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SEED SURFACE SCAN AND SEED MORPHOLOGY OF SOME DICOT. TAXA

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ABSTRACT

The present work is devoted to investigate the surface scan attribute by using light and scanning electron microscope, as well as, micromorphological characters of seeds of three different species, each include two varieties or cultivars, representing three genera and three families.

The main results could be summarized as follows:-

1- According to seed exomorphology, the variation in the seed shape, color and size were of great importance and could be considered as good diagnostic features that make the identification and separation among the studied cultivars and varieties easier and more effective.

2-The combination of both seed surface patterns and micromorphological characteristics could be useful in delimitation of the studied taxa.

3-The examination of seed surface scan, by using SEM showed differences or namentation of the seed surface which could be considered major significant diagnostic attributes to facilitate the separation of taxonomic unites.

4-The cuticle thickness is considered as diagnostic features that could be depended on identification of some taxa rather than classificatory purposes.

5-According to anticlinal and periclinal walls, it was noticed that this feature is of a great importance and could be considered as a good diagnostic feature which make the differentiation and identification of the studied taxa more clear and effective.

INTRODUCTION

Plant taxonomy has drawn great attention of many scientists dealing with this scope of study. Different trends dealing with the basis of plant taxonomy, especially, those related to plant families.

That is why we find many researches dealing with the basis of taxonomy, especially, those based on the following characteristics:-

The morphological surface scan characters (using light and scanning electron microscope) and micromorphological attributes of seeds are significantly employed as a criterion for taxonomic treatments Stace (1980). Also these features are of great importance and could be considered as good diagnostic features that make the differentiation and separation between the cultivars more effective and easier as well Abo-Baker (2004).

The main object of the present investigation is to throw light on the morphological surface scan characters (using light and scanning electron microscope) and micromorphological attributes of seeds of three species, included two even cultivars or varieties for each specie (representing 3 families and 3 genera).

Hence, the present work aimed to apply morphological attributes to facilitate identification and separation of the studied taxa, as well as, studying their use as criteria for taxonomic aspects.

MATERIALS AND METHODS

I. Plant materials:

In this work, seeds of three different species of three families [i.e., Malvaceae, Solanaceae and Brassicaceae were taken as plant materials. For each species; seeds of two economical varieties or cultivars were secured from Seed Bank Of The CAIM-Herbarium of Flora and Phytotaxonomy Department, Horticulture Research Institute (HRI), Agricultural Research Center (ARC), Agricultural Museum, Dokki, Giza. The studied seeds belong to three genera namely: *Gossypium, Lycopersicon* and *Brassica* according to Hutchinson's classification (1973).

Table (1): The species under investigation showing the different families and genera according to Hutchinson's (1973).

Table (1): The different species taken as plant material in the present study.

Family	Genera	Varieties or Cultivars
Malvaceae	*Gossypium*	*barbadense* L. cv. Giza 86
	Gossypium	*barbadense* L. cv. Giza 66
Solanaceae	*Lycopersicon*	*esculentum* Mill. var. Pyriforme
	Lycopersicon	*esculentum* Mill. var. Grandifolium
Brassicaceae	*Brassica*	*napus* L. cv. Pactol
	Brassica	*napus* L. var. Tower

II. Methods :

1. Seed exomorphology and surface scan aspects:

Seed dimensions were measured by binocular stereo-microscope using ocular micrometer. The general exomorphological features of the seeds were examined by the same microscope.

The (SEM) micrographs were taken after the mounting of the completely mature dry seeds with (SPI) supplies; conducting carbon paint, on copper stubs coated with a thin layer of gold palladium in Edwards Sputter Coater; S 150 B, and examined in different positions using different magnifications by JEOL-JSM-T100 Scanning Electron Microscope at the Faculty of Science, Zagazig University.

The SEM-micrographs were used to facilitate the description of seed exomorphology. The magnification power was expressed by (X) for each SEM-photograph. In this connection, it must be mentioned that the magnification power of the SEM was changed from 35 X to 1500 X, among the taxa investigated to clarify the finest details, as well as the characters of more interest.

In case of large-sized seeds which were out of SEM field (cotton seed). The stereo microscope photographs were taken in the National research center, Dokki, Giza.

Glossary to describe terms for the seed surface scan followed Murley (1951), as cited by Stearn (1983)"

.Reticulate: With a raised network of narrow and sharply angled lines frequently presenting ageometric appearance, each area or depression outlined by reticulum being an interscope.

Favulariate : With the surface finally ribbed; the ribs are separated by zigzag furrows.

Rugose: Wrinkled, the irregular elevation making up the wrinkles and running mostly in one direction.

Undulate: Appear as sea waves.

2. Seed micromorphology:

Comparative microscopical examination of seeds of all genera and cultivars (Gossypium, Lycopersicon and Brassica) was done as follows:

1- The dry seeds were soaked in water and then killed and fixed in F.A.A. solution (5ml formalin, 5ml glacial acetic acid and 90 ml ethyl alchohol 70%) for at least 48 h., washed in 50% ethyl alchohol, dehydrated in series of ethyl alcohols 70, 80, 90, 95 and 100%, infiltrated in xylene, embedded in paraffin wax of a melting point of 60-63°C, sectioned 20 microns in thickness (Sass, 1951).

2- The sections were stained with the double stain method (safranin and fast green), cleared in xylene and mounted in canada-balsam (Johanson, 1940).

Drawings were made at bench level by aid of leitz camera Lucida. The magnification was given by beck-stage micrometer scaled 0.01 to 0.1 mm.

RESULTS AND DISCUSSION

1- Seed exomorphology (Table, 2 & Plate,1):

a-Seed shape(Table 2 & Plate VII):

- Obovate: *Gossypium barbadense* L. cv. Giza 86 and *Gossypium barbadense* L. cv. Giza 66.
- Oval with tapering end: *Lycopersicon esculentum* Mill. var. *pyriforme* and *Lycopersicon esculentum Mill.* var. *grandifolium.*
- Slightly globose: *Brassica napus* L. cv. Pactol.
- Oblong shiny and minute coarse: *Brassica napus* L. var. *Tower.*

b-Seed colour (Table 2):

The colour of seeds of the studied cultivars and varieties was either dark brown to black as in *Gossypium barbadense* cultivars, straw yellow in *Lycopersicon esculentum* Mill. var. *pyriforme*, pale yellow in *Lycopersicon esculentum* Mill. var. *grandifolium*, dark brown in *Brassica napus* L. cv. Pactol or light brown in *Brassica napus* L. var. *tower.*

Of the main workers are Chang and Heckard (1972) who depended on seed colour in some delimitations. On the other hand, the seed colour is of rather limited taxonomic value (Hausain *et al.* 1990). Furthermore, the justification of this rejection is supported by the fact that the seed colour is an attribute which depends largely on the metabolic activities within the plant on one hand and on the effect of the enviromental conditions on the other hand (Karakish, 1993). This character has no effective consideration among the other good ones for its possible fluctuation with the same taxon at different durations (Hussein, 1995).

c- Seed size (Table.2):

- Small-sized seeds: Less than 2mm long as in *Brassica napus* L. var. *Tower.*
- Large-sized seeds: i.e., more than 4 mm long as *Gossypium barbadense* cultivars.
- Median-sized seeds: 2-4mm long as in *Lycopersicon esculentum* varieties.

Heinisch (1955) recorded some characters and measurerments of the seeds of some species of the *Trifolium* genus. Peinado *et al.* (1971) provided a key to 13 of *Trifolium* species based on size and weight of their seeds. Stebbins, (1974) emphasized that the precise adjustment of seed size is often highly adaptive and the reproductive success is dependent upon strong buffering and canalization of the processes involved in seed development. Thompson (1981) stated that such attributes are subjected to ecological and physiological variations. Mourad (1988) and Karakish (1993) stated that the seed size is unreliable for both identification or differentiation.

2. Surface scan:

a-General features of epidermis (Table, 3 & Plate, 2) :

- Favulariate : *Gossypium baradense* L. cv. Giza 86 and *Brassica napus* L. and cv. Pactol
- Undulate: *Gossypium baradense* L. cv. Giza 66
- Micro reticulate: *Lycopersicon esculentum* Mill. var. *Pyriforme.*
- Rugose: *Lycopersicon esculentum* Mill. var. *grandifolium.*
- Reticulate : *Brassica napus* L. var. *Tower.*

b-Anticlinal walls:

A- Level: (Table, 3):

- Raised: *Gossypium barbadense* cultivars, *lycopersicon esculentum* L. var. *pyriforme*, *Brassica napus* cv. Pactol and *Tower* variety.
- Depressed: *Lycopersicon esculentum* Mill. var. *grandifolium.*

A- Undulation: (Table, 3)

- Widely lobed: *Gossypium barbadense* L. cv. Giza 86.
- Slightly Lobed: *Gossypium barbadense* L. cv. Giza 66, *Lycopersicon esculentum* Mill. var. *pyriforme*, and *Brassica napus* L. var. *Tower.*
- Slightly depressed: *Lycopersicon esculentum* Mill var. *grandifolium.*
- Elevated: *Brassica napus* L. cv. Pactol.

A- Texture: (Table, 3):

- Rigid: *Gossypium barbadense* cultivars.
- Rough: *Lycopersicon esculentum* varieties.
- Smooth: *Brassica napus* cv. Pactol and *Tower* variety.

a- Appearance (Table, 3):

- Wavy and straieted: *Gossypium barbadense* L. cv. Giza 86.
- Straight: *Gossypium barbadense* L. cv. Giza 86.
- Hairy: *Lycopersicon esculentum* Mill. var. *Pyriforme.*
- Ageometric: *Lycopersicon esculentum* Mill. var. *grandifolium.*
- Irregular protruded: *Brassica napus* L. cv. Pactol.
- Flapped: *Brassica napus* L. var. *Tower.*

Fig. (1): X =20

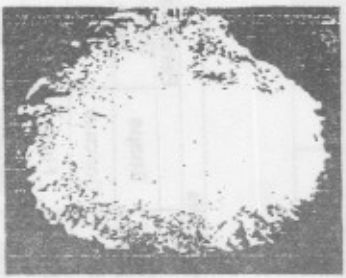

Fig. (3): X =35

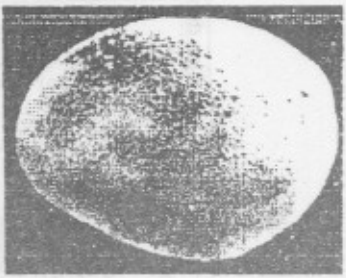

Fig. (5): X =50

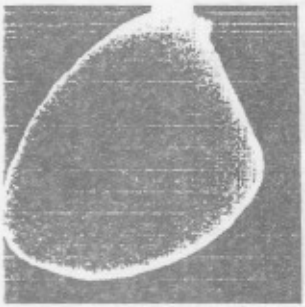

Fig. (2): X =20

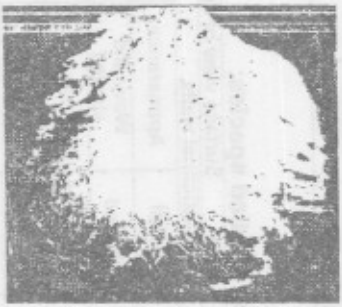

Fig. (4): X =35

Fig. (6): X =50

Plate (1): The different shapes of the studied seeds (Text Figs 1-6)
Figs. (1 and 2) : obovate: e.g., *Gossypium barbadense.*
Figs. (3 and 4): oval with tapering end: e.g., *Lycopersicon esculentum.*
Figs.(5): slightly globose: e.g., *Brassica napus* L. cv. Pactol.
Figs.(6):oblong, shiny and minute coarse: e.g., *Brassica napus* L. var. *Tower.*

1- Outerpericlinal wall: (Table 3):

-Concave and microreticulate: *Gossypiumbarbadense* L. cv. Giza 86.
- Flate and microreticulate: *Gossypium barbadense* L. cv. Giza 66.
- Concave and striate curled: *Lycopersicon esculentum* Mill. var. *Pyriforme.*
- Highly elevated and irregularly: *Lycopersicon esculentum* Mill. var. *grandifolium.*
- Highly elevated and discharged: *Brassica. Napus* L. cv. Pactol.
Flate and microreticulate: *Brassica napus* L. var. *Tower.*

Table (2): The morphological Aspects of the seeds of the studied species:

Species	Shape of seeds	Faces	Colour	Size dimension (mm.)		
				Mean length	Mean width	Grade
Gossypium barbadense L. cv. *Giza 86*	Obovate	Convex	Dark brown to black	4.85	1.7	L
Gossypium barbadense L. cv. *Giza 66*	Obovate	Convex	Dark brown to black	5.2	1.95	L
Lycopersicon esculentum Mill. var. *Pyriforme*	Oval /tapering end	Convex	Straw yellow	3.2	1.48	M
Lycopersicon esculentum Mill. var. *grandifolium*	Oval /tapering end	Convex	Pale yellow	3	1.33	M
Brassica napus L. cv. *Pactol*	Slightly globose, granulate	Flat	Dark brown	2.1	1.3	M
Brassica napus L. var. *Tower*	Oblong, shiny and minute coarse	Convex flat	Light brown	1.9	1.2	S

L= Large M= Medium S= Small

Table (3): The surface view aspects of the seeds of the studied species:

Species	Shape (S.V.)	Surface view of epidermal cells							
		Undulation	Anticlinal walls				Periclinal walls		
			Thickness	Level	Appearance	Surface texture	Shape	Thick-ness	Appearance
Gossypium barbadense L. cv. *Giza 86*	Favulariate	Widely lobed	Very thick	Raised	Wavy and striated	Rigid	Concave	Thin	Micro reticulate
Gossypium barbadense L. cv. *Giza 66*	Undulate	Slightly lobed	Thick	Raised	Straight	Rigid	Flate	Thin	Micro reticulate
Lycopersicon esculentum Mill. var. *Pyriforme*	Micro reticulate	Slightly lobed	*Thick*	Raised	Hairy	Rough	Concave	Thin	Striate curled
Lycopersicon esculentum Mill. var. *grandifolium*	Rugose	Slightly depressed	Thick	Depressed	Ageometric	Rough	Highly elevated	Thin	Irreguraly
Brassica napus L. cv. *Pactol*	Favulariate	Elevated	Thick	Raised	Irregular protruded	Smooth	Highly elevated	Thin	Discharged
Brassica napus L. var. *Tower*	Reticulate	Slightly lobed	Thick	Raised	Flapped	Smooth	Flate	Thin	Micro reticulate

3. Seed coat micromorphology:

a- *Gossypium barbadense* cultivars: (plate 3 – Fig. 1&2):

The malvaceous seed coat develops from both the outer and inner integuments of the ovule, yet the mechanical tissue develops from the inner integument and particularly from its outer epidermis, thus the inner integument constitutes the major portion of the seed coat. The outer integument of the ovule shares only in the formation of the outer epidermis of the seed coat which may be one layer of cells or more. The outer epidermis of the inner integument forms the highly characteristic palisade cells. The cells laying below the palisade tissue (which constitute the mesophyll of the inner integument) are 3-4 layers thick and filled with a dark brown pigment. Beneath the pigmented layer, is a relatively compact 2 rows of cells (fringe layer) constituting the inner epidermis of the integument.

Seed coat micromorphology of *Gossypium barbadense* cv. 86 and cv 66 are similar, except that the middle layer of tegment of cv. Giza 66 forms 3 rows of cells, but in cv. Giza 86 it forms 4 rows of cells.

In the taxa studied, the seed coat micromorphology gives more evidence of the family as being a homogenous natural group. These findings agreed with Khushk and Vaughan (1985) who studied the general morphology and structure of the seeds of 3 genera and 8 species (*Gossypium arboreum, G. barbadense, G. herbaceum, G. hirsutum, G. wightianum, Lebronnecia Kokioides, Thespesia populnea and Thespesia populneoides*) of the Hibisceae. According to them, the study provides strong evidence for the inclusion of Gossypiun and other genera in the tribe Gossypieae.

Khushk and Vaughan (1986) studied the general morphology and structure of the seeds of 17 species of the Abutileae (Malvaceae), using light and scanning electron microscopy. Relevance of seed structure to the tribal division of the family was previously discussed. Nee *et al.* (1986) who studied the seed coat components of *Hibiscus abelmoschus*. Yatsu *et al.* (1986)

made some chemical and microscopic studies on the matrix substance in pigment glands of *Gossypium hirsutum* seeds. Loutfy (1992) stated that the seed coat anatomy of Malvaceae gives more evidence of the family as being a homogeneous natural group, by using the light microscopic examination, variations between the taxa were generally nonsignificant.

b- *Lycopersicon esculentum* Mill. var. pyriforme and var. grandifolium (plate 3- Fig.3&4):

The apparent "hairs" on the outside of the testa are in fact the thickened radial walls of the epidermal cells. The epidermal cells present a characteristic sinuous appearance. With the epidermis are two or three layers of crushed parenchyma cells which form the remains of the outer integument. The outer integument is limited internally by a single layer of cells with brown pigment. The inner integument consists of five or six layers of parenchyma cells.

These findings agreed with (Soueges, 1907) who used various histological characters of the seed integument to classify the main genera of family Solanaceae and extended them to the different species. However, Soueges (1907) reported that solanum has proved to be of taxonomic value below the sectional level; and of more value above the Solanaceae Moreover, the same author suggested that these data clearly demonstrated that *S. nigrum* could be the identical type species of the genus.

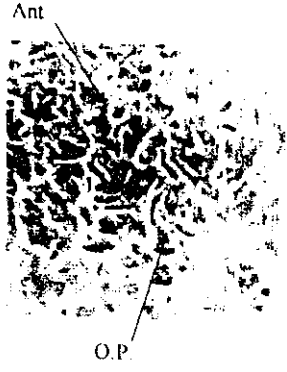

Fig. (1): X = 350

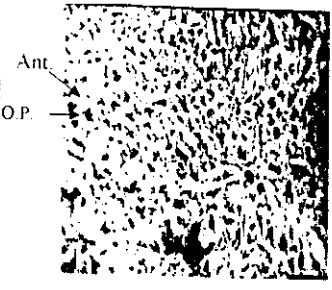

Fig. (3): X = 200

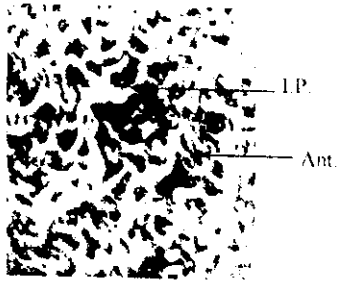

Fig. (5): X = 750

Fig. (2): X = 350

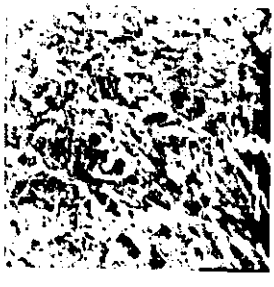

Fig. (4): X = 200

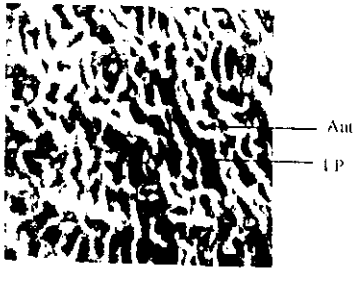

Fig. (6): X = 750

Plate (2)
Surface scan of the seed surface
(Text Figures 1-6)
Fig. 1: Favulariate: *Gossypium barbadense* L. cv. Giza 86.
Fig. 2: Undulate: *Gossypium barbadense* L. cv. Giza 66.
Fig. 3: Micro reticulate: *Lycopersicon esculentum* Mill. var. *Pyriforme*.
Fig. 4:Rugose: *Lycopersicon esculentum* Mill. var. *grandifolium*.
Fig. 5: Favulariate: *Bassica napus* L. cv. Pactol.
Fig. 6: Reticulate: *Brassica napus* L. var. *Tower*.

Abbreviation:
Ant: Anticlinial wall **OP: Outer periclinal wall** **IP: Inner periclinal wall**

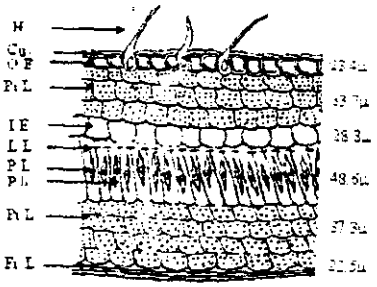

Fig. (1): X = 100

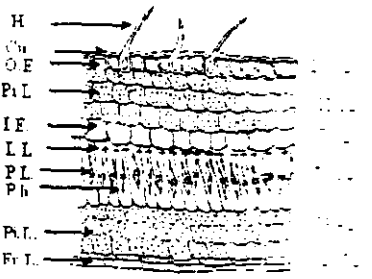

Fig. (2): X = 100

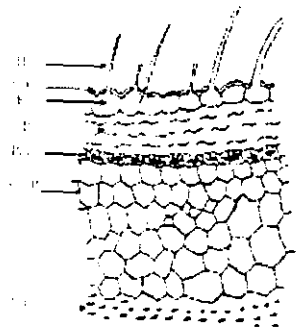

Fig. (3): X = 100

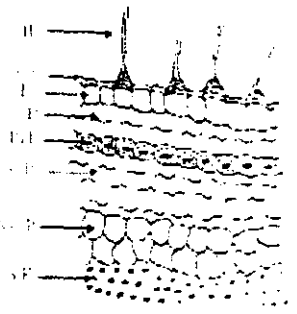

Fig. (4): X = 100

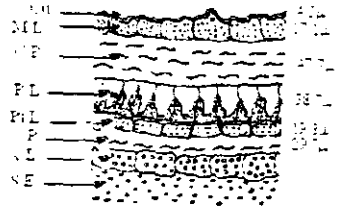

Fig. (5): X = 100

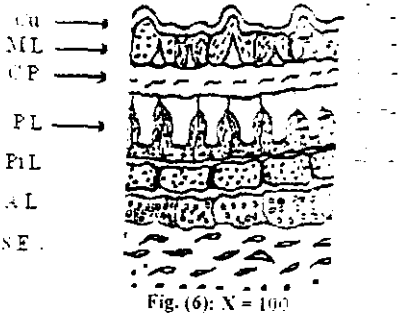

Fig. (6): X = 100

Plate (3): Seed coat micromorphology of the cultivars and varieties under study (Text figures 1-6), X100

Fig. 1: *Gossypium barbadense* L. cv. Giza 86

Fig. 2: *Gossypium barbadense* L. cv. Giza 66

Fig. 3: *Lycopersicon esculentum* Mill. var. *Pyriforme*

Fig. 4: *Lycopersicon esculentum* Mill. var. *grandifolium*

Fig. 5: *Brassica napus* L. cv. Pactol

Fig. 6: *Brassica napus* L. var. *Tower*

Abbreviation:

ML. Mucilage layer A. Alerone LL. Light line Scp. Seed coat parenchyma Cp. Crushed parenchyma

H. Hair Cu. Cuticle Of. Outer epidermis Pp.l. Pigmented layer

I.E. Inner epidermis PL. Palisade layer

Pb Pigment body Pi.l Pigment layer *Fr.l. Fringe layer*

The previous discussion of the seed coat micromorphology opens the door for making a pathway for the identification between *Lycopersicon esculentum* L. var. *pyriforme* and var. *grandifolium*. The differences between the two varieties under study could be described as follows:

- The epidermis of the seed coat in var. pyriforme covered with thin cuticle but, in var. *grandifolium* the cuticle is thick.
- The epidermis reprints a wide zone of crushed parenchyma in var. pyriforme but in var. *grandifolium* a narrow zone was found.
- The inner epidermis followed by 5 rows of hexagonal parenchyma cells in var. *pyriforme* but, in var. *grandifolium* the inner epidermis is followed by a wide zone of crushed parenchyma cells, lying immediately below 2 rows of polyhedral parenchyma cells.
- The previous discussion showed that the micromorphology of the seed coat is of great importance and could be considered as a good diagnostic in feature that make the identification and separation between the studied varieties more effective and easier (Corner, 1976), also in previous studies that mentioned, the seed coat micromorphology gives more evidence of the family Solanaceae as being a natural group (Preisner, 1985).

c- Brassica napus cv. Pactol and Tower var. plates 3 –Fig. 5&6):

Micromorphological characteristics of seed coat are very conservative features that allow a better understanding of natural relationship, and may be more useful for classification (Meyer and Engel, 1991).

In the present work, it was found that there are two integuments (outer and inner).

The outer one consists of mucilaginous layer with heavy cellulosic deposit on the inner tangential wall of the epidermis, forms radially oriented bars, one in each cell. The inner layer of the outer integument consists of palisade-like layer, for the cell develop lignified thickening on radial and inner tangential walls. The inner integument dies and then compressed. The inner epidermis of this integument becomes the pigment layer. This agreed with Esau, (1977) and Hammouda, (2004) who stated that the bitegmic ovules of the Brassicaceae have rather thick integument. The outer has two to five cell layers, the inner up to ten. In many species, the epidermal cells of the outer integument become almost filled with mucilaginous material. The mucilage which consists of pectin and cellulose, swells when it comes in contact with water and, in some species, bursts the outer wall of the cell and forms a gelatinous film over the surface of the seed. However, the subsequent salient attributes will be discuss as in the following:

The seed coat of *Brassica napus* L. cv. Pactol is characterized by the epidermal cells of the outer integument covered with thin cuticle layer and followed by a wide zone of crushed cells, but in *Tower* var., cuticle is thick and the epidermis followed by a narrow zone of crushed cells. The other difference is that the presence of a zone of crushed parenchyma cells below the pigmented layer, in the seed coat of cv. Pactol. It's not found in seed coat anatomy of var. *Tower*

The obtained results from the seed micromorphology gave a good evidence that the Brassicaceae as a family forms a natural taxon. Besides, seed anatomy also provides taxonomic confirmation at the family level. Also it was found that the seed coat anatomy is of great importance and could considered as a good diagnostic features that make the identification and separation between the studied vars. and cvs. of more effective and easier in application (Wojciechowska, 1972).

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خصائص البذرة المظهرية والمسح السطحي لها فى بعض الفئات التصنيفيه لذوات الفلقتين

على حسن شاهين* و احمد لطفى ونس* وفاتن حسن محمود إسماعيل* و عبدالمجيد على عبدالمجيد و محمد محمد عبدالعال***

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يهدف هذا البحث إلى إجراء دراسة لخصائص البذرة المظهرية والمسح السطحى لها باستخدام المجهر الضوئى والمجهر الماسح الإلكترونى وكذلك دراسة الصفات التشريحية لبذور ثلاثة أنواع نباتية ، تشمل من كل نوع صنفين ، تمثل هذه الأنواع ثلاثة أجناس وثلاثة عائلات هى كالتالى:
من العائلة الخبازية القطن صنف جيزة ٨٦ وصنف جيزة ٦٦
ومن العائلة الباذنجانية الطماطم صنف بيرفورم وصنف جراندفوليم ومن العائلة الصليبية الكانولا صنف باكتول وصنف توير.

وقد تم الحصول على بذور النباتات السابقة الذكر من بنك البذور بقسم بحوث الفلورا وتصنيف النباتات (CAIM). معهد بحوث البساتين – مركز البحوث الزراعية.

في هذا الصدد يهدف هذا البحث إلى إلقاء الضوء على الخصائص المورفولوجية البذرية في عمليات التمييز والتعريف للوحدات التصنيفية تحت الدراسة. كما تؤكد على تسهيل أهمية استعمال هذه الخصائص كمعايير ودلائل في الدراسات التصنيفية.

وقد تعرض هذا البحث للخصائص المورفولوجية المظهرية للبذور ولصفات المسح السطحي لها وكذلك الصفات التشريحية للبذور تحت الدراسة في جداول ولوحات مجمعه وأيضا أمكن إيضاح هذه الخصائص بالصور الفوتوغرافية الدقيقة والرسومات اليدوية للقطاعات العرضية للبذور في محاولة جادة لتسهيل ملاحظة الاختلافات والتشابهات والترابط والتمييز بين الوحدات المدروسة ويمكن تلخيص أهم النتائج التي توصل إليها هذا البحث فيما يلي:

- بالنسبة لخصائص البذور المظهرية والمسح السطحي لها بالإضافة إلى الصفات التشريحية للأنواع المدروسة:

١-تبعاً للمورفولوجيا المظهرية للبذور وجد أن لشكل ولون وحجم البذرة أهمية عظيمة ومميزات تشخيصية جيدة حيث تجعل التفريق والتمييز بين الأصناف تحت الدراسة أكثر فعالية وأسهل.

٢-أمكن الدمج بين كل من خصائص المسح السطحي للبذور والصفات التشريحية لتحقيق الفصل المرضي بين الوحدات المدروسة.

٣-وجد باستخدام المجهر الماسح الإلكتروني لفحص سطح البذور أن صفات الزخرف في القصرة تعد صفات تصنيفية تشخيصية رائدة لها أهميتها لفصل الوحدات التصنيفية المدروسة.

٤-أمكن اعتبار درجة سمك الأدمة من الخصائص التشخيصية التي يمكن الاعتماد عليها في عمليات التعريف أكثر من استخدامها في عمليات التقسيم.

٥-بالنسبة للجدر المماسية والقطرية أظهرت الفحوصات بالنسبة لهذه الصفة أنه يسهل التمييز والتعرف على الأصناف المختلفة.