

# ANATOMICAL FINGER PRINT AND BIOCONSTITUENTS AS SYSTEMATICAL TOOLS IN SOME DICOT. SEEDS

## 1-Seed surface scan and seed morphology.

by

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### ABSTRACT

The present thesis is devoted to investigate the exomorphological and surface scan – of some seeds - attribute by using light and scanning electron microscope, as well as, micromorphological characters and chemical composition of seeds of three different species, each include two varieties or cultivars, representing three genera and three families as follows:-

(From Malvaceae, cotton seed cvs. Giza 86 and Giza 66, Solanaceae, tomato seed vars. *Pyriforme* and *grandifolium* and Brassicaceae, rape seed cv. Pactal and var. *Tower*). The seeds under study even cultivars or varieties were obtained from The Seed Bank Of Flora and Phytotoxonomy Research Department (CAIM), Horticultural Research Institute (HRI), Agricultural Research Center (ARC). The aim of the present work was to clarity the importance and the significance of the seed morphological attributes and chemical composition to facilitate the process of differentiation and identification of these cultivars and varieties, as well as, using these attributes and chemical composition as criteria in taxonomic aspects. The different seed exomorphological, surface scan, anatomical attributes and chemical composition of the studied taxa are presented in forms of cumulative tables and plates, as well as, microphotographic pictures and hand drawings of the cross section of seeds in order to facilitate observation of variations, similarities, correlations and differences among the taxa.

#### **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 differentiation 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 different ornamentation of the seed surface which could be considered major significant diagnostic attributes to facilitate the separation of taxonomic unites.

- 4-From the anatomical point of view, there are anatomical characters that could be useful in delimitation of the studied taxa.
- 5-The cuticle thickness is considered as diagnostic features that could be depended on identification of some taxa rather than classificatory purposes.
- 6-A ccording 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 material 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 exomorphological, surface scan characters (using light and scanning electron microscope) and micromorphological attributes of seeds. These features are significantly employed as acriterion 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 exomorphological, 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 intended to apply morphological attributes to facilitate identification and separation of the studied taxa, as well as, studying their use as criteria in taxonomic aspects.

## MATERIALS AND METHODS

### 1. Plant materials:

In this work, seeds of three different species of three families [i.e., Malvaceae, Solanaceae and Cruciferae (Brassicaceae)] were taken as plant materials in this study. 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 (HIR), (ARC), Agricultural Museum, Dokki, Giza. The studied taxa 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	<i>Gossypium</i>	<i>barbadense</i> L. cv. Giza 86
	<i>Gossypium</i>	<i>barbadense</i> L. cv. Giza 66
Solanaceae	<i>Lycopersicon</i>	<i>esculentum</i> L. var. Pyriforme
	<i>Lycopersicon</i>	<i>esculentum</i> L. var. Grandifolium
Cruciferae (Brassicaceae)	<i>Brassica</i>	<i>napus</i> L. cv. Pactol
	<i>Brassica</i>	<i>napus</i> L. var. Tower

## 2. Methods :

### 2.1. Seed surface scan, macro and micro-morphology:

#### 2.1.1. Gross macromorphological “Phenology” aspects:

The general morphological description of each species was compiled from the current text books of plant taxonomy such as **Tindall (1993) and Boulos (1999)**.

#### 2.1.2. Seed exomorphology and surface scan aspects:

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

Scanning Electron Microscope (SEM), at 25 Kv. using different magnifications. 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 were 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 descriptive terms for the seed surface scan after **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.

### 2.1.3. Seed micromorphology:

Comparative microscopical examination of seeds of all genera and cultivars (cotton, tomato and rape) took place.

- 1- The dry seeds were soaked in water and then killed and fixed in F.A.A. (5ml formalin, 5ml glacial acetic acid and 90 ml ethyl alcohol 70%), washed in 50% ethyl alcohol, 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

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

#### 1- Shape of seeds (Table 2 & Plate VII):

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

#### 2- 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* L. var. *pyriforme*, pale yellow in *Lycopersicon esculentum* L. 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 the effect of the environmental 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 duration (**Hussein, 1995**).

### 3- Size of the seed 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 measurements of the seeds of some species of a 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.

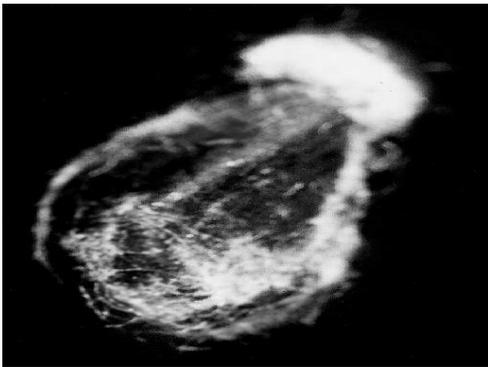


Fig. (1): X =20

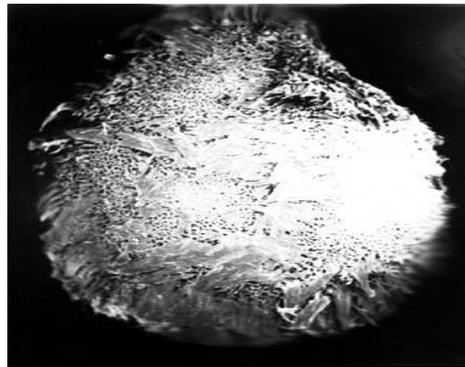


Fig. (3): X =35

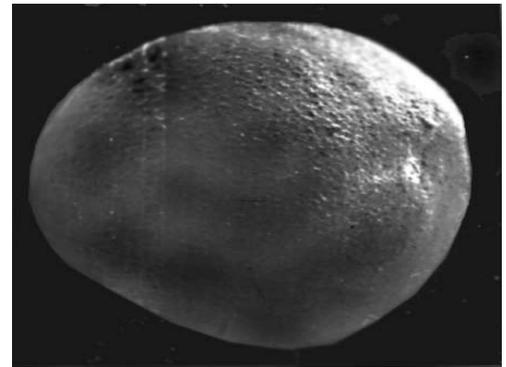


Fig. (5): X =50

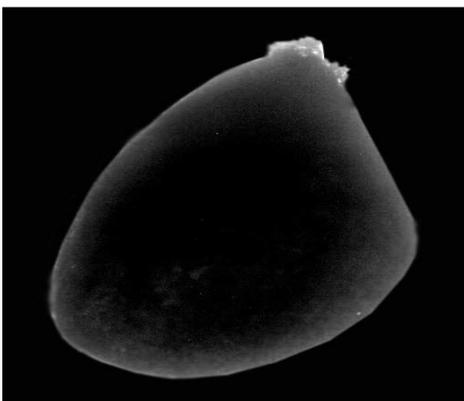


Fig. (2): X =20

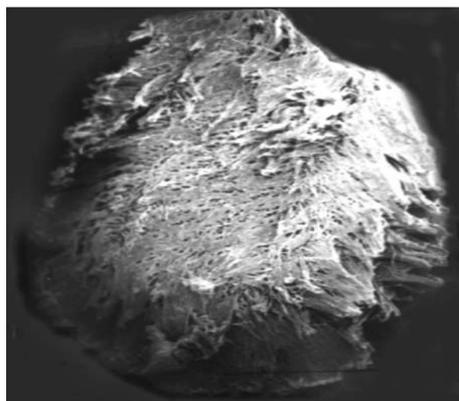


Fig. (4): X =35

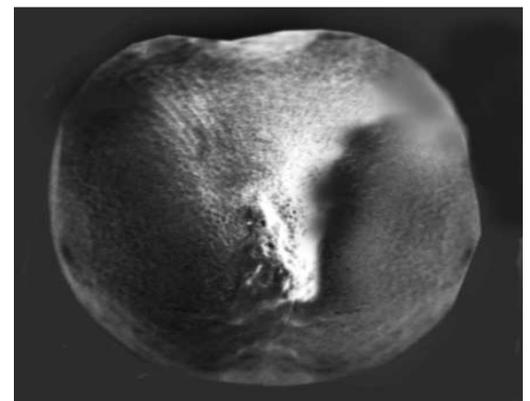


Fig. (6): X =50

**Plate (1): The different shapes of the seed (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*.

## 1.2. Surface scan:

### 1- 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* L. var. *Pyriforme*.
- Rugose: *Lycopersicon esculentum* L. var. *grandifolium*.
- Reticulate : *Brassica napus* L. var. *Tower*.

### 2- 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* L. var. *grandifolium*.

#### B- Undulation: (Table, 3)

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

#### C- Texture: (Table, 3):

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

#### D- Appearance (Table, 3):

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

### 3- 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* L. var. *Pyriforme*.
- Highly elevated and irregularly: *Lycopersicon esculentum* L. var. *grandifolium*.
- Highly elevated and discharged: *Brassica. Napus* L. cv. Pactol.

Flate and microreticulate: *Brassica napus* L. var. *Tower*.

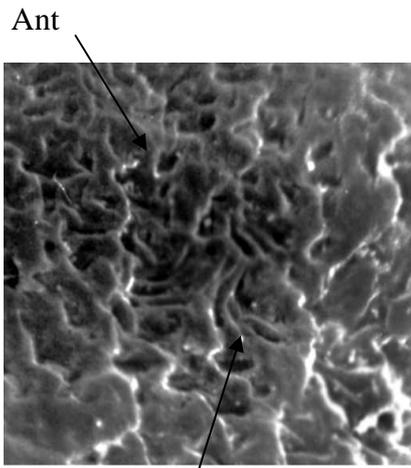


Fig. (1): X = 350  
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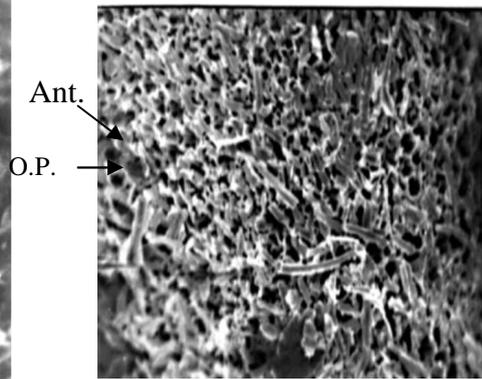


Fig. (3): X = 200

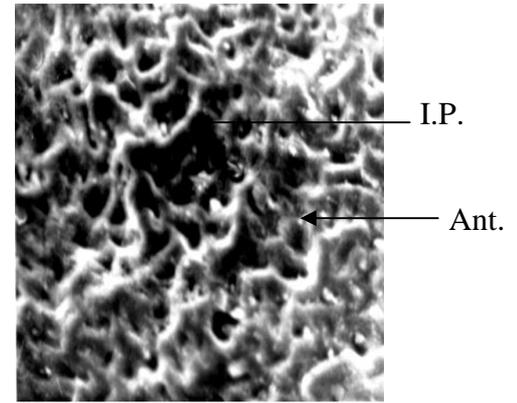


Fig. (5): X = 750

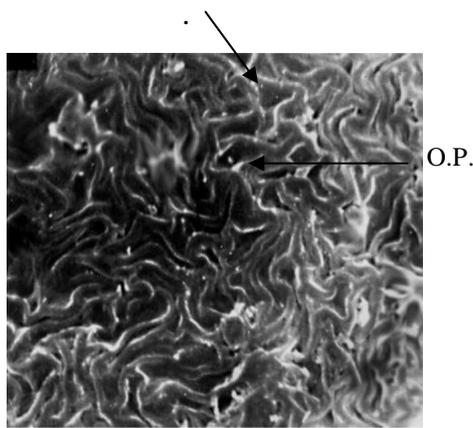


Fig. (2): X = 350

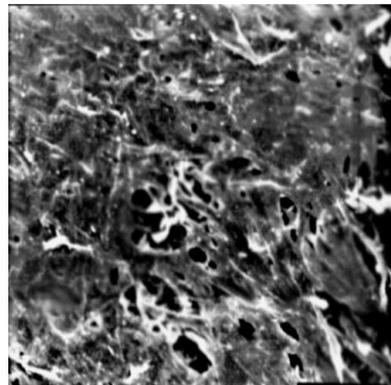


Fig. (4): X = 200

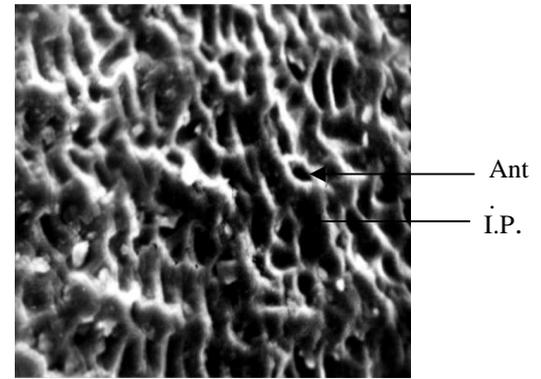


Fig. (6): X = 750

## 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* L. var. *Pyriforme*.

Fig. 4: Rugose: *Lycopersicon esculentum* L. var. *grandifolium*.

Fig. 5: Favulariate: *Bassica napus* L. cv. Pactol.

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

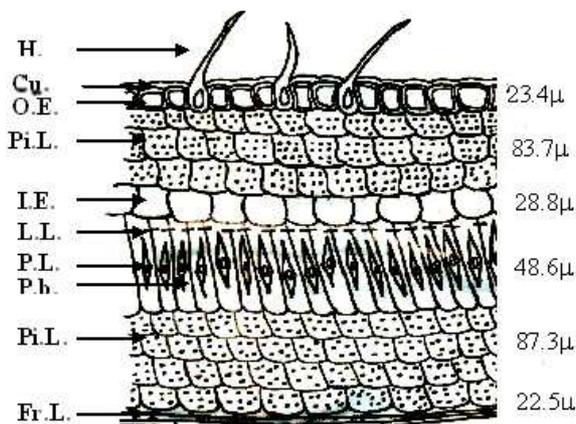


Fig. (1): X = 100

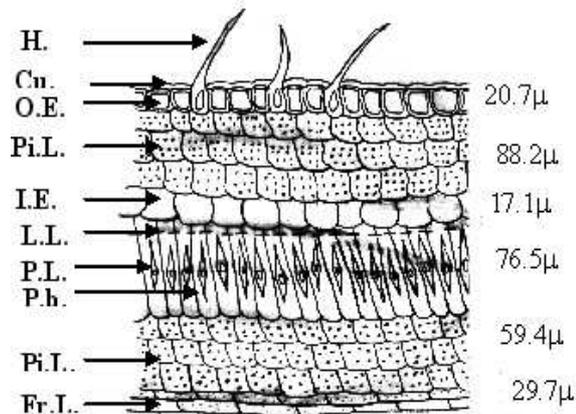


Fig. (2): X = 100

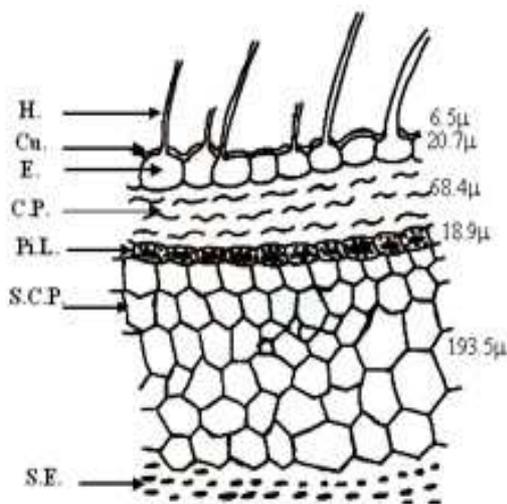


Fig. (3): X = 100

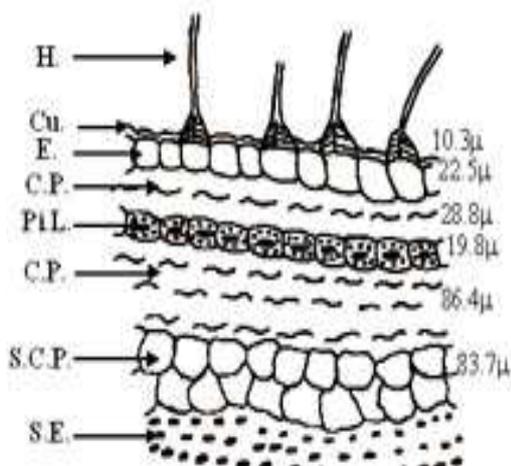


Fig. (4): X = 100

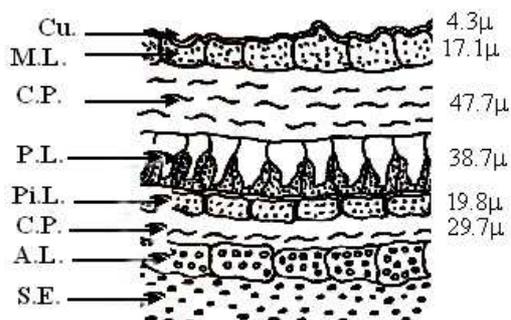


Fig. (5): X = 100

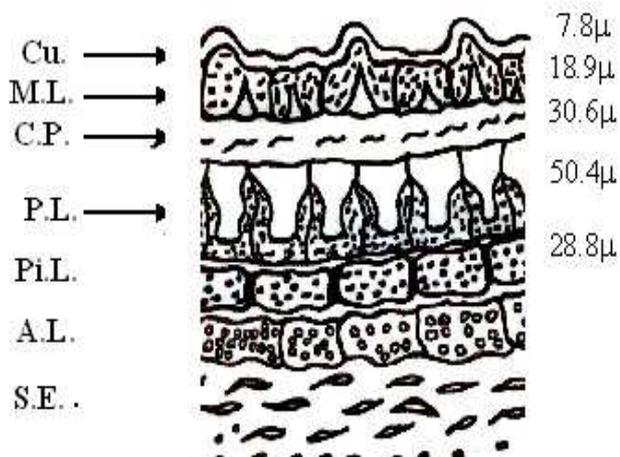


Fig. (6): X = 100

**Plate (3): Seed coat anatomy of the cultivars and varieties under study (Text figures 1-6)**

Fig. 1: Seed coat anatomy of *Gossypium barbadense* L. cv. Giza 86

Fig. 2: Seed coat anatomy of *Gossypium barbadense* L. cv. Giza 66

Fig. 3: Seed coat anatomy of *Lycopersicon esculentum* L. var. *Pyriforme*

Fig. 4: Seed coat anatomy of *Lycopersicon esculentum* L. var. *grandifolium*

Fig. 5: Seed coat anatomy of *Brassica napus* L. cv. *Pactol*

Fig. 6: Seed coat anatomy of *Brassica napus* L. var. *Tower*

### **1.3. Seed coat anatomy:**

#### **1.3.1. Seed coat anatomy (micromorphology) of *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 anatomy of *Gossypium barbadense* cv. 86 and cv 66 are similar, except that the middle layer of tegmen of cv. Giza 66 form 3 rows of cells, but in cv. Giza 86 it form 4 rows of cells.

In the taxa studied, the seed coat anatomy 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 Gossypion 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 microscope examination, variations between the taxa were generally nonsignificant.

#### **1.3.2. Seed coat anatomy (micromorphology) of *Lycopersicon esculentum* L. 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, in case of genus solanum (**Soueges 1907**) 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.

**Crete (1959)** studied the structure and characters of seed coat of *Nicandra physaloides*. The structure and development of the seed coat of *Nicotiana tabacum* and *N. glutinosa* were studied by **Jos and Singh (1968)**.

**Paspad, (1970)** studied the developing seed coat of *Nicandra physaloides* and found that it is formed of an epidermis. Outer middle layers and an inner zone of middle layers which undergo gelatinization. He also observed that at maturity the seed coat consists of thick – walled epidermis. A few pressed outer middle layers and thin walled endothelium, followed by the layers of the inner integument. The epidermis is covered with a thick mucilaginous layer.

The previous discussion of the seed coat anatomy opens the door for making a pathway for the differentiation 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 anatomy of the seed coat is of great importance and could be considered as a good diagnostic in feature that make the differentiation and separation between the studied varieties more effective and easier (**Corner, 1976**), also in previous studies that mentioned, the seed coat anatomy gives more evidence of the family Solanaceae as being a natural group (**Preisner, 1985**).

### 1.3.3. Seed coat anatomy (micromorphology) of *Brassica napus* cv. *Pactol* and *Tower* var. plates 3 –Fig. 5&6):

Anatomical 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**).

The endomorphological studies in the present work showed 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, form radially oriented bars, one in each cell. The inner 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 which appears in layers. 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 discussed 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 third 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 *Tower* var.

The obtained results from the seed anatomy gave a good evidence that the Brassicaceae as a family forms a natural taxon. Besides, seed anatomy also provides taxonomic confirmation at the familial 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 differentiation and separation between the studied vars. and cvs. of more effective and easier in application (**Wojciechowska, 1972**).

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

Species	Shape of seeds	Faces	Colour	Size (mm.)		
				Mean length	Mean width	Grade
<i>Gossypium barbadense</i> L. cv. <i>Giza 86</i>	Obovate	Convex	Dark brown to black	4.85	1.7	L
<i>Gossypium barbadense</i> L. cv. <i>Giza 66</i>	Obovate	Convex	Dark brown to black	5.2	1.95	L
<i>Lycopersicon esculentum</i> L. var. <i>Pyriforme</i>	Oval /tapering end	Convex	Straw yellow	3.2	1.48	M
<i>Lycopersicon esculentum</i> L. var. <i>grandifolium</i>	Oval /tapering end	Convex	Pale yellow	3	1.33	M
<i>Brassica napus</i> L. cv. <i>Pactol</i>	Slightly globose, granulate	Flat	Dark brown	2.1	1.3	M
<i>Brassica napus</i> L. var. <i>Tower</i>	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		
			Thick ness	Level	Appearance	Surface texture	Shape	Thickness	Appearance
<i>Gossypium barbadense</i> L. cv. <i>Giza 86</i>	Favulariate	Widely lobed	Very thick	Raised	Wavy and striated	Rigid	Concave	Thin	Micro reticulate
<i>Gossypium barbadense</i> L. cv. <i>Giza 66</i>	Undulate	Slightly lobed	Thick	Raised	Straight	Rigid	Flate	Thin	Micro reticulate
<i>Lycopersicon esculentum</i> L. var. <i>Pyriforme</i>	Micro reticulate	Slightly lobed	<i>Thick</i>	Raised	Hairy	Rough	Concave	Thin	Striate curled
<i>Ly copersicon esculentum</i> L. var. <i>grandifolium</i>	Rugose	Slightly depressed	Thick	Depressed	Ageometric	Rough	Highly elevated	Thin	Irreguraly
<i>Brassica napus</i> L. cv. <i>Pactol</i>	Favulariate	Elevated	Thick	Raised	Irregular protruded	Smooth	Highly elevated	Thin	Discharged
<i>Brassica napus</i> L. var. <i>Tower</i>	Reticulate	Slightly lobed	Thick	Raised	Flapped	Smooth	Flate	Thin	Micro reticulate

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Pactol

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