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Palynological Study Within Tribe *Trifolieae* (Leguminosae)

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Abstract: The pollen morphology of 41 species belonging to tribe *Trifolieae*, 4 taxa to *Ononis*, 8 to *Trigonella*, 13 to *Medicago*, 4 to *Melilotus* and 12 to *Trifolium* has been studied using both light and scanning electron microscopes. The results revealed that this tribe is stenopalynous with different pollen types. According to present data the species studied have been classified into three pollen types with five subtypes. These types not in accordance with the taxonomic divisions of these genera. General pollen descriptions of the genera as well as a discussion about the classification of the genera has been done. An evolutionary line according to pollen characters has been postulated.

Key words: Palynological study, classification, plant taxonomy, evolution

INTRODUCTION

The pollen grains of the leguminous plants have been studied^[1-7]. These works, among others, dealt mainly with the description of the pollen grains of certain genera or sometimes tribes. Tribe *trifolieae* as proposed by Berchtold and Presl^[8] characterized by having trifoliate leaves and stipules adnate to the petioles, include seven genera. These genera have been faced with a lot of taxonomic opinions after the progression in using modern tools in taxonomy. In the early nineteens, Schulz^[9] gathered the genera *Trifolium*, *Parochetus* and *Ononis* in a tribe called *trifolieae*, according to seed characters. Hutchinson^[10] separated the genus *Ononis* in a monogeneric tribe *ononideae* and the rest of the genera i.e. *Trigonella*, *Parochetus*, *Melilotus*, *Medicago*, *Factorovskya* and *Trifolium* in tribe *Tridfolieae*. This treatment of the genera has been accepted^[11-14]. The separation of the genus *Ononis* while the other six genera have been subdivided into two subtribes; subtribe *Trifoliinae* with the genus *Trifolium* only and subtribe *Trigonellinae* with the rest of the genera^[15-18]. This arrangement of the genera has been accepted by Taia (unpublished) with the addition of separating genus *Medicago* in another monogeneric subtribe *Medicagonae* according to seed characters. Meanwhile Chaudhary and Sanjappa^[19] separated the genus *Parochetus* in a new subtribe *Parochetinae* with the approval of arrangement of the other genera^[15].

For that, genera within tribe *Trifolieae* still in need to more taxonomical studies using the modern tools in order to achieve a more natural classification of the group. Step forward in this direction, this work has been done to understand the relationships between these genera, their taxonomic position and their evolutionary line.

MATERIALS AND METHODS

The pollen grains of forty one species, belonging to the Egyptian *Trifolieae*, distributed over five genera; *Ononis*, *Trigonella*, *Medicago*, *Melilotus* and *Trifolium*; have been gathered and studied carefully by both light and scanning electron microscopes. A list of the materials obtained, their locations and collectors are summerised in Table 1. The pollen grains were acetolysed according to Erdtman^[20] and mounted in glycerine jelly for light microscope investigation, at least 30 pollen grains are measured from each species. Nonacetolysed, dry pollen grains were sputtered onto clean stubs, coated with 30 nm. Gold, for scanning electron microscope examination. At least 30 pollen grains from each taxa are measured under the light microscope. Samples of mature, dry anthers were adhered to clean stubs, coated with gold and scanned with the SEM. The terminology used here are according to Faegri^[21]. This work has been done during 1999 and 2002.

Table 1: Taxa investigated, source, locality, collector and date

Source/Locality/Collector and Date	Taxa
AUH/ Matruh-Siwa road/Ayyad <i>et al.</i> , 21-3- 1989	<i>Ononis vaginalis</i> Vahl.
AUH/Burg El-Arab/El-Ghazaly <i>et al.</i> , 2-4-1982	<i>O. reclinata</i> L.v.minor
AUH/Matruh, wadi El-Ramla/Tadros, 14-4-1948	<i>O. sicula</i> Guss.
AUH/Rosetta/Rizk <i>et al.</i> , 28-3-1992	<i>O. serrata</i> Forssk.
AUH/El-Arish/Boulos, 18-3-1955	<i>Trigonella arabica</i> Del.
AUH/Burg El-Arab/Ayyad <i>et al.</i> , 27-2-1988	<i>T. anguina</i> Del.
CAI/Helwan desert/Ghabour and Mostafa, 13-3-1959	<i>T. occulta</i> Del.ex DC.
AUH/ Mariut/ Zidan, 15-3-1994	<i>T. monspeliaca</i> L.v.nuda
CAI/Sinai, EL-Hasnaa/Shamso, 3-4-1988	<i>T. stellata</i> Forssk.
AUH/ Burg El-Arab/ Rizk <i>et al.</i> , 24-3-1981	<i>T. laciniata</i> L.v.subsessilis
AUH/Siwa oasis / Ayyad <i>et al.</i> , 4-4-1978	<i>T. maritime</i> Del.
AUH/ El-Arish/ Boulos, 18-3-1955	<i>T. hamosa</i> L.
AUH/ Burg El-Arab/ Ayyad <i>et al.</i> , 3-4-1986	<i>Medicago marina</i> L.
AUH/ Botanical garden, Faculty of Science, Alexandria/Ahmed, 10-8-1987	<i>M. lupulina</i> L.
AUH/Matruh- El-Sallum road/ Ahmed, 24-4-1993	<i>M. coronata</i> (L.) Bartal.
AUH/ El-Omayed/ Zedan, 14-3-1988	<i>M. orbicularis</i> (L.) Bartal.
AUH/ Portawfiq, Suez/ Taia, 14-4-1992	<i>M. polymorpha</i> L.
AUH/ Siwa oasis/ Ayyad <i>et al.</i> , 4-4-1978	<i>M. granadensis</i> Willd
AUH/ El-Omayed/ Zedan, 14-3-1988	<i>M. littoralis</i> Rohde ex Loisel
AUH/ El-Omayed/ Zidan, 14-3-1988	<i>M. truncatula</i> Gaertn.
CAI/ Giza, near the pyramids/ El-Hadidi <i>et al.</i> , 20-3-1989	<i>M. turbinata</i> (L.) All.
AUH/ Burg El-Arab/ Fakhry, 21-4-1991	<i>M. intertexta</i> (L.) Mill.
AUH/ Matruh-Siwa road/ Ahmed, 12-4-1987	<i>M. minima</i> (L.) Bartal
AUH/ Burg-El-Arab / Fakhry, 21-4-1991	<i>M. laciniata</i> (L.) Mill.
K/ Montpellier / Tadros, 11-8-1948	<i>M. sativa</i> L.
CAI/ Ras El-Hekma/ Taclholm <i>et al.</i> , 30-4-1955	<i>Melilotus alba</i> Medic
CAI/ El-Sharkiya / Amer, 8-2-1982	<i>M. sulcata</i> Desf.
AUH/ El-Mansoura / Boulos, 5-2-1981	<i>M. siculus</i> (Turra)B.D.Jacks.
AUH/ El-Bousily/ Rizk <i>et al.</i> , 21-3-1989	<i>M. indica</i> (L.) All.
AUH/ Burg El-Arab/ El-Ghazaly <i>et al.</i> , 7-2-1981	<i>Trifolium fragiferum</i> L.
AUH/ El-Bousily / Rizk <i>et al.</i> , 21-3-1989	<i>T. resupinatum</i> L.
AUH/ Alexandria-Matrouh road/ Rashad, 20-3-1975	<i>T. tomentosum</i> L.
AUH/ El-Omayed / Zedan, 14-3-1988	<i>T. procumbens</i> L.
CAI/ Sinai, El-Hasnaa/ Shamso, 3-4-1988	<i>T. phlilistaeum</i> Gruenb. et Zoh.
AUH/ El-Omayed, in sand dunes/ Zedan, 14-3-1988	<i>T. nervulosum</i> Boiss. & Heldr.
AUH/ Alexandria-Matrouh road/Rashad, 20-3-1975	<i>T. repens</i> L.
AUH/ Samouha/ Abdel-Aziz, 6-1-1948	<i>T. alexandrinum</i> L.
AUH/ Matruh-Siwa road/ Ayyad <i>et al.</i> , 21-3-1989	<i>T. scabrum</i> L.
AUH/ Burg El-Arab/ Fakhry, 23-3-1992	<i>T. stellatum</i> L.
AUH/ Saint Katren/ Ayyad <i>et al.</i> , 15-4-1982	<i>T. purpureum</i> Loisel
AUH/ Matrouh-Siwa road/ Ayyad <i>et al.</i> , 12-4-1987	<i>T. desvauxii</i> Boiss. et Bl.

(The arrangement of the genera and species is according to Tackholm, 1974)

Abbreviations used: AUH= Alexandria University Herbarium, CAI= Cairo University Herbarium, K= Kew gardens

RESULTS

The pollen grains in tribe *trifolieae* are heterogenous which exhibit considerable range of variations. They varies in size, shape, number and type of apertures and even in exine ornamentation. These variations, especially those of apertures, considered the bases of distinguishing three pollen types and five subtypes within the tribe.

General pollen morphology for the genera

Genus *Ononis*: Pollen grains small to medium sized ranging from: P = (20-) 33 (-42); E = (-12) 24 (-30) Um. Subprolate or prolate, tricolporate or tricolpate. Colpi extending onto the poles, tapered towards the poles with finely granulated or smooth membrane and either ornamented or smooth margins. The apocolpi often narrow and either smooth or ornamented. Endoapertures,

if present, covered with exinous bridge. Exine foveolate or reticulate with irregular lumina and deep muri (Fig. 1-3). In the studied *Ononis* species the anthers differ in sizes and opened by apical pores but this does not affect pollen sizes (Fig. 4 and 5).

Genus *Trigonella*: Pollen grains small, rarely medium, sized ranging from: P = (17-) 30 (-40); E = (15-) 20 (-28) Um. Prolate-spheroidal, subprolate or prolate occasionally perprolate, colporate or colpate. The colpi are long extending onto the poles with tapering ends, coarsely granulated membranes and either smooth or ornamented margins. The apocolpi always ornamented, rarely smooth. Endoaperture, if present, well developed, oval in shape and protruding at the equator. Exine reticulate, foveolate granulate or rarely tectate beset with fine granules. The lumina in the reticulate exine are regular while they are

Fig. 1 - 48: Showing the pollen grains of the studied species by scanning electron microscope

Table 2: Palynological characters as shown by both light and scanning electron microscopes

Taxa\Charac.	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>O. vaginialis</i>	4	3	2	20-23 21.0	12-17 14.0	1.3-1.5 1.4	18-20 19.0	2	2	2	3	5	1a
<i>O. reclinata</i>	3	3	2	30-33 31.0	22-26 24.0	1.3-1.4 1.36	26-28 26.5	1	2	2	3	2	1a
<i>O. sicula</i>	4	3	2	38-42 40.5	25-30 28.5	1.4-1.5 1.45	35-38 36.0	2	2	1	3	5	1a
<i>O. serrata</i>	5	3	1	23-27 24.0	13-18 14.5	1.5-1.8 1.75	20-23 21.0	1	1	2	0	2	2
<i>T. arabica</i>	4	3	2	22-24 22.5	15-17 15.5	1.4-1.5 1.47	20-22 20.5	3	1	2	1	5	1b
<i>T. anguina</i>	5	3	1	35-40 38.5	18-22 21.0	1.8-1.9 1.85	22-27 24.0	3	1	1	0	3	2
<i>T. occulta</i>	4	3	2	25-27 26.5	17-19 17.5	1.4-1.5 1.46	22-25 23.0	2	1	2	1	5	1b
<i>T. monspeliaca</i>	1	3	1	30-34 31.0	25-28 26.5	1.1-1.2 1.12	25-27 26.5	2	2	2	0	4	2
<i>T. stellata</i>	3	3	2	20-23 22.0	17-19 18.0	1.1-1.2 1.15	18-20 19.0	3	1	2	1	5	1b
<i>T. laciniata</i>	1	3	2	17-20 18.5	15-18 16.5	1.1-1.2 1.15	13-17 14.5	3	2	2	1	5	1b
<i>T. maritima</i>	1	3	2	20-23 21.0	18-20 18.5	1.1-1.3 1.25	18-20 19.5	3	2	2	1	5	1b
<i>T. hamosa</i>	3	3	1	23-27 24.0	18-21 20.0	1.2-1.3 1.25	21-25 22.0	3	2	2	0	4	2
<i>M. marina</i>	3	3	1	24-26 24.5	18-21 19.0	1.3	19-21 20.0	2	2	2	0	2	2
<i>M. lupulina</i>	1	3	2	18-22 19.5	18-20 19.5	1.0-1.1 1.05	13-17 15.5	1	2	2	3	2	1a
<i>M. coronata</i>	1	3	2	21-24 22	20-23 21.5	1.05	20-22 21.0	2	2	2	1	5	1b
<i>M. orbicularis</i>	3	3	1	31-34 32.5	26-28 24.5	1.2	24-27 25.5	2	2	2	0	2	2
<i>M. polymorpha</i>	3	3	1	27-32 30.0	20-24 22.5	1.35	23-26 24.0	2	2	2	0	2	2
<i>M. granadensis</i>	1	3	2	28-32 30.5	23-28 25.5	1.1-1.2 1.15	20-24 22.5	1	1	2	2	2	1c
<i>M. littoralis</i>	1	3	2	20-23 21.0	18-21 20.0	1.1	16-19 18.0	2	2	2	3	3	1a
<i>M. truncatula</i>	3	3	1	25-28 26.5	21-23 21.5	1.2	18-21 19.5	1	2	2	0	2	2
<i>M. turbinate</i>	3	3	1	31-34 32.5	24-27 26.0	1.3	20-23 21.0	1	2	2	0	2	2
<i>M. intertexta</i>	2	4 6	1	32-38 35.0	32-38 35.0	1.0	17-25 22.5	1	1	2	0	2	3a
<i>M. minima</i>	3	3	1	28-32 30.5	23-27 24.5	1.2	17-23 21.5	1	1	1	0	2	2
<i>M. laciniata</i>	1	3	2	32-36 34.0	30-34 32.0	1.06	22-24 23.0	1	2	2	3	2	1a
<i>M. sativa</i>	3	3	2	21-24 22.5	16-19 16.5	1.3	15-18 16.5	1	1	2	2	2	1c
<i>M. alba</i>	5	3	1	33-36 34.0	17-20 19.0	1.85	30-32 30.5	3	2	2	0	4	2
<i>M. sulcata</i>	5	3	1	34-37 35.0	13-16 15.0	2.3-2.5 2.35	30-32 30.5	3	2	2	0	5	2
<i>M. siculus</i>	5	3	1	35-39 36.5	19-20 19.5	1.8-2.0 1.9	29-32 30.0	1	2	2	0	5	2
<i>M. indica</i>	3	3	1	28-32 30.5	23-25 24.0	1.2-1.3 1.26	23-26 24.0	2	2	2	0	6	2
<i>T. fragiferum</i>	3	3	2	35-38 36.0	25-28 26.5	1.4	26-30 27.5	1	1	2	1	9	1b
<i>T. resupinatum</i>	1	3	1	32-36 33.5	28-30 28.5	1.1-1.2 1.14	23-26 24.5	3	2	2	0	9	2
<i>T. tomentosum</i>	1	3	2	30-32 31.5	25-28 26.0	1.1-1.2 1.14	28-30 28.5	3	2	2	1	7	1b
<i>T. procumbens</i>	3	9	1	29-32 30.5	24-26 25.0	1.2	25-27 25.5	3	2	2	0	1	3b

Table 2: Continued

Taxa\Charac.	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>T. philistæum</i>	3	9	1	26-28 27.0	20-22 20.5	1.3	18-21 19.0	3	2	2	0	1	3b
<i>T. nervulosum</i>	1	9	1	28-30 28.5	25-27 25.5	1.1	22-25 23.0	3	2	2	0	1	3b
<i>T. repens</i>	1	3	2	26-28 27.5	24-26 25.5	1.1	22-25 23.5	3	1	2	1	5	1b
<i>T. alexandrenum</i>	3	3	2	29-32 31.0	23-25 24.0	1.3	23-26 24.0	2	1	2	1	9	1b
<i>T. scabrum</i>	5	3	1	38-41 39.0	20-22 21.5	1.9	30-33 31.5	2	2	2	0	8	2
<i>T. stellatum</i>	3	3	2	25-28 27.0	20-23 22.0	1.2-1.3 1.22	21-23 21.5	2	2	2	1	3	1b
<i>T. purpureum</i>	5	3	1	27-30 28.5	14-17 15.0	1.7-1.9 1.82	20-23 21.0	2	2	2	0	3	2
<i>T. desvauxii</i>	3	3	2	20-22 20.5	17-19 17.5	1.2	16-18 17.5	1	1	2	1	5	1b

1-Pollen shape: 1=prolate spheroidal, 2=rhomboidal, 3=subprolate, 4=prolate, 5=perprolate, 2-Number of apertures
 3-Type of apertures: 1=colpate 2=colporate
 4-Polar axis length (Um)
 5-Equatorial axis length (Um)
 6-Ratio Polar axis / Equatorial axis
 7-Colpus length (Um)
 8-Colpus membrane : 1=smooth, 2=finely granulate
 9-Colpus margin : 1=smooth 2=ornamented
 3=coarsely granulate
 10-apocolpi : 1=smooth, 2=ornamented
 11-Endoaperture: 0=absent, 1=oval, 2=lolongate, 3=bridged
 12-Exine ornamentation : 1=tectate perforate, 2=foveolate, 3=foveolate-granulate, 4=granulate, 5=reticulate
 6=tectate echinate, 7=rugate, 8=rugate granulate
 9=microreticulate granulate
 13-Pollen type

irregular in the foveolate ones. The muri are usually deep and concave (Fig. 6-11, 16 and 17).

Genus *Medicago*: This genus is a stenopalynous genus in which the pollen grains small to medium sized ranging from: P= (18-) 27 (-38); E= (18-) 24 (-38) Um. Prolate-spheroidal, subprolate or rhomboidal, tricolporate or tricolpate, sometimes hexacolpate. The colpi long reaching the poles with tapering ends, smooth margins and either finely granulated or smooth membranes. The apocolpi considerably narrow and either ornamented or smooth. Endoapertures, when present, are either lolongate or bridged. Exine foveolate, foveolate granulate or reticulate. The lumina irregularly shaped and the muri shallow and thickened toward the poles forming a tectum perforatum (Fig. 18-35).

Genus *Melilotus*: Pollen grains medium sized ranging from: P= (28-) 35 (-39); E= (13-) 19 (-25) Um. Perprolate rarely subprolate, tricolpate with long, narrow and grooved colpi with smooth membranes and ornamented margins. Exine reticulate or tectate beset with either granules or echinae. The lumina in the reticulate exine are regular throughout the poles and equators with shallow muri (Fig. 12-15).

Genus *Trifolium*: This genus is stenopalynous genus with different pollen shapes, apertures and exinous ornamentation. The pollen grains medium sized ranging from: P= (20-) 30 (-38); E= (17-) 26 (-30) Um. Prolate-spheroidal, subprolate or perprolate, tricolporate, tricolpate or polycolpate. The colpi usually long, tapered

or slightly rounded at the ends and reaching the poles. They have either finely or coarsely membranes and smooth margins forming an area like the lips, rarely without. In polycolpate species, three of the colpi are well developed while the rest are short and like shallow grooves. Exine tectate-perforate, foveolate-granulate, rugate, rugate-granulate or microreticulate-granulate. The lumina regular throughout the whole pollen and the muri are shallow (Fig. 36-48).

Pollen types

Type 1: Pollen grains with different shapes; prolate-spheroidal, subprolate, prolate or perprolate; having tricolporate apertures.

Subtype A: The endoapertures hidden by exinous extension like the bridge. This subtype includes the following species: *Ononis vaginalis*, *O. reclinata*, *O. sicula*, *Medicago lupulina*, *M. littoralis* and *M. laciniata* (Fig. 2, 21, 31 and 32).

Subtype B: The endoaperture oval in shape and protruding towards the equators. This subtype includes the following species: *Trigonella arabica*, *T. occulta*, *T. stellata*, *T. laciniata*, *T. maritime*, *Medicago coronata*, *Trifolium fragiferum*, *T. tomentosum*, *T. repens*, *T. alexandrenum*, *T. stellatum* and *T. desvauxii* (Fig. 5-9, 33, 35-37 and 39-41).

Subtype C: The endoapertures lolongate. This subtype includes the following species: *Medicago granadensis* and *M. sativa* (Fig. 25 and 30).

Type 2: Pollen grains with different shapes; prolate-spheroidal, subprolate or perprolate; having tricolpate apertures. This type includes the following species: *Ononis serrata*, *Trigonella anguina*, *T. monspeliaca*, *T. hamosa*, *Medicago marina*, *M. orbicularis*, *M. polymorpha*, *M. truncatula*, *M. turbinata*, *M. minima*, *Melilotus alba*, *M. sulcata*, *M. siculus*, *M. indica*, *Trifolium resupinatum*, *T. scabrum* and *T. purpureum* (Fig. 1, 10-18, 20, 29, 30, 34, 42 and 44).

Type 3: Polyaperture pollen grains

Subtype A: Pollen grains rhomboidal in shape with tetra- or hexacolpate aperture and faintly foveolate or tectate perforate exine. This subtype include one species; *Medicago intertexta* (Fig. 22, 24 and 25).

Subtype B: Pollen grains prolate-spheroidal or subprolate in shape with polycolpate aperture (more than six) and tectate perforate exine. This subtype includes the following species: *Trifolium procumbens*, *T. philistaeum* and *T. nervulosum* (Fig. 43, 45 and 46).

DISCUSSION

The results obtained show that this group of genera has great dissimilarities in their pollen grains as illustrated from the characters 1, 2, 3, 11 and 12. According to this dissimilarities, it becomes very difficult to separate any of these genera according to the pollen characters only. In spite of that, we can notice the variations between taxa which can help in their segregation. The genera *Ononis* and *Trifolium* which separated each in a separate group according to morphological and seed characters^[15-18] and *Taia* (unpubl.) can be supported by their pollen grains morphology. Meanwhile gathering the rest of the genera in another third group is not supported by their pollen characters.

From these results, the genus *Medicago* easily separate as proposed by *Taia* (unpubl.), in spite of the different pollen types it has. Species of both *Trigonella* and *Melilotus* seems to be similar in their pollen grains which support their position together.

According to the type and number of apertures, we find three types of pollen grains in the taxa of this tribe; tricolporate, tricolpate and polycolpate; this not in agreement with Ferguson and Skvarla^[22] who said that the leguminous plants mostly fall into two categories of pollen apertures; tricolporate with well obvious endoaperture and tricolporate with operculate and diffused endoapertures. Here in tribe *Trifolieae* the apertures show great variations which can help in postulating a line of evolutionary trend within the group

(Fig. 49). In this phylogenetic postulation we can consider type 3 with the polycolpate apertures the most primitive within the studied taxa, while the tricolporate apertures, type 1, the most advanced. New trend in evolution in which the apertures evolute from tricolporate to tricolpate^[2,22-24]. Present results show the evolutionary line in the studied taxa move from the polycolpate, tectate perforate or even psilate to tricolpate, foveolate or reticulate and end with the tricolporate with echinate and granulate tectum. The last group moves from the oval endoaperture in two parallel lines toward the lolongate and the bridged endoapertures (Fig. 49).

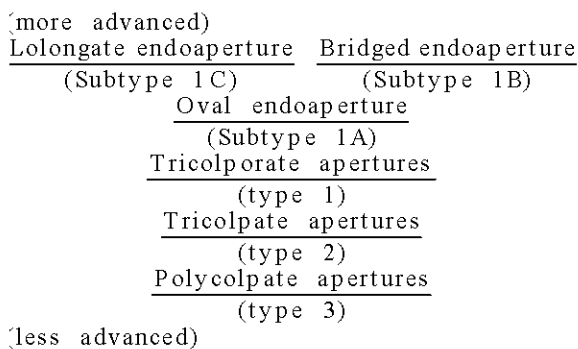


Fig. 49: Evolutionary line within tribe *Trifolieae*

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