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Cladistic analysis of the genera: *Trifolium, Trigonella* and *Melilotus* (Fabaceae: Papilionaceae) in Egypt.

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ABSTRACT

The morphological characters of the three genera *(Trifolium, Trigonella* and *Melilotus*) of the tribe *Trifolieae* were analysed and used to construct their phylogenetic relationships. The cladistic analysis reveals strong similarities between the species of genus *Trifolium* which clustred in one clade, and also for species of genus *Trigonella.* For *Melilotus*, all species are strongly related to each other except for *M. albus* which is evolved early and its taxonomic position is unclear. This species is very rare in Egypt (recorded once), the number of flowers per raceme and length of inflorescence in relation to subtending leaves play an important role in the identification of the species. Leaflets are oblong obovate to lanceolate, pods are obliquely ovate to elliptic with sharp acute beak. The seed protein pattern of the most common species of the previous genera was analyzed using Sodium Dodecylsulphate –Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique. The seed protein data showed that all species of genus *Trifolium* form one clad, the second clad includes all species of *Trigonella,* the third clad contains all species of *Melilous.* These data are compatible with taxonomic position of species of the three genera.

KEYWORDS: Cladistics, *Trifolium, Trigonella* and *Melilotus,* Papilionaceae, Egypt

INTRODUCTION

The genera *Trifolium, Trigonella, Melilotus* and *Medicago* are in the tribe Trifolieae of subfamily Papilionoideae (Family Fabaceae). These three genera are closely related to each other and are belonging to tribes *Trigonelleae* and *Trifolieae,* (Schulz 1901). The *Trifolieae* was described by Hyen (1981). Trautvetter (1841) stated that it is difficult to establish clear cut limits between the genera of *Trifolieae.* Linnaeus (1753) classified *Melilotus* as one group under *Trifolium.* Seringe (1825) placed the two genera *Melilotus* and *Trigonella* in a special section known as *Grammocarpus* of genus *Trigonella.* Battacharyya (1958) concluded that *Trigonella* and *Melilotus* might be treated as two subgenera. Heyn (1966) reported the presence of intermediate species between the three genera *Medicago, Trigonella* and *Melilotus.* Therefore, some species of *Medicago* viz. *M.ruthenica* and *M.polycarpa* are considered by most botanists to be intermediates between *Trigonella* and *Medicago.*

The tribe *Trifolieae* seems to be natural units. Its four genera do not constitute a phyletic group; though, there are still some morphological links between its genera (Zohary & Heller 1984). Molecular phylogenetic relashionships of species of genus *Medicago* was reviewed by Bena (1998), who concluded that genus *Medicago* is closely related to the other studied three genera, and it was chosen by this study as outgroup. Small *et al.* (1987) stated that the highly evolved syndrome of floral adaptations provides in *Medicago* an appropriate means of circumscribing the genus. Accordingly, 23 *Trigonella* species were transfered to *Medicago*. Some intermediate characters such as the horn, the stigma and the flower tripping mechanism characterize these species. Moreover, Greuter *et al.* (1989) regarded *Trigonella polyceratia* as a member of genus *Medicago,* which is recognized by Tächkolm (1974) as *Trigonella polyceratia.* Endo & Ohashi (1997) carried out a cladistic analysis of tribes Cicereae, Trifolieae and Vicieae; of which Cicereae and Vicieae were found to be a monophyletic group,

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and Trifolieae was its sister group. Steel *et al* (1997) studied the phylogenetic relationships of the tribes Trifolieae and Vicieae. They pointed out that *Medicago* supported a monophyletic group distinct from that formed by *Melilotus,* which is nested within *Trigonella* and *Trifolium* basal to the remainder of *Trifolieae*.

Due to the existing overlap in identifying the genera of the tribe *Trifolieae* on morphological basis, more accurate and precise criterion; viz electrophoretic analysis of storage proteins, was applied to achieve more convenient generic identification (Robnson & Megarrity 1975; Dallying et *al*: 1979; Wrigley *et al*: 1982; Ferguson & Grobe 1986). The present work represents the first attempt to study the phylogenetic relationship between the three genera in Egypt using morphological characters and variability of seed storage proteins.

MATERIALS AND METHODS

Morphology: A total of 34 species belonging to the genera *Trifolium, Trigonella* and *Melilotus* of tribe Trifolieae were the subject of systematic revision by Kamel (1992), Hassan (1992), and El-Bous (1995). The morphological characters of their species (31charaters) are of taxonomic importance and were scored (Table 1). Many of these characters are used for the first time to construct the phylogenetic relationships.

Seed protein analysis: seeds belonging to sixteen species belonging to. *Trifolium, Trigonella and Melilotus* of different origins were obtained from herbarium of Suez Canal University. Characterization and molecular mass determination of seed storage proteins was carried out using one-dimensional SDS-polyacrylamide gel electrophoresis. Samples were prepared for electrophoresis by extracting protein from 0.5g seed powder in 1cm^3 of 0.2M Tris/Hcl, pH 8, and 1mM phenylmethylsulphonyl fluoride (PMSF) for 2h. in a refrigerator. The extract was centrifuged at 5000g for 20 min. and proteins in the supernatant were precipitated with 5 volumes of cold acetone at –20 ^{0}C for 2h. Pellets obtained after centrifugation were dissolved in 0.02 ml of sample buffer (0.125 M Tris/Hcl, pH6, 8, 2% m/v SDS, 10% m/v sucrose, 1% v/v β-mercaptoethanonl, 0.1% m/v bromophenol blue) and denaturated by heating at 80 ^{0}C for 2-3 min. 17% SDS-polyacrylamide gel slabs were prepared as described by Laemmli (1970). Equal amounts of protein were loaded per lane. Electrophoresis was carried out in Tris/glycine. SDS running buffer (0.25M Tris, 1.88M glycine 0.1% SDS) using vertical gel electrophoresis units (Mini-protein cell, BioRad, USA) at 140 V for the first 15 min followed by 150 V until the indicator dye reached the bottom of the gel. Gels were stained over night in 20 cm^3 of 0.25% acetic acid, 50% (v/v) methanol, 7% (v/v) glacial acetic acid and destained by shaking overnight in 50% methanol and 7% glacial acetic acid. The relative mobility of the band was calculated and the presence or absence of each band was treated as a binary character (Table 2).

The cladistic analysis of the protein and the morphological characters are followed the principles of phylogenetic reasoning elaborated by Hennig (1966). The results were analyzed by computer using Henning 80 program of Farris. The data were entered in computer with the assumption that species *Medicago sativa* was considered to be the outgroup because it is a native species to the Old World as mentioned by Small (1987).

RESULTS

Analysis of the morphological characters (Table 1)

1. **Life Span:** annual (0), perennial (1).
2. **Stem:** procumbent (0), erect or decumbent (1), ascending (2), prostrate (3).
3. **Indumentum:** Hairy (0), and glabrous (1).
4. **Stipules shape:** Triangular oblong (0), ovate (1), and lanceolate (2).
5. **Stipules margin:** Entire (0), dentate (1), and incised (2).

6. Leaf shape: obovate (0), Elliptical (1), and lanceolate (2).
7. Leaf margin: Entire (0), dentate to denticulate (1), and serrate to serrulate (2)
8. Leaf apex: acute to acuminate (0), rounded to truncate (1), emarginate or mucrounate (2).
9. Calyx teeth: shorter than calyx tube (0), as long as calyx tube (1), longer than calyx tube (2).
10. Corolla colour: white -to cream (0), yellow (1), and pink to mauve (2).
11. Stander length: longer than keel and wing (0), shorter than keel and wing (1).
12. Stander: distinct into limb and claw (0), not distinct into limb and claw (1).
13. Apex: notched (0), and obtuse (1).
14. Wing shape: oblong (0), oblanceolate (1).
15. Wing claw: shorter than Limb (0), or claw as long as limb (1), also claw longer than limb(2).
16. Wing horn: present (0), absent (1).
17. Keel shape: oblong (0), ovate (1).
18. Keel apex: obtuse or rounded (0), Acute (1).
19. Filament of Stamens: connate for nearly; 2/3 of its length (0), connate for 1/2 of its length (1), or 3/4 of its length (2).
20. Shape of Ovary: Linear-oblong (0), or subglobular to ovate (1), cylindrical (2).

Table 1: Data matrix of the 31 morphological characters used to construct the phylogeny of the three genera (*Trifollium, Trigonella & Melilotus*) of family Papilionaceae of Egypt

	Characters
Species	1 1 2 3 0 0 0
Medicago sativa	1202011202011000001001103100000
Trifolium alexandrinum	0202011210000020010100001020211
Trifolium tomentosum	0201101112000020110000002032112
Trifolium repens	1311002210001011001010013031012
Trifolium stellatum	0201101220001010011100001022111
Trifolium resupinatum	0200001112000000010000001030011
Trifolium campestre	0201001121011100010101103020000
Trifolium argutum	0200020222000000010100011030000
Trifolium incarnatum	0201101222001020010100011032112
Trifolium nigrescens	0312001110000000000010003032011
Trifolium glanduliferum	0211201222000000000100002032110
Trifolium angustifolium	0310002202101011000101001030010
Trifolium fragiferum	1312221110000111000000002032012
Trifolium philistaeum	0211011022011120010101103022111
Trifolium scabrum	0201201110001020000000001030210
Trifolium lappaceum	0210010222001020010100011030010
Trifolium purpureum	0202021222001000010100011030110
Trifolium dasyurum	0201210222101020000100011022011
Trifolium dichroanthum	0200011122001020010100011021011
Trigonella maritima	0111201201000000110021000112100
Trigonella arabica	0211200200000000100021000012010
Trigonella stellate	0010001201100000010010103212100
Trigonella cylindracea	0102001201000000001201103212100
Trigonella glabra	0111201201000000002210103132000
Trigonella laciniata	0102201211010000000111101120000
Trigonella occulta	0000101211000120100101111130000
Trigonella foenum-graecum	0110011201000000102121100232010
Trigonella polyceratia	0110001001000020102010100132000
Trigonella anguina	0312201211110010102121003122000
Melilotus indicus	0102001201011000000101003120010
Melilotus segetalis	0100201201000000001101012221010
Melilotus sulcatus	0212201101100000000101002201010
Melilotus messanensis	0200101111000000002101001212000
Melilotus albus Medicus	1202002100011000001010001110010
Melilotus serratifolius	0200212011000001021010022110000

21. Number of ovules: from one to two (0), from three to eight (1), or from five to twenty (2).
22. Ovary: glabrous (0), hairy (1).
23. Style length: longer than ovary (0), or shorter (1).
24. Style: curved (0), straight at apex (1).
25. Pod shape: or linear (0), ovate to elliptical (1), obovate (2), curved oblong-circular (3).
26. Pod surface: membranous (0), with concentric striation (1).
27. Pod apex: obtuse (0), sharp acute (1), mucronate (2), or tapering (3).
28. Seed shape in outline: ovate (0), oblong to circular (1), or oblong ovate (2).
29. Seed colour: brown (0), or yellow (1), orange red (2).
30. Surface of seed coat: smooth (0), wrinkled (1).
31. Length of peduncle: from 0-4 cm (0), from 4-10 cm (1), of from 10-33 cm (2).

Cladogram (Figure 1)

The cladogram is based on 31 morphological characters, each number at a node or branching point representing a hypothetical ancestor and with the numbers of lines correspond to the characters tabulated in table 1.

Only one tree resulted as shown in (Figure 1), with two main clades. The first clade includes all *Trifolium* species. *Trifolium argutum* evolved early based on characters: leaf margine serrate to sirrulate, calyx teeth is shorter than calyx tube and ovary hairy. Other *Trifolium* species are separated in two groups or two subgenera separated at nodes 59, 60. The first node at node 59 uses the characters: leaf obviate, calyx teeth as long as calyx tube and style up curved at apex, while node 60 carries different characters: leaf lanceolate, calyx teeth longer than calyx tube and style straight. The first subgenus can also be separated into two

subgroups at node 55, including *Trifolium alexandrinum*, *T. scabrum*, *T. stellatum, T. incarnatum, T. tomentosum* and *T. glanduliferum*. *Trifolium alexandrinum* considered as basal group to the other members in this group by having stipule shape ovate and its margin dentate, *Trifolium glanduliferum* is a sister to *Trifolium tomentosum*. *Trifolium glanduliferum* having hairy plants, margin of stipules incised, leaf apex rounded, calyx teeth as long as calyx tube, claw of wing longer than limb, keel shape ovate, keel apex acute and length of peduncle from 0-4 cm. *Trifolium tomentosum* is a glabrous plant or glabrescent or with sub apprised to patent hairs, stipules margin dentate, leaf apex rounded, and length of peduncle 10 – 33 cm.
The second subgroup at node 56, includes *T. resupinatum, T. nigrescens, T. repens, T. frgiferum. Trifolium resupinatum* in this group is basal to others since it has stem ascending, plant hairy, corolla yellow, keel apex acute and seed shape is ovate. *T. repens* is sister to *T.fragiferum*. The first has stipules ovate, with entire margine, leaf obovate, with serrate to serrulate margine, leaf apex retuse, stander apex obtuse, staminal tube of androecium connate for half of its length, style straight, pod curved oblong and seed shape oblong to circular. *Trifolium fragiferum* has lanceolate stipules, and its margin incised, leaf shape lanceolate, with dentate margin and apex rounded, stander apex notched, staminal tube of androecium connate for 2\3 of its length, style curved at apex, pod shape obovate and seed shape oblong ovate.
The second group of genus *Trifolium* is separated at node 60 to including *T. purpureum, T. angustifolium, T. lappaceum, T. dasyurum, T. dichroanthum, T. campestre and T. philistaeum. Trifolium purpureum* is basal group having calyx teeth longer than calyx tube, ovary glabrous and style straight. *Trifolium campestre and T. philistaeum.* Are closely related species, *Trifolium campestre* is hairy plants, obovate leaf and a rounded apex; the claw of wing is shorter than limb, seed shape ovate, seed colour brown, and surface of seed coat smooth and length of peduncle 0-4cm. *Trifolium philistaeum,* glabrous, leaf shape is elliptic, and its apex is acute, the claw of wing is longer than limb, seed shape oblong ovate, seed colour yellow, surface of seed coat wrinked and length of peduncle 4-10 cm.

The second largest clade includes *Trigonella* and *Melilotus* species, which is separated from other clade at node 65 which has the characters: stem erect to decumbent, corolla yellow, stander are not distinct into limb and claw, pod ovate elliptical. At node 61, *Trigonella occulta* has evolved early based on having: stipules margin dentate, wing shape oblanceolate, style straight, seed shape ovate and is regarded as basal species for other *Trigonella* and *Melilotus* species.

At node 58, *Trg. cylindracea, Trg. stellata* and *Trg. maritima, Trg. arabica* clustered together based on the characters: staminal tube connected for nearly 2/3 for its length, gynecium linear oblong, pod surface *reticulate. Trg. cylinderacea* and *Trg. stellata* are closely related to each other, while *Trg. maritima* and *Trg. arabica* are forming another sister species. At node 54, *Trg. polycerata*, *Trg. foenum – graecum and Trg. glabra* clustered together on: number of ovules 3 – 8, style shorter than ovary, pod apex tapering. On the other hand, *Trg. foenum _ graecum and Trg. polyceratia* are sister species having stipules margine dentate and pod shap linear

For genus *Melilotus, M. segetalis* is considered as sister species to other three species (*M. messanensis, M. sulcatus,* and *M. serratifolius)* which has the characters: stem erect to decumbent, leaf apex retused emarinated mucronate and pod apex tapering. *Melilotus segetatis* is considered as basal species at node 35 having standard length shorter than keel and wing, seed shape oblong to circular. *Melilotus albus* comes early in this tree due to its biennial, mature leaflets are oblong obovate to lanceolate, the number of flowers per raceme and length of inflorescence in relation to subtending leaves play an important role in the identification of species (30-60 flowers), corolla is white, pods are obliquely ovate to elliptic with sharp acute beak.

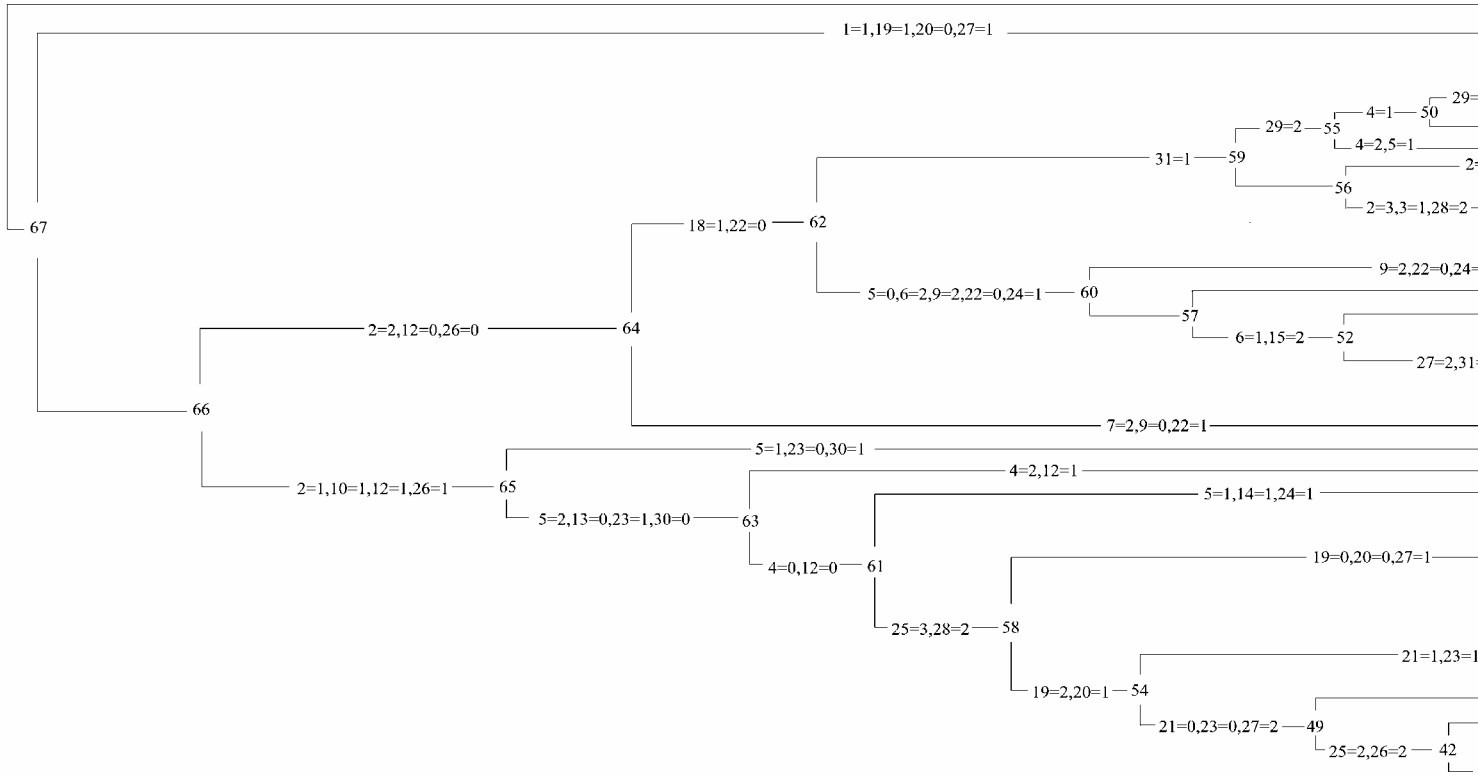

Figure. 1: Morphological cladogram for the Egyptian three genera (Subfamily: Papilionidae) showing the relationship between them (Length=241, Ci=

Me. Medicago, Mel. Melilotus, Tri. Trifolium, Trg. Trigonella

Protein analysis (Table 2 and Figure 2)

1- Band with molecular weight 160 KD: in all species of *Melilotus*, two *Trigonella* (*stellata* and *cylindraceae*), and in two *Trifolium* (*stellatum* and *resupinatum*).
2- Band with molecular weight 130 KD: in all *Trigonella* and *Trifolium*.
3- Band with molecular weight 110 KD: in two *Trifolium* (*stellatum* and *angustifolium*), in all *Melilotus* except *messanensis*
4- Band with molecular weight 95 KD: in *Trifolium carnatum* and in three *Trigonella* (*maritime*, *stellata* and *cylindraceae*).
5- Band with molecular weight 90 KD: in all *Trigonellae* and two *Trifolium* (*stellatum* and *incarnatum*).
6- Band with molecular weight 80 KD: in all *Melilotus* except species *messanensis*, and in five *Trifolium* (*alexandrinum, tomentosum*, *repens*, *resupinatum* and *campestre*).
7- Band with molecular weight 75 KD: in three *Trifolium* (*stellatum*, *angustifolium* and *incarnatum*), also in two *Trigonella* (*maritima* and *stellata*), and in all *Melilotus* except *messanensis*.
8- Band with molecular weight 73 KD: in three *Trifolium* (*stellatum*, *angustifolium* and *incarnatum*), also in *Trigonella stellata*, and in three *Melilotus* (*indicus*, *segetalis* and *messanensis*).
9- Band with molecular weight 71 KD: only in three *Trifolium* (*tomentosum*, *repens* and *campestre*)
10- Band with molecular weight 69 KD: in two *Trifolium* (*tomentosum* and *repens*), and in *Melilotus indicus*.
11- Band with molecular weight 67 KD: in three Melilotus (*segetalis*, *sulcatus* and *messanensis*)
12- Band with molecular weigh 65 KD: only in *Melilotus indicus*.
13- Band with molecular weight 62 KD: in two *Trifolium* (*angustifolium* and *incarnatum*), also in two *Trigonella* (*arabica* and *cylindraceae*)
14- Band with molecular weight 60 KD: in all *Trifolium* and in two *Trigonella* (*maritima* and *cylindraceae*)
15- Band with molecular weight 56 KD: in all *Trigonella*
16- Band with molecular weight 52 KD: in three *Trigonella* (*maritima*, *arabica* and *stellata*)
17- Band with molecular weight 48 KD: only in *Trigonella cylindraceae*.
18- Band with molecular weight 46 KD: in all *Trifolium* and in two *Melilotus* (*indicus* and *sulcatus*)
19- Band with molecular weight 44 KD: only in *Trigonella maritime*.
20- Band with molecular weight 42 KD: in all *Trigonella*.
21- Band with molecular weight 40 KD; in all *Trigonella.*
22- Band with molecular weight 38 KD: in two *Melilotus* (*sulcatus* and *messanensis*).
23- Band with molecular weight 36 KD: in two *Trifolium* (*angustifolium* and *incarnatum)*
24- Band with molecular weight 35 KD: in *Trifolium angustifolium* and *incarnatum.*
25- Band with molecular weight 33 KD: in *Melilotus segetalis and sulcatus.*
26- Band with molecular weight 31 KD: in *Melilotus segetalis and sulcatus.*
27- Band with molecular weight 30 KD: in all *Melilotus.*
28- Band with molecular weight 29 KD: in all *Melilotus.*
29- Band with molecular weight 28 KD: in five *Trifolium (alexandrinum, tomentosum, repens, stellatum, and resupinatum).*
30- Band with molecular weight 26 KD: only in *Trifolium compestre.*
31- Band with molecular weight 24 KD: in all *Trifolium.*
32- Band with molecular weight 22 KD: in all *Trifolium* except (*repens* and *angustifolium*)
33- Band with molecular weight 21 KD: in three *Trifolium (alexandrinum, angustifolium* and *incarnatum)*
34- Band with molecular weight 20 KD: in *Trifolium alexandrinum* and *stellatum.*
35- Band with molecular weight 19 KD: in *Trifolium and Trigonella, also Melilotus indicus.*
36- Band with molecular weight 18 KD: in *Trifolium* and *Trigonella*, also in *Melilotus* except *indicus.*
37- Band with molecular weight 17 KD: in four *Trifolium (alexandrinum, stellatum, angustifolium, and incarnatum).*
38- Band with molecular weight 16 KD: in all *Trifolium* species.
39- Band with molecular weight 15 KD: in all *Trifolium* species.
40- Band with molecular weight 14 KD: in all *Melilotus* species.
41- Band with molecular weight 13 KD: in all *Melilotus* species.
42- Band with molecular weight 12 KD: in all *Trigonella* species.
43- Band with molecular weight 10 KD: in all *Trigonella* species.

Table 2: Data matrix of the 43 protein pattern characters used to construct the phylogeny of the three genera (*Trifollium, Trigonella & Melilotus*) of family Papilionaceae of Egypt. (0= band absent, 1= band present).

Species	Characters 1	1	2	3	4
	0	0	0	0	0
Medicago sativa	00000000000000000000000000000000000000000000				
Trifolium alexandrinum	01000100000001000100000000001011111111110000				
Trifolium tomentosum	01000100110001000100000000001011001101100000				
Trifolium repens	01000100110001000100000000001010001101100000				
Trifolium stellatum	11101011000001000100000000001011011111100000				
Trifolium resupinatum	11000100000001000100000000001011001101100000				
Trifolium campestre	01000100100001000100000000000011000110110000				
Trifolium argutum	01100011000011000100011000000111011111100000				
Trifolium incarnatum	01011011000011000100011000000111011111100000				
Trigonella maritima	01011010000001110011100000000000000110000011				
Trigonella arabica	01001000000010110001110000000000000110000011				
Trigonella stellata	11011011000001110001110000000000000110000011				
Trigonella cylindraceae	11011000000010101001100000000000000110000011				
Melilotus indicus	10100111010100000100000000110000000100001100				
Melilotus segetalis	10000010001000000000000001111000000010001100				
Melilotus sulcatus	10100111001000000100000011110000000011001100				
Melilotus messanensis	10100111001000000000000000110000000011001100				

Cladogram (Figure 3)

The electrophoretic pattern of the most common species of the three genera is shown in Fig.2. Protein marker with wide range (2-212 KD) which contains 13 bands as analyzed by SDS-PAGE in which 3 bands (97,36, and 20) have high intensity. A great homology of protein patterns within range 160-73 KDa can be recognized amongst all studied genera except *Melilotus* and *Trifolium* which showed the absence of one band 95 KD and two extra bands at the range of 110-80 KD, where *Melilotus* shows the absence of the two bands at 90 and 130 KD respectively. Another area of great similarity in protein profiles was observed in the range 19-18 KD, where are agreed similarity in protein profiles was observed, between *Melilotus* and *Trigonella* in 11 bands ranged from 71-15 KD. The similarity between *Trigonella* and *Trifolium* is also observed within the range 130-13 KD, where 7 bands were shared between *Melilotus* and *Trifolium* ranged from 56-38 KD

Electrophoretic protein profiles corresponding to different accessions of the different species in *Melilotus* showed 7 indentical patterns (67, 33, 31, 30, 29,14 and 12 KD). There are 5 bands that characterized *Trigonella* species at 56, 46, 42, 40, 38 KD, while in *Trifolium* the electrophoretic protein profiles showed 6 identical patterns (71,28,24,20, 16,15 KD).

On the basis of 43 protein storage characters, only one tree resulted from the cladogram of the 16 Egyptian species (Figure 3), which it distinguished into two main monophyletic groups based on nodes 30, 29.

The first clade includes genus *Melilotus*; *M. segetalis* is a sister group to the monophyletic group due to the absence of bands 3, 6 and 8. These bands with molecular weight 110 KD, 80 KD and 37 KD which are present in all other member of the genus. *Melilotus sulcutus* with character 36 has a band with 23 KD is a sister to species *M. indicus*.

The second largest clade includes two sub-clades: the first includes all *Trigonella* group, *Trigonella arabica* is considered as a basal species by the absence band with molecular weight 62 KD. *Trigonella stellata* is sister to *T. maritima* because they share bands of molecular weight 60 & 44 KD.

On the other hand, *T. cylinderica* separated having band with molecular weight 60 KD. The other sub-clade which nested all *Trifolium* species, *T. campestre* is considered as a basal group which has bands of molecular weight 50, 46 26, 24, 16 & 15 KD. *Trifolium argutum* is sister to *T. incarnatum* having bands with molecular wight 35 & 21KD.

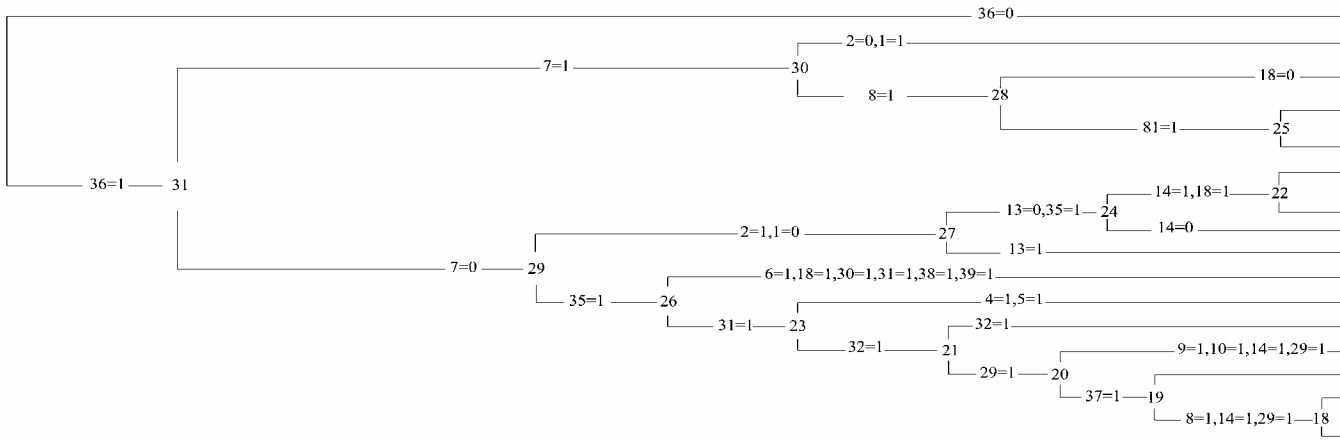

Figure 3 : Cladogram of the Egyptian genera (*Trif., Trg., Mel.*) of the tribe Trifolieae, Subfamily: Papilionideae, showing the relationship between th
Me. Medicago, Mel. Melilotus, Trg. Trigonella, Tri. Trifolium.

DISCUSSION

The present study aimed to analyze 31 morphological characters and 43 seed storage protein characters. The first analysis based on morphological characters suggests that there are two clades in one tree among all species of the three genera examined. The first clade reveals strong similarities between *Melilotus* and *Trigonella* (Sering 1825; Battacharyya 1958; Heyn 1966). These two sister genera based on sharing the characters: stipules margin incised, stander apex notched, style longer than ovary and surface of seed coat smooth. In this clade *Melilotus indicus* comes out early as a basal group to the other members at the node 65 which has the characters: stipule margin entire, stander apex obtuse, style longer than ovary and surface of seed coat wrinked. *Melilotus albus* evolved early depending on the characters: perennial plant, staminal tube connate for half of its length, gynoecium linear oblong and pod apex sharp acute. Our results agree completely with those of Shulz (1901) and Survorov (1950) who classified *Melilotus* into two subgenera; one includes *M. albus* and the other

subgenus included two sections; one including *M. indicus* and the other including all the other species investigated.

The second clade includes all species of the genus *Trifolium* sharing these characters: stem ascending, corolla pinks to mauve, stander distinct into limbe, claw and pod surface membranes. According to Steel *et al*. (1997) *Trifolium* is basal to the remainder of Trifolieae.

The protein analysis suggests one tree with 3 clades. The first clade includes genus *Melilotus* sharing bands with molecular weight 160 and 75 KD, *M. segetalis* evolved early based on the presence of the band with molecular weight 18 KD and the absence of bands with molecular weight 110, 80 and 73 KD. The second clade at the node 27 comprised all species of *Trigonella, T. stellata, maritima* and *cylindracea* which are evolved together depending on the presence of the bands 95, 75, 60 KD.

Trigonella arabica is basal group in this clade by having a band with molecular weight 62 KD. While *T. stellata* and *maritima* are sister species depending on having bands with molecular weights 95, 80, 71, 60, 24, 16 and 15 KD.

The third clade, at the node 26 is then separated on the basis of bands with molecular weight 80, 24, 16, 15 KD. *Trifolium campestre* has evolved from the hypothetical ancestor number 23 based on having the band with molecular weight 80, 46, 26, 24 KD. *Trifolium angustifolium* and *incarnatum* are sister species, due to sharing the intensity of bands with molecular weight 130 and 21 KD.

In conclusion, the morphological tree reflects that *Melilotus* is nested within *Trigonella* and that *Trifolium* is basal in this tree Steel *et al*. (1997) while *M. albus* has a special taxonomic position and collected only once from one locality El-Hadidi (1956). *Melilotus albus* is believed adventive in Egypt (El-Bous 1995).

The seed protein profiles are powerful discrimination tool for the identification of the three genera. In addition, seed protein profiles may provide evidence of the genetic relationships between *Trifolium*, *Trigonella*, and *Melilotus*.

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الملخص العربي

دراسة وتحليل تطور أجناس ترافوليام - ترايجونيللا- مليلوتس (تحت الفصيلة الفراشية) في مصر

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تم دراسة تطور جميع الأنواع التابعة لأجناس (ترافوليام – ترايجونيللا – مليلوتس) من قبيلة ترافويلي. بالتحليل الإحصائي للصفات المورفولوجية باستخدام برنامج هينج والذي يساهم في رسم وبيان العلاقات التطورية بين الأنواع المختلفة, أوضحت الدراسة أنه يوجد أرتباط قوي بين جميع الأنواع داخل جنس ترافوليام وكذلك الأنواع داخل جنس ترايجونيللا بينما في جنس مليلوتس فقد أنفصل مبكرا نوع واحد فقط هو مليلوتس البس ويعزي هذا الانفصال الي وضعة التصنيفي الغير واضح بالإضافة إلى صفاته التصنيفية المختلفة عن باقي الأنواع داخل هذا الجنس.

كما تم تحليل التركيب البروتيني لبذور الأنواع الشائعة من الأجناس الثلاثة وذلك باستخدام طريقة الفصل الكهربائي والتي أوضحت أن كل الأنواع التابعة لجنس الترافوليم مرتبطة من الناحية التطورية وكذلك الأنواع التابعة لجنس ترايجونيللا ومليلوتس.