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TESTA STRUCTURE AND IDENTIFICATION OF SOME *Vicia* SPECIES

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ABSTRACT

Testa structure of some *Vicia* species *(V. amphicarpa, V. dasycarpa, V. ervilia, V. narbonensis and V. villosa)* was studied by Scanning Electron Microscope and Light Microscope. The following results were obtained:

Spermoderm pattern is papillose. Papillae arrangement; shape of their apex; compactness, presence of the striations on their surface; papillae size, shape of hilum, symmetry of central groove, shape of micropyle, close at its middle, presence of Malpighian cells and shape of tracheid bar at hilar region differed in the studied *Vicia* species.

Apex of papillae covered with wax; rounded papillae apex and presence of fibrous hour-glass cells can be considered as taxonomic markers for *V. dasycarpa*. In addition, blocked central groove of hilum and presence of tracheid bar at aperature of hilar groove are characteristic for *V. narbonensis*.

The highest values of hilum length and width; width: length ratio, length and width of central groove, maximum width of micropyle, average thickness of the palisade -like cells, the palisade layer at hilar region, thickness of the counter palisade layer, summation of thickness of the palisade layer + thickness of counter palisade layer, thickness of aperature hilar groove, maximum length of tracheid bar and its maximum width its were recorded in *V. narbonensis*.

Key words: *seed testa structure Vicia species*

1. INTRODUCTION

Lersten and Gunn (1982) observed papillose testa, elongated hilum, virtual absence of hilar rim and a micropyle adnate to hilum in tribe *Vicieae*. Whereas, Kaur and Pal (1989) examined the central region of *Vicia* seeds, on lateral sides by using SEM and found that it was tuberculate pattern with slight variations. These tubercles were arranged in somewhat irregular fashion *in V. tetrasperma, V. sativa, V. cracca, V. sepium, V. unijuga and V. cretica*, whereas; it was in more regular way in *V. hirsuta.* Moreover, they found prominent striations radiate around the main body of tubercles in *V. dumetorum, V. cracca, V. unijuga, V. sativa* and *V. hirsuta* Striations of the tubercles of *V. cracca, V. hirsuta and V. angustifolia* extend and join those of adjacent tubercles. These tubercles were arranged more or less in a compact manner in *V. dumetorum, V. unijuga, V. tetrasperma and V. hirsuta,* whereas they were more sparsely arranged in *V. sativa, V. cracca, V. angustifolia* and *V. cretica.* On the other hand, Hassan (1997) studied the spermoderm pattern of *Vicia* species and found that the papillae shape was star-like in *V. cinerea,* octopus in *V. cordata,* troughs and crests in *V. faba* cv. Giza 2, conical to triangular in *V. monantha,* triangular in *V. nigra* and sea anemone-like in both *V. peregrina* and *V. sativa*; its arrangement was in regular rows as in *V. cordata, V. monantha, V. nigra, V. peregrina* and *V. sativa*; its apex was truncate in *V. cinerea,* obtuse in both *V. cordata* and *V. monantha,* acute to hooked in *V nigra,* acute to obtuse in *V. peregrina* and rounded to truncate in *V. sativa.*

Gunn (1970) and Lersten and Gunn (1982) stated that all of the studied hila of *Vicia* species have a fine central groove (the hilar groove). The groove was readily visible. They recognized five hilum shapes (circumlinear, linear, oblong, wedge and oval); a circumlinear hilum was more than 10 times longer than wide. A linear hilum which was 5 to 7 times as long as wide, has parallel margins; an oblong hilum which is less 5 times, usually, greater than twice, as long as wide, has slightly curved margins. An oval hilum length was twice than its width and rounded in outline. Moreover, Kaur and Pal (1989)

found that the hilum was oblong-elliptical in *V. faba, V. tetrasperma* and *V. angustifolia,* oval in *V. cretica* and linear in all other *Vicia* species. The hilum has a prominent, narrow and elongated groove in all the ten species. Length of the hilum varied from very long in *V. dumetorum* to very short in *V. cretica.* In addition, Hassan (1997) pointed out that the maximum values for average length and width (mm) of hilum (4.5 and 1.5, respectively) in *V. faba* cv. Giza 2; while, minimum values were 1.1 and 0.3 mm for average length in *V. sativa* and for average width in both *V. nigra* and *V. sativa,* respectively. Hilum shape was oblong in *V. cinerea, V. monantha* and *V. sativa,* linear in both *V. cordata* and *V. nigra,* elliptic in *V. faba* cv. Giza 2 and linear to oblong in *V. peregrina.*

Roti-Michelozzi and Serrato-Valenti (1981) examined the *Vicia* seed structure and observed an external palisade layer of prismatic malpighian cells overlying on outer hypodermis consisting of hour-glass cells, separated by wide intercellular spaces, and three to four layers of unspecialised cells, the hour glass cell layer consists of a sclerified tissue, composed by osteo-or lagenosclereids. In addition, Lersten and Gunn (1982) described the tracheid bar of the hilum to be broadly elliptical in a transectional view in most *Vicia* species, but it approaches the cuticular in *Vicia sylvatica, V. faba* and *V. bithynica;* and it was more narrowly elliptical in *V. semiglabra* and *V. sativa* species. The tracheid bar was noticeably smaller in the transectional area in *V. unijugea, V. cassubica* and *V. nigricans* species. Moreover, Kaur and Pal (1989) and Sanchez-Yelamo *et al.,* (1992) found that the macrsclereid layer was followed by a layer of thick-walled; hour glass cells which were dumbell-shaped having air spaces between them; remaining part of the seed coat consists of 2-7 layers of degenerating parenchyma and dark crushed mass of cells in all the studied *Vicia* species. The hilar region was composed of palisade and counter palisade layer. The counter palisade cells were found in the area of the hilar region only . Also, they noted a hilar groove in all the studied *Vicia* species. Furthermore, Hassan (1997) studied the seed structure of *Vicia* species and found that the variations in average thickness of the palisade-like cells, the hour glass cells, the parenchymatous tissue and average parenchyma cell, as well as, Number of each parenchymatous cells and palisade-like cell rows

were significant among most examined *Vicia* species.

The present research was carried out to study testa structure of some *Vicia* species and their identification by structural characters of testa.

2. MATERIALS AND METHODS

A field experiment was performed, on 15 October during the growing season of 1998/1999 at the Experimental Farm of Suez Canal University in Ismailia to study testa structure of five *Vicia* spp.; seeds of all 5 spp. were imported from the Molecular Biology Section Botany Institute 1, JLU, GieBen, Germany. These species are:

1. *Vicia amphicarpa.*
2. *Vicia dasycarpa.*
3. *Vicia ervilia.*
4. *Vicia narbonensis.*
5. *Vicia villosa.*

The seeds of the different five *Vicia* species were planted in plots in a complete randomized design with four replicates. The plot area of each replicate was 20 m^2 having nine rows of 4 m in length and 50 cm width. Usually seeds of some *Vicia* species do not germinate under natural conditions; therefore mechanical scarification for testa was carried out according to Sharma and Lavania (1979). Seeds of each species were sown in hills 25 cm apart in sandy soil. Nitrogen, phosphorous and potassium were incorporated in the soil at the rate of 30, 35, and 48 unit/feddan, respectively in split doses at 20 days after seed emergence and at the beginning of flowering. Supplementary irrigation was provided whenever necessary.

2.1.The following data were recorded

2.1.1. For studying of spermoderm pattern, hilum and micropyle characters of various *Vicia* species, the method described by Trivedi *et al.*, (1978) was followed. Samples of air dried seeds were taken, adhesived on the stubs of the Scanning Electron Microscope (Cambridge S_4) and then coated with gold. The apparatus was supplied with photocopy unit.

2.1.2. For studying the anatomical structure of seed coat, the seed samples were taken before harvest time, killed and fixed in 70% FAA

solution, dehydrated with n-butyl alcohol and embedded in pure paraffiwax (M.P. 56-58°C) as described by Willey (1971). Using a rotary microtome, sections (12μ) were obtained and stained with safranin and anilin blue according to Gerlach (1977). Sections, in such cases were microscopically examined.

3. RESULTS AND DISCUSSION

3.1. Morphological studies

3.1.1. Spermoderm pattern

Table (1) and Figure (1) show that papillae arrangement in rows was irregular in *V. amphicarpa*, *V. dasycarpa* and *V. villosa* (Figure 1 A, B and E); whereas; it was regular in *V. ervilia* and *V. narbonensis* (Figure 1 C and D). Papillae apex covered with wax was observed in *V. dasycarpa* (Figure 1 B); while; it was absent in other *Vicia* species under investigation. Shape of papillae apex was rounded in *V. dasycarpa* (Figure 1 B); while it was acute in *V. amphicarpa, V. ervilia, V. narbonensis* and *V. villosa* (Figure 1A, C, D and E). Papillae were more compacted in both *V. dasycarpa* and *V. villosa* (Figure 1B and E respectively). Whereas; it showed medium compactness in both *V. amphicarpa* and *V. ervilia* (Figure 1A and C). It was less compacted in *V. narbonensis* (Figure 1D). Presence of the striations on the papillae surface was observed in *V. amphicarpa, V. ervilia, V. narbonensis* and *V. villosa* (Figure 1A, C, D and E). Whereas, it was absent in *V. dasycarpa* (Figure 1B). Papillae size was large in *V. narbonensis* (Figure 1D). At the same time, it was medium in both *V. amphicarpa* and *V. ervilia* (Figure 1A and C, respectively). Whereas, it was small in both *V. dasycarpa* and *V. villosa* (Figure 1B and E, respectively). Such results are strengthened by Roti-Michelozzi and Serrato-Valenti (1981), Kaur and Pal (1989) and Hassan (1997) who pointed out that *Vicia* species spermoderm is papillose. These papillae are regular and their apices are rounded in *V. sativa*. Moreover, papillae compactness differed according to the studied species.

3.1.2. Hilum

Table (1) and Figure (2) show that the highest values of

Table(1): Morphological studies of spermoderm pattern, hilum and micropyle of seed coat of some *Vicia* species using SEM.

Characters	Species	*V. amphicarpa*	*V. dasycarpa*	*V. ervilia*	*V. narbonensis*	*V. villosa*
Spermoderm pattern	Papillae arrangement in rows	Irregular	Irregular	Regular	Regular	Irregular
	Papillae apex covered with wax	-	+	-	-	-
	Shape of papillae apex	Acute	Rounded	Acute	Acute	Acute
	Papillae compactness	Medium	More comp.	Medium	Less comp.	More comp.
	Striations presence on the papillae surface	+	-	+	+	+
	Papillae size	Medium	Small	Medium	Large	Small
Hilum	Average length (mm)	1.57	1.63	0.95	2.17	1.63
	Average width (mm)	0.43	0.43	0.46	1.17	0.37
	Shape	Oblong to linear	Oblong to linear	Oval	Oval	Linear
	Width/length ratio	0.27	0.26	0.48	0.53	0.22
Central groove	Symmetry	+	+	+	-	+
	Average length (mm)	1.28	1.48	0.78	1.71	1.37
	Average maximum width	0.014	0.028	0.03	0.23	0.028
	Blocked	-	-	-	+	-
Micropyle	Shape	Bridge	Oblong	Bridge	Pear	Bridge
	Closed at its middle	+	-	+	-	+
	Average maximum width (μ m)	24	24	6	30	9

+ = present - = absent

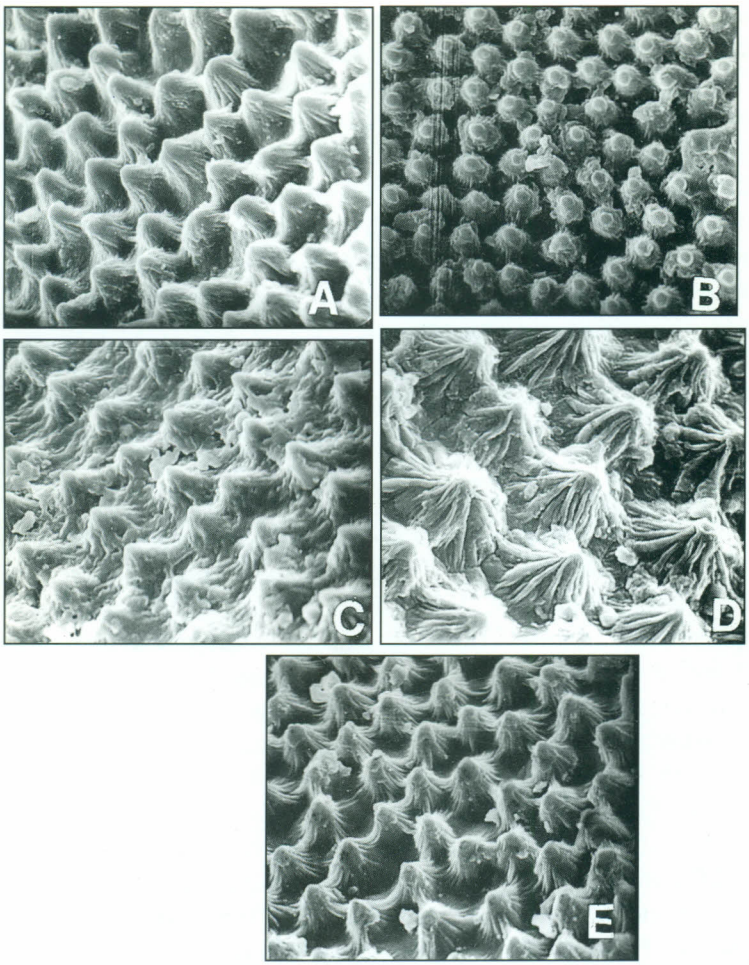


Figure (1): Scanning electron micrographs of testa surface showing spermoderm pattern of:
A-*V. amphicarpa* (X1320)
B-V. dasycarpa (X 1320)
C-*V. ervilia* (X1320)
D-*V. narbonensis* (X1320)
E-*V. villosa* (X1320)
A –E ——— 20μm

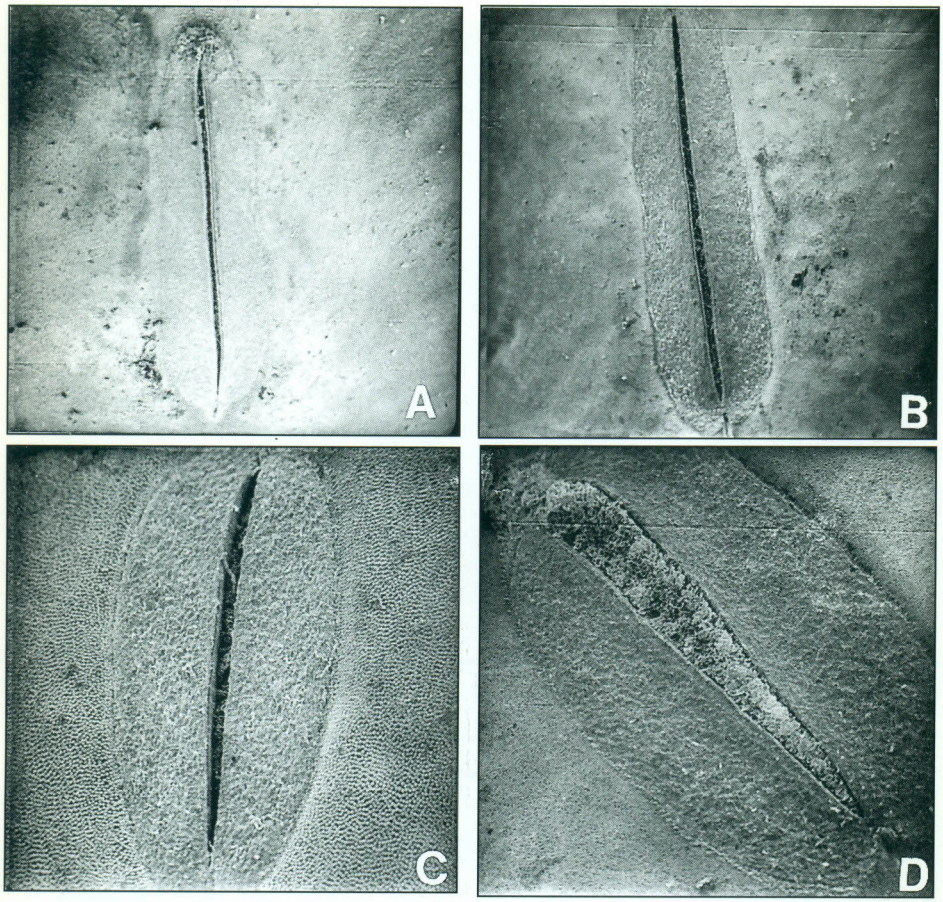
average length of hilum (mm), average width of hilum (mm) and width: length of hilum ratio were recorded in *V. narbonensis* (2.17, 1.17 and 0.53, respectively) as shown in Figure (2D), while, the lowest ones were found in *V. ervilia* (0.95 mm) for average hilum length (Figure 2 C); *V. villosa* (0.37 and 0.22) for both average hilum width (mm) and width/length of hilum ratio (Figure 2E). Shape of hilum was oblong to linear in both *V. amphicarpa* and *V. dasycarpa* (Figure 2 A and B); but; it was oval in both *V. ervilia* and *V. narbonensis* (Figure 2 C and D) whereas; it was linear in *V. villosa* (Figure 2E). These results are in agreement with those obtained by Hassan (1997) who mentioned that average of hilum length ranged from 1.1 to 4.5 mm; while; average of hilum width ranged from 0.3 to 1.5 mm in studied *Vicia* species.

3.1.3. Central groove of hilum

It is noticed from (Table 1 and Figure 2) that the central groove of hilum is symmetrical in *V. amphicarpa, V. dasycarpa, V. ervilia* and *V. villosa* (Figure 2A, B, C and E), whereas, it is asymmetrical in *V. narbonensis* (Figure 2D). The highest values for average length (mm) and average width (mm) of central groove were found in *V. narbonensis* (1.71 and 0.23; respectively) as shown in (Figure 2 D). The lowest ones for the two above mentioned characters were observed in *V. amphicarpa* (1.28 and 0.014 mm; respectively) as given in (Figure 2A). Blocked central groove was noticed in *V. narbonensis* (Figure 2 D). whereas, central groove was not blocked in other different *Vicia* species studied (Figure 2 A, B, C and E). These results are similar with Lersten and Gunn (1982) who reported that the hilum of *Vicia* species have a fine central groove.

3.1.4. Micropyle

Data in Table (1) and Figure (3) show that the micropyle is bridge-shaped in *V. amphicarpa*, *V. ervilia* and *V. villosa* (Figure 3 A, C and E), while, it was oblongshaped in *V. dasycarpa* (Figure 3 B). Moreover, it is pearshaped as in *V. narbonensis* (Figure 3 D). Micropyle is closed at its middle in *V. amphicarpa* , *V. ervilia* and *V. villosa* (Figure 3 A, C and E), while; it was open in the other *Vicia* species studied (Figure 3 B and D). Maximum width of micropyle



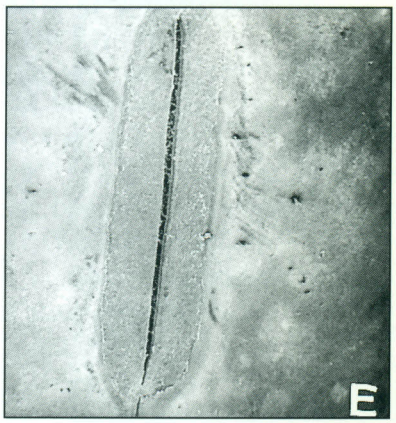


Figure (2): Scanning electron micrographs of testa surface showing hilum shape of:
A-*V. amphicarpa* (X35)
B-V. dasycarpa (X 35)
C-*V. ervilia* (X65)
D-*V. narbonensis*(X35)
E-*V. villosa* (X35)

C ——————————

A, B, D and E ———— 500 µm

species studied (Figure 3 B and D).Maximum width of micropyle (μm) was observed in *V. narbonensis* (30μm) as shown in Figure (3 D). The minimal value was found in *V. ervilia* (6μm) as given in Figure (3C). These results are in agreement with those of Hassan (1997), who reported that micropyle was pear-shaped in *V. cordata*, oval to oblong in *V. nigra* and their average width was 10 μm in *V. monantha*.

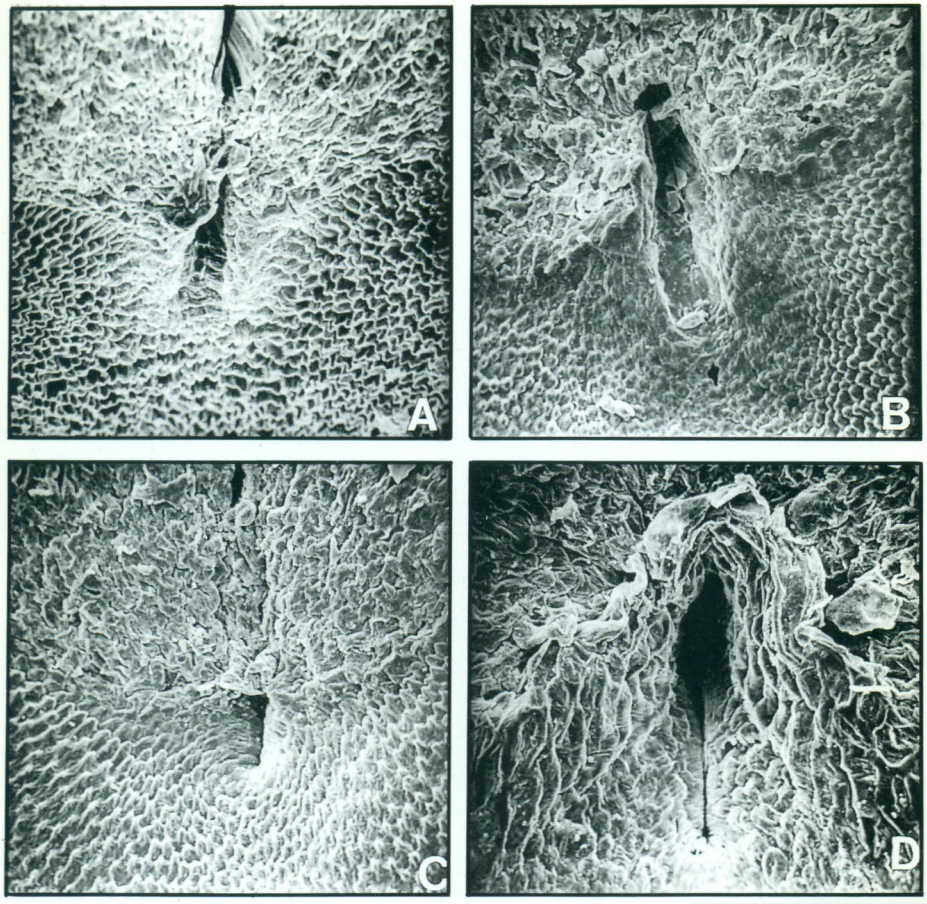
3.2. Anatomical studies

3.2.1. Seed testa

Table (2) and Figure (4) show that the highest values for average thickness of the palisade-like cells (μ) and average thickness of the hour glass cells (μ) were found in both *V. narbonensis* (180) and *V. villosa* (68) (Figure 4 D and E), respectively; in addition; the lowest ones for the two above mentioned characters were recorded in *V. ervilia* (72 and 16 (μ) respectively (Figure 4 C). Presence of malpighian cells was observed in *V. amphicarpa, V. dasycarpa* and *V. ervilia* Figure(4 A, B and C), while, they were absent in both *V. narbonensis* and *V. villosa* (Figure 4 D and E). Presence of fibrous hour glass cells was recorded in *V. dasycarpa* (Figure 4 B); while; it was absent in other studied *Vicia* species (Figure 4 A, C, D and E). Such results are strengthened by Corner (1976), Roti-Michelozzi and Serrato-Valenti (1981), Kaur and Pal (1989) and Sanchez-Yelmamo *et al.*, (1992) who found that the macrsclereid layer was followed by a layer of thick-walled; hour-glass cells.

3.2.2. Seed testa at hilar region

It is clear from Table (3) and Figure (5) that the shape of tracheid bar was small elliptical in *V. amphicarpa* (Figure 5 A); narrowly elliptical in both *V. dasycarpa* and *V. villosa* (Figure 5 B and E) and broadly elliptical in both *V. ervilia* and *V. narbonensis* (Figure 5 C and D). Presence of tracheid bar at aperature of hilar groove was found in *V. narbonensis* (Figure 5 D); whereas; it was absent in other *Vicia* species studied (Figure 5 A, B, C and E). Maximum values of thickness of the palisade layer (μ), thickness of the counter palisade layer (μ), summation of the palisade layer + the counter palisade layer (μ), thickness of aperature hilar groove (μ),



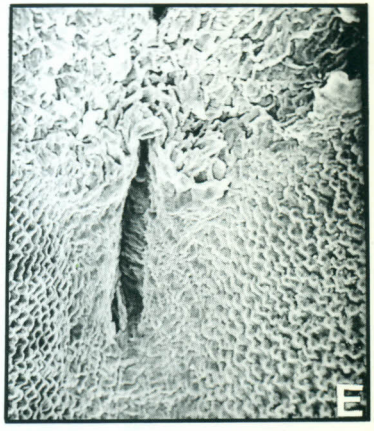


Figure (3): Scanning electron micrographs of testa surface showing micropyle shape of:
A-*V. amphicarpa* (X325)
B-V. dasycarpa (X 325)
C-*V. ervilia* (X325)
D-*V. narbonensis*(X325)
E-*V. villosa* (X325)
A – E——————— 100 µm

Table (2): Anatomical characters of five *Vicia* species seed testa.

Characters \ Species	*V. amphicarpa*	*V. dasycarpa*	*V. ervilia*	*V. narbonensis*	*V. villosa*	L.S.D at 0.05
Average thickness of the palisede like cells (μ)	80	96	72	180	116	7.52
Average thickness of the hour glass cells (μ)	32	56	16	36	68	0.49
Presence of Malpighian cells	+	+	+	-	-	-
Presence of fibrous hour glass cells	-	+	-	-	-	-

Table (3): Anatomical characters of the five *Vicia* species seed testa at hilar region.

Characters \ Species	*V. amphicarpa*	*V. dasycarpa*	*V. ervilia*	*V. narbonensis*	*V. villosa*	L.S.D at 0.05
Shape of tracheid bar	Small elliptical	Narrowly elliptical	Broadly elliptical	Broadly elliptical	Narrowly elliptical	
Presence of tracheid bar at aperature of hilar groove	-	-	-	+	-	
Thickness of the palisade layer (μ)	46	61	62	107	92	5.55
Thickness of the counter palisade layer (μ)	76	61	68	92	76	4.13
Summation of the palisade layer + the counter palisade layer (μ)	122	122	130	199	168	3.51
Thickness of aperature hilar groove (μ)	15	61	18	292	15	0.84
Maximum length of tracheid bar (μ)	184	307	212	615	338	1.29
Maximum width of tracheid bar (μ)	107	169	168	477	169	3.11

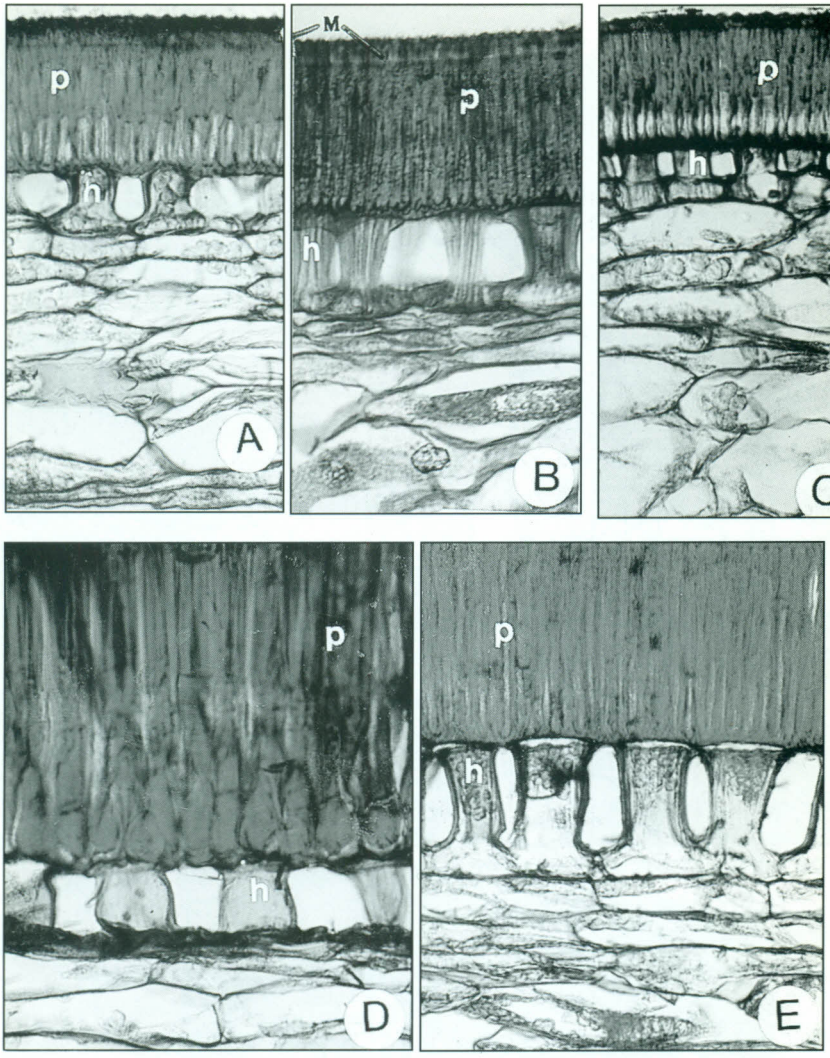


Figure (4):Seed testa cross sections of:

A-*V. amphicarpa* (X250) D-*V. narbonensis* (X250)

B-*V. dasycarpa* (X250) E-*V. villosa* (X250)

C-*V. ervilia* (X250)

Abbreviations : (P, palisade-like cells ;h, hour glass cells and M; malpighian cell)

A –F 200 µm

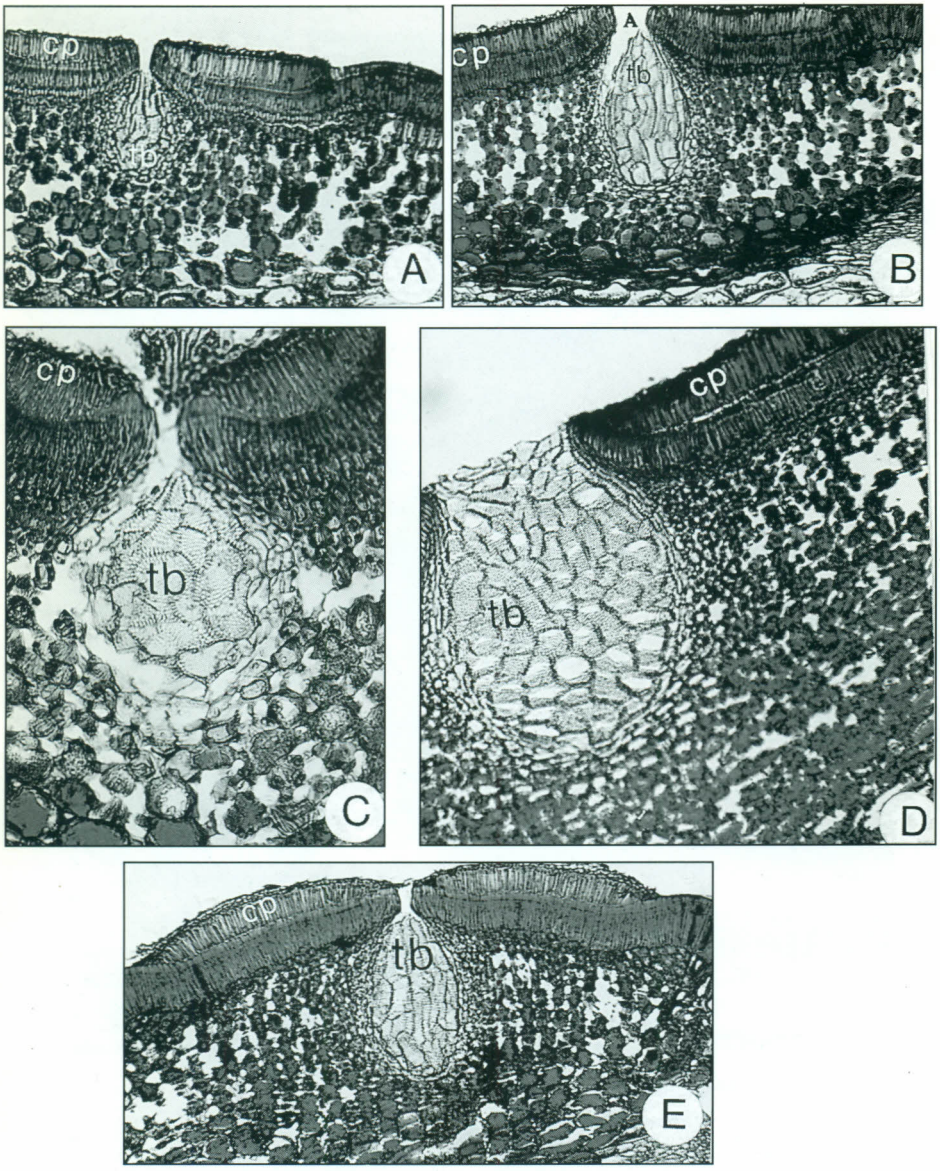


Figure (5):Seed testa longitudinal sections at hilar region of:

A-*V. amphicarpa* (X65) D-*V. narbonensis* (X65)

B-*V. dasycarpa* (X 65) E-*V. villosa* (X65)

C-*V. ervilia* (X160)

Abbreviations : (tb, trachied bar ; cp, counter palisade cells and A; aperature of hilar groove) C———————

A, B, D and E ———— 200 µm

maximum length of trachied bar (μ) and maximum width of tracheid bar (μ) were recorded in *V. narbonensis* (107, 92, 199, 292, 615 and 477) respectively Figure(5 D). The lowest values of thickness of the palisade layer (μ), summation of the palisade layer + the counter palisade layer (μ), thickness of aperature hilar groove (μ), maximum length of tracheid bar (μ) and maximum width of tracheid bar (μ) were noticed in *V. amphicarpa* (46, 122, 15, 184 and 107) respectively; Figure (5 A). These results in agreement with those obtained by Lersten and Gunn (1982) who described the tracheid bar of the some *Vicia* species where; it was broadly elliptical in most *V. species*, narrowly elliptical in *V. semiglabra* and *V. sativa* and smaller in *V. unijugea*, *V. cassubica* and *V. nigricans*.

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تركيب القصرة والتعرف على بعض أنواع الفول

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ملخص

درس تركيب القصرة لبعض أنواع الفول *V. aasycarpa* و *V. amphicarpa* و *V.villosa* و *V. narbonensis* و *V. ervilia* بواسطة الميكروسكوب الإلكتروني الماسح والميكروسكوب الضوئي وقد أمكن التوصل للنتائج التالية:

كان نظام التضاريس لقصرة أنواع الفول المدروسة حلميا. وقد اختلفت الأنواع المدروسة فى انتظام الحلمات وشكل قمتها ، كثافتها ، وجود الاخاديد على سطحها ، كذلك حجم الحلمات ، شكل السرة ، تماثل الأخدود المركزي لها ، شكل النقير ، الانغلاق عند منتصفة ، وجود خلايا ملبيجى وشكل Tracheid bar عند منطقة السرة.

تعتبر قمة الحلمات المغطاة بالشمع ، القمة المستديرة ، الخلايا العظمية الليفية الشبيهة بزجاجات الساعة علامات تقسيمية مميزة للنوع *V. dasycarpa* ، بالإضافة لذلك فان وجود الأخدود المركزي المسدود للسرة ووجود Tracheid bar عند فتحة السرة تعتبر علامات تقسيمية مميزة للنوع *V. narbonensis*

سجلت أعلى القيم لطول السرة وعرضها ونسبة الطول إلى العرض ، طول الأخدود المركزي وعرضة ، أقصى عرض للنقير ، متوسط سمك الخلايا

الشبيهة بالخلايا العمادية فى منطقة السرة ، سمك الطبقة العمادية المقابلة ، مجموع سمك الطبقة العمادية + سمك الطبقة العمادية المقابلة ، سمك فتحة أخدود السرة ، و أقصي طول لـ Tracheid bar و أقصى عرض له في النوع *V. narbonensis*

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (51) العدد الأول يناير (2000): **55-72.**