp. Biol.*, **9**: 105-113.
- Gillespie, D.J. & McComb, J.A.** 1991. Morphology and distribution of species in the *Medicago murex* complex. – *Canad. J. Bot.*, **69**: 2655-2662.
- Heyn, C.C.** 1963. The annual species of *Medicago*. – *Scripta Hierosolymitana*, **12**: 1-154.
- Husain, S.Z., Aziz, K., Syeda, S.T. & Jahan, N.** 1994. Micromorphological studies of seven species of the genus *Medicago* L. (*Fabaceae*) from Pakistan. – *Pakistan J. Bot.*, **26**(2): 409-419.
- Kirkbride, J.H., Gunn, C.R. & Weitzman, A.L.** 2003. Fruits and Seeds of Genera in the Subfamily *Faboideae* (*Fabaceae*). United States Department of Agriculture, Technical Bulletin 1208 No. 1890.
- Lersten, N.R.** 1981. Testa topography in *Leguminosae* subfamily *Papilionoideae*. – *Proc. Iowa Acad. Sci.*, **88**(4): 180-191.
- Lewis, G.P., Schrire, B. D., Mackinder, B. A., Lock M., (ed.)** 2005. Legumes of the World. Royal Botanic Garden, Kew, UK.
- Negre, R.** 1956. Les luzernes du Maroc. Travaux l'Inst. – *Sci. Cherif. Maroc.*, Ser. Bot., **3**: xxii, 1-120.
- Schulz, O.E.** 1901. Monographie der Gattung *Melilotus*. – *Bot. Jahrb.*, **29**: 660-735.
- Small, E.** 1990a. *Medicago syriaca*, a new species. – *Canad. J. Bot.*, **68**: 1473-1478.
- Small, E.** 1990b. *Medicago rigiduloides*, a new species segregated from *M. rigidula*. – *Canad. J. Bot.*, **68**: 2614-2617.
- Small, E.** 2010. Alfalfa and Relatives: Evolution and Classification of *Medicago*. NRC Research Press, Canada.
- Small, E. & Brookes, B.** 1990. A numerical taxonomic analysis of the *Medicago littoralis* – *M. truncatula* complex. – *Canad. J. Bot.*, **68**(8): 1667-1674.
- Small, E. & Jomphe, M.** 1989. A synopsis of the genus *Medicago* (*Leguminosae*). – *Can. J. Bot.*, **67**: 3260-3294.
- Stearn, W.T.** 1992. *Botanical Latin*. 4th ed. Timber Press, Oregon, USA.
- Täckholm, V.** 1974. Students' Flora of Egypt. Ed. 2, Cairo Univ., Egypt.
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