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INVESTIGATION OF OVULE INTEGUMENTS OF SOME *VICIA* TAXA BY TRANSMISSION ELECTRON MICROSCOPY (TEM) AT ANTHESIS.

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ABSTRACT

Electron-microscopic preparations of some *Vicia* species ovule integuments (*V.cinerea, V.cordata, V.faba* cv.Giza 2, *V.monantha, V.nigra, V.peregrina* and *V.sativa*) were studied at anthesis. Cuticle shape, average thickness of cuticle + cell wall (nm), presence of the space between the inner and outer ovule integuments, shape of mesoparenchyma cells of both outer and inner integuments and maximum width (nm) of the space between them; and mesoparenchyma cell outline shape, vacuoles, starch grains, plastids, mesoparenchyma cells of both inner and outer ovule integuments; differed according to the studied taxa.

Integer cuticle shape, dentatus outer and inner integument margins and irregular-rombicus cell outline shape of mesoparenchyma cells of inner ovule integument are characteristic for *V.cordata* species. Furthermore, absence of the space between the inner and outer integuments are characteristic for *V.nigra* species. Serrated cuticle margin, obovatus mesoparenchyma cell of outer integument shape and pentagonal mesoparenchyma cell of inner integument shape are taxonomical markers for *V.sativa* species. In the same time, deltoid and reniform ovule inner integument cells and presence of osmiophilic bodies are characteristic for both *V.monantha* and *V.peregrina* species; respectively.

Key words: *ovule integuments, transmission electron microscopy, Vicia taxa..*

1. INTRODUCTION

Wojciechowska and Erdelska (1983) studied the bud development from the green bud to the overblown flower stages in *Vicia faba, V.sativa, V.villosa, Lathyrus pratensis, L.silvester* (*L.sylvestris*) and *Pisum sativum*. Megasporogensis in *Vicia sativa* and *Lathyrus silvester* was normal. *Vicia sativa* had one or two tetrads in the ovule and *Lathyrus silvester* had anisobilateral or linear tetrads. Each species had a unique bud developmental rhythm. In addition, Johansson and Walles (1993a) studied the structure adaptations promoting apoplastic transport to the embryo sac in broad beans (*Vicia faba*) during histogenesis from ovule primordia to seed formation. Nucellus and the inner integument disappear at an early stage. The outer integument then adjoins the embryo sac boundary. In the thin part distal to the funiculus the cells of the persisting integument are smaller than in other parts and develop PAS-positive wall ingrowths opposite the embryo. At the late globular stage, the embryo establishes contact with the embryo sac boundary. In the contact zone, embryo cells develop wall ingrowths. Wall ingrowths are further formed on both sides of that part of the endosperm that is inserted between the outside of the cotyledons and the embryo sac boundary. The wall proliferations characterize all these cells as transfer cells. They concluded that the embryo sac is supported with nutrients from digested maternal tissues (nucellus, chalaza, inner integument, and part of the outer integument). These tissues are generally rich in starch grains. After the embryo has established contact with the embryo sac boundary, it is supported by transport of solutes from transfer cells in the outer integument. In the same time, Johansson and Walles (1993b) investigated ultrastructural features of broad bean (*Vicia faba*) ovules for nutrient transport to the embryo sac at various ontogenetic stages up to 10 days after pollination. In unpollinated flowers a notable homogeneous or fibrillar material is deposited in the endostome, between the two integuments and on the nucellus. Osmiophilic globules accumulate at the plasmalemma and in the walls at the micropylar end of the inner integument. These

globules increase in number after fertilization and appear also in other cells near the embryo sac. The central cell, which has some wall ingrowths typical for transfer cells, shows intrusive growth between cells of the nucellar cap. After fertilization wall thickenings occur in cells close to the embryo sac. At 10 days after pollination the inner integument has degenerated entirely. Also the nucellus, including the nucellar cap, is digested. In the endosperm free- nuclear divisions start and the cytoplasm increases in amount. Wall ingrowths are formed along the whole embryo sac boundary. The suspensor consists of two pairs of multinucleate cells: the pair adjacent to the embryo proper have rounded cells: the other pair have elongated ones. The suspensor cells that are attached to the embryo sac boundary become transfer cells. Their plastids have prolamellar bodies, and these structures are not seen anywhere else in the ovule. This study confirms that transfer cells are common at junctions between the different generations in the ovules, that the transport to the embryo sac is apoplastic, and that symplastic transport is possible between endosperm and embryo and further between suspensor and embryo proper. On the other hand, Johansson and Walles (1994) studied *Vicia faba* cv.Green Hangdown ovules by light microscopy, TEM and SEM from 10 days after pollination (DAP) until seed maturity, to disclose ultrastructural features which can facilitate nutrient transport to the embryo sac. Fertilization occurred during the first 24 hours after pollination. The endosperm was a coenocyte, which was eventually consumed by the embryo. By 10 DAP the inner integument was degraded and the outer integument adjoined the embryo sac boundary. The heart-shaped embryo approached the embryo sac boundary at two sites, which were named contact zones. Small integument cells in the neighbourhood of the first formed contact zones became separated by prominent intercellular spaces. A heterogenous scattering material, probably representing secretion products accumulated in these spaces. By 14-16 DAP the integument exudate disappeared, and the suspensor degenerated. As the contact zones increased in size, wall ingrowths formed a bridging network in the narrow space between the embryo sac boundary and the extra-embryonic part of the endosperm wall. The epidermal cells of the embryo separated adjacent to these zones, and developed conspicuous wall ingrowths. At 20 DAP vacuoles showing various stages in formation of protein bodies appeared in the

cells of the embryo.

The present research was carried out to study the ultrastructural variations among ovules of some *Vicia* taxa at anthesis.

2. MATERIALS AND METHODS

Seeds of the different *Vicia* species (*V.cinerea* M.Bieb., *V.cordata* Wulfen ex Hoppe, *V.faba* L. cv. Giza2, *V.monantha* Retz., *V.nigra* L., *V.peregrina* L. and *V.sativa* L.) were planted, on 15 October during the growing season of 2000 / 2001 at the Experimental Farm of Suez Canal University, 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. Mechanical scarification of seed testa for all *Vicia* species except *V.faba* cv.Giza2 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 fertilization were incorporated in the soil at 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. The different studied *Vicia* taxa flowered at various stages.

For studying the transverse sections of six different *Vicia* taxa and *V.faba* cv. Giza2 ovules by Transmission Electron Microscopy (TEM), ovules were dissected from the samples at anthesis. Ovules to be sectioned were fixed 12 –24 hours at 4 °C in 4 – 5 % glutaraldhyde buffered to pH 7.0 –7.4 by 0.05 – 0.1 M phosphate buffer (di-sodium hydrogen phosphate dihydrate + potassium dihydrogen phosphate). After three buffer rinses for 20 minutes each, ovules were postfixed in 1 % OsO_4 (Osmium tetraoxide) in the same buffer for 1- 1.5 hours, washed in the buffer (4 times) and distilled water, 20 minutes. Each, dehydrated in ethanol series, 50 %, 70 %, 85 %, 90 %, 95 % and 100 % for 15 – 30 minutes. Each; then transition in ethanol – spur resin mixture for 4 hours and resin pure twice for 24 hours, then embedding in LR-White resin at 50° C. Silver sections (90 nm) were cut with Reichert Ultramicrotome with a diamond Knife. After uranyl acetate and lead citrate staining, the thin sections were viewed with a Philips 300 Transmission Electron Microscope at 60 KV (Dute *et al.*, 1989).

3. RESULTS AND DISCUSSION

3.1. Ovule outer integument

Data in Table (1) and Figure (1) show that cuticle shape is erosus-integer in both *Vicia cinerea* (Fig.1A) and *V.monantha* (Fig. 1D), erosus in *V.faba* cv.Giza2 (Fig. 1C), *V.nigra* (Fig. 1E) and *V.peregrina* (Fig. 1F) and it is integer (Fig. 1B) and serrated (Fig. 1G) in both *V.cordata* and *V.sativa*. Maximum value of average thickness of cuticle + outer epidermal cell walls (992 nm) was recorded in *V.sativa* (Fig. 1G); whereas, the lowest ones (370 nm) was found in *V.cinerea* (Figure 1A), *V.cordata* (Fig. 1B) and *V.nigra* (Fig. 1E).

3.2. Mesoparenchyma cells of ovule outer integument

Data in Table (1) and Figure (2) indicate that mesoparenchyma of ovule outer integument cell outline shape is rombicus in *V.cinerea* (Fig. 2A), *V.monantha* (Fig. 2D), *V.nigra* (Fig. 2E) and *V.peregrina* (Fig. 2F), deltoid in both *V.cordata* (Fig. 2B) and *V.faba* cv.Giza2 (Fig. 2C). In addition, it is obovatus in *V.sativa* (Fig. 2G). Highest value for vacuoles number per cell (12) was found in *V.monantha* (Fig. 2D); whereas, the lowest one (2) was shown in both *V.faba* cv.Giza2 (Fig. 2C) and *V.sativa* (Fig. 2G). Starch grains are present in *V.cinerea* (Fig. 2A), *V.nigra* (Fig. 2E) and *V.sativa* (Fig. 2G); while, they are absent in the other studied genotypes (Figs. 2B, C, D and F). Highest number of starch grains (10) and plastids per cell (7) were found in both *V.cinerea* (Fig. 2A) and *V.faba* cv.Giza2 (Fig. 2C) respectively. Moreover, plastids are absent in *V.cinerea* (Figure 2A), *V.cordata* (Fig. 2B) and *V.nigra* (Fig. 2E). Whereas, they are present in the remaining studied genotypes (Figs. 2C, D, F and G). Presence of starch grains in the outer integument cells of ovule in *V.cinerea*, *V.nigra* and *V.sativa* is in agreement with the data obtained by Johansson and Walles (1993a). Furthermore, absence of plastids of ovule outer integument cells in *V.cinerea*, *V.cordata* and *V.nigra* is harmony with those of Johansson and Walles (1993b) who observed that plastids are not seen anywhere else in the ovule.

3.3. The space between the inner and outer integument of ovule

Table (2) and Figures (3 and 4) show that the absence of the space between the inner and outer integument is characteristic for

Table (1): Ultrastructural of mesoparenchyma cell of outer integument ovule of some *Vicia* taxa at anthesis in 2000 / 2001 season.

Recorded data / Taxa	Ovule outer integument		Mesoparenchyma cell of outer integument					
	Cuticle shape	Average thickness of cuticle + cell wall (nm)	Cell outline shape	Vacuoles number per cell	Presence of starch grains	Number of starch grains per cell	Presence of plastids	Number of plastids per cell
Vicia cinerea	Erosus – integer	370	Rombicus	7	+	10	-	-
V.cordata	Integer	370	Deltoid	4	-	-	-	-
*V.faba cv.*Giza2	Erosus	450	Deltoid	2	-	-	+	7
V.monantha	Erosus – Integer	580	Rombicus	12	-	-	+	1
V.nigra	Erosus	370	Rombicus	7	+	2	-	-
V.peregrina	Erosus	812	Rombicus	9	-	-	+	2
V.sativa	Serrated	992	Obovatus	2	+	4	+	1

Table (2): Some anatomical characters of the space between the inner and outer integument and mesoparenchyma cell of ovule inner integument of some *Vicia* taxa at anthesis in 2000 / 2001 season.

Recorded Data / Taxa	The space between the inner and outer integument				Mesoparenchyma cell of inner integument							
	Presence of space	Shape of outer integument margin	Shape of inner integument margin	Maximum width of it (nm)	Cell outline shape	Small vacuoles number	Presence of large vacuoles	Number of large vacuoles per cell	Presence of starch grains	Number of starch grains per cell	Presence of plastids	Presence of osmiophilic bodies
Vicia cinerea	+	Erosus	Undulated	562	Rombicus	Medium	-	-	-	-	+	+
V.cordata	+	Dentatus	Dentatus	562	Irregular - rombicus	Medium	+	1	+	5	-	-
*V.faba cv.*Giza2	+	Undulated	Undulated	375	Rombicus	Few	+	2	-	-	+	-
V.monantha	+	Undulated	Undulated	687	Deltoid	Numerous	-	-	-	-	+	-
V.nigra	-	-	-	-	Rombicus	Few	-	-	+	10	-	+
V.peregrina	+	Undulated	Undulated	375	Reniforms	Numerous	+	1	-	-	-	+
V.sativa	+	Erosus	Undulated	500	Pentagonal	Few	+	1	-	-	+	-

+= Present - = Absent V=*Vicia*

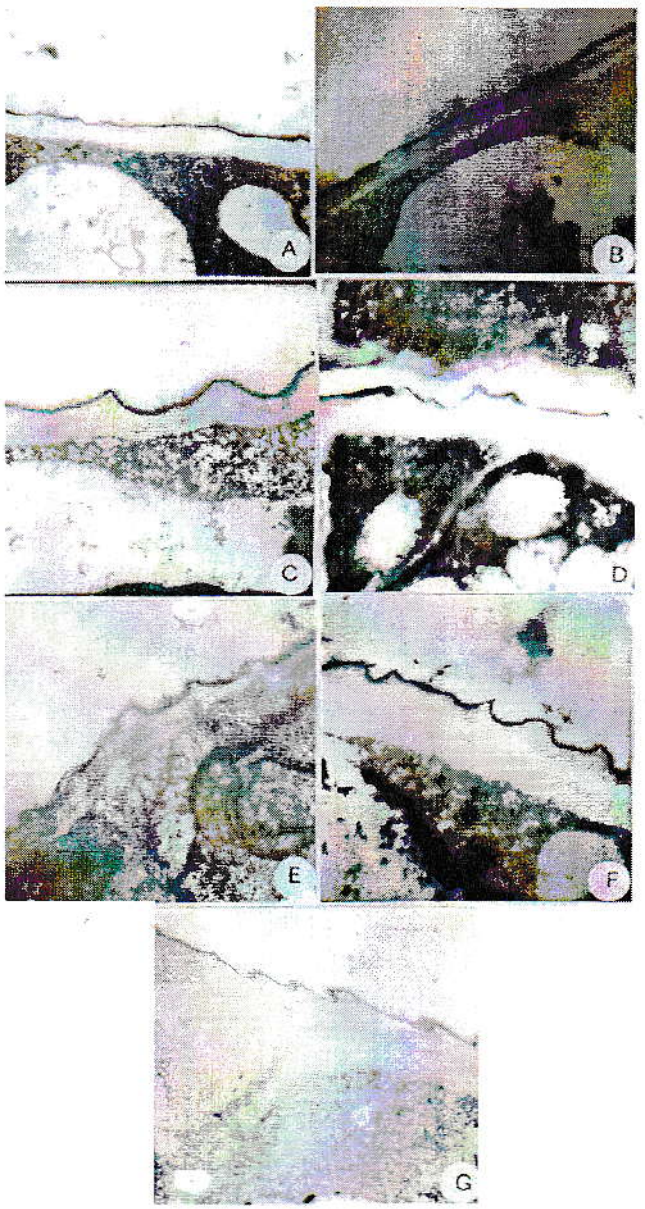

Figure (1):- Transmission electron micrographs of the outer integument of the ovule showing cell wall and cuticle of the outer epidermal cells.

A- *V.cinerea*	(x 16000)	E- *V.nigra*	(x 16000)
B- *V.cordata*	(x 16000)	F- *V.peregrina*	(x 16000)
C- *V.faba* cv.Giza 2	(x 16000)	G- *V.sativa*	(x 13100)
D- *V.monantha*	(x 16000)		

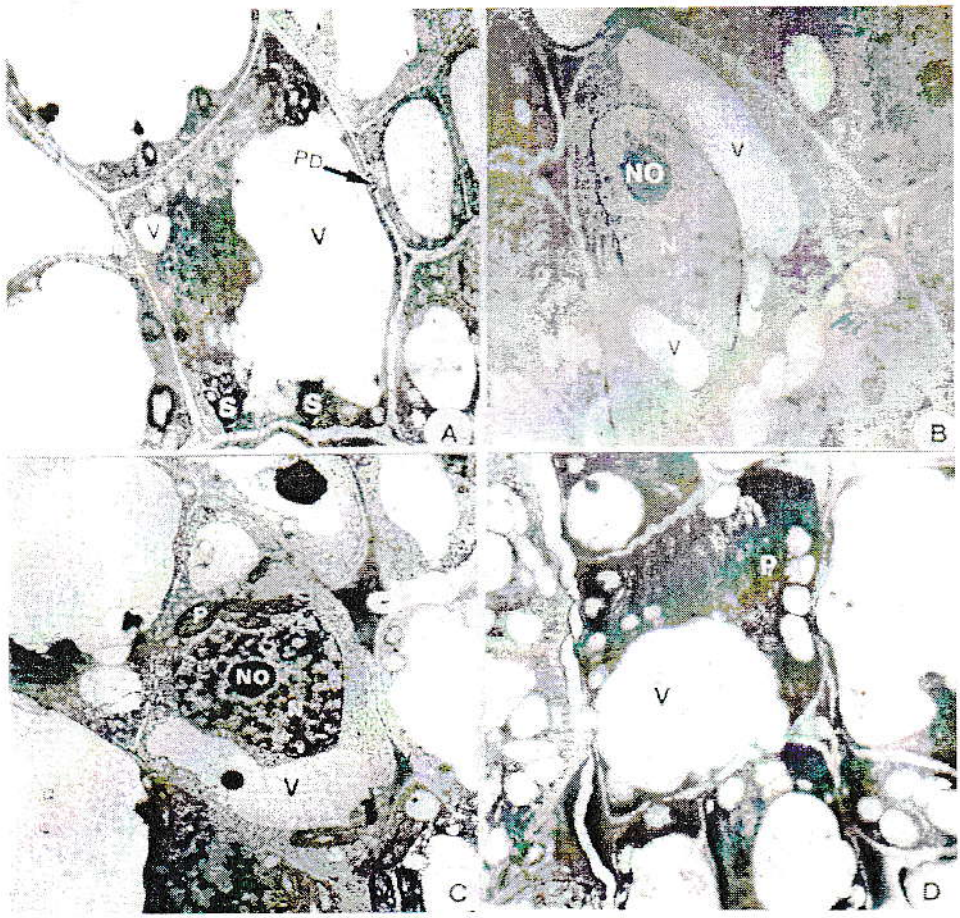

Figure (2):- Transmission electron micrographs of the mesoparenchyma cell of ovule outer integument:-

A- *V.cinerea*	(x 6860)	B- *V.cordata*	(x 6860)
C- *V.faba* cv.Giza 2	(x 4740)	D- *V.monantha*	(x 6860)

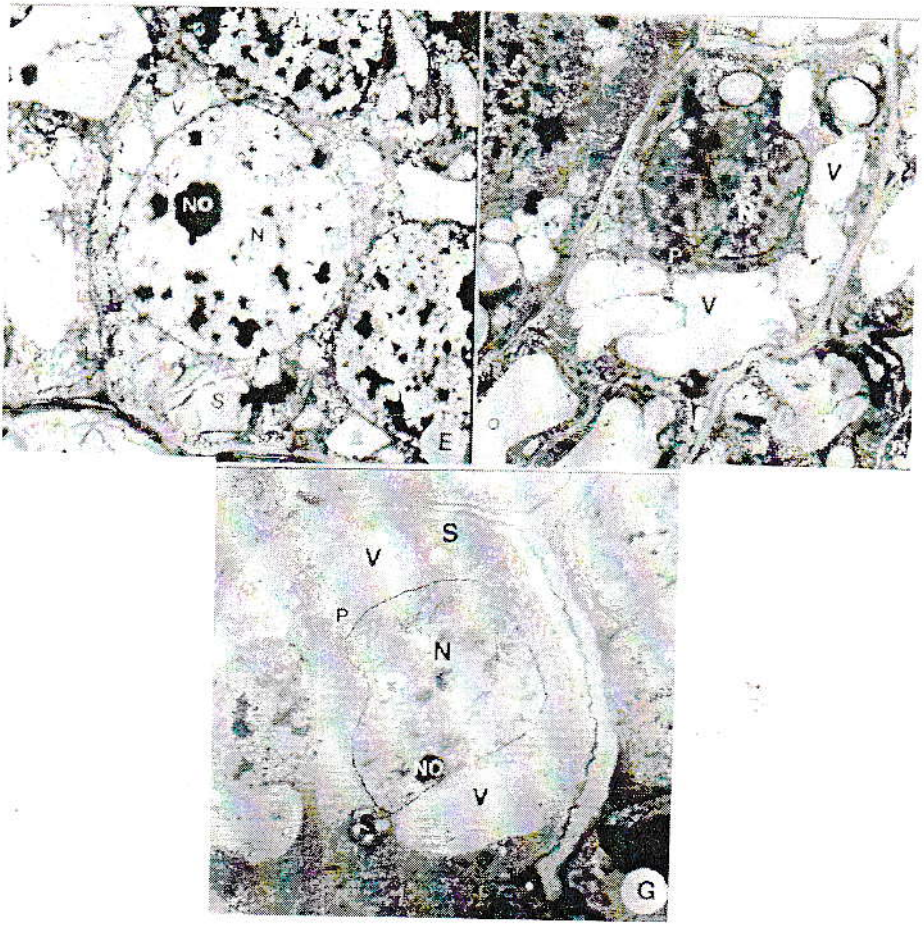

Figure (2):- Cont.

E- *V.nigra*	(x 8680)
F- *V.peregrina*	(x 5800)
G- *V.sativa*	(x 6860)

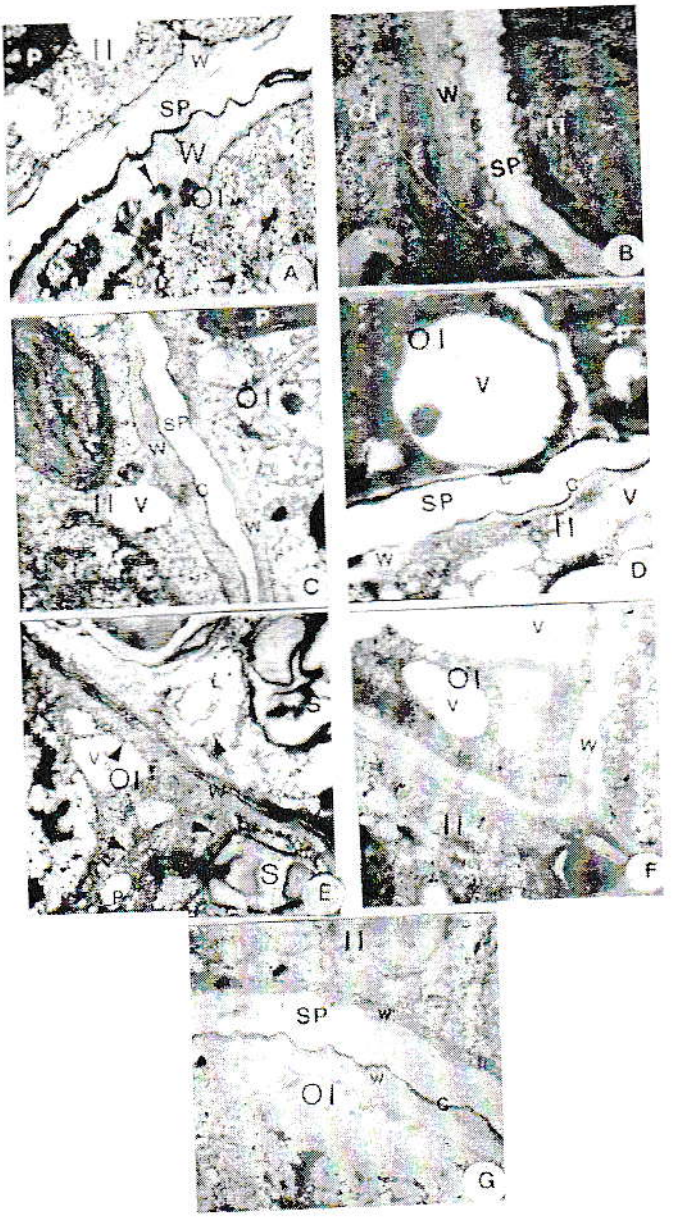

Figure (3):- Transmission electron micrographs of the space between the inner and outer integuments of the ovule of:-

A- *V.cinerea*	(x 16000)	E- *V.nigra*	(x 13100)
B- *V.cordata*	(x 16000)	F- *V.peregrina*	(x 16000)
C- *V.faba* cv.Giza 2	(x 16000)	G- *V.sativa*	(x 16000)
D- *V.monantha*	(x 16000)		

Vicia nigra (Fig. 3E), whereas it was found in the other studied genotypes (Figs. 3A, B, C, D, F and G). Shape of outer integument margin is erosus in both *V.cinerea* (Figure 3A) and *V.sativa* (Fig. 3G), dentatus outer and inner integument margins are characteristic for *V.cordata* (Fig. 3B) and undulated margin is predominant in outer and inner integument margins of *V.faba* cv.Giza2 (Fig. 3C), *V.monantha* (Fig. 3D) and *V.peregrina* (Fig. 3F) and in inner integument margins of *V.cinerea* (Fig. 3A) and *V.sativa* (Fig. 3G). Maximum width for the space between the inner and outer integument was found in *V.monantha* ovule (687 nm) as given in (Fig. 3D). Absence of the space between the inner and outer integument of *Vicia nigra* may be that the two integuments are adherent.

3.4. Mesoparenchyma cell of ovule inner integument

Table (2) and Figures (3 and 4) indicate that outline shape of mesoparenchyma cell of ovule inner integument is rombicus in *V.cinerea* (Fig. 3A), *V.nigra* (Fig. 3E) and *V.faba* cv.Giza2 (Fig. 3C), whereas, irregular rombicus, deltoid, reniform and pentagonal shape are characteristic for *V.cordata* (Fig. 3B), *V.monantha* (Fig. 3D), *V.peregrina* (Fig. 3F) and *V.sativa* (Fig. 3G), respectively. Small vacuoles number is medium in both *V.cinerea* (Fig. 4A) and *V.cordata* (Fig. 4B), few in *V.faba* cv.Giza2 (Fig. 4C), *V.nigra* (Fig. 4E) and *V.sativa* (Fig. 4G) and numerous in both *V.monantha* (Fig. 4D) and *V.peregrina* (Fig. 4F). On the other hand, presence of large vacuoles and number of them were shown in *V.cordata* (1) as given in (Fig. 4B), *V.faba* cv.Giza2 (2) as shown in (Fig. 4C), *V.peregrina* (1) and *V.sativa* (1) as observed in (Figs. 4F and G). In the same time, large vacuoles are absent in the remaining studied *Vicia* genotypes (Figs. 4A, D and E). Presence of starch grains and number of them per cell were noticed in both *V.cordata* (5) as given in (Fig. 4B) and *V.nigra* (10) as shown in (Fig. 4E). Plastids are present in *V.cinerea* (Fig. 4A), *V.faba* cv.Giza2 (Fig. 4C), *V.monantha* (Fig. (4D) and *V.sativa* (Fig. 4G), whereas, they are absent in the other studied *Vicia* genotypes (Figs. 4B, E and F). Osmiophilic bodies are taxonomical marker for *V.cinerea* (Fig. 3A), *V.nigra* (Fig. 3E) and *V.peregrina* (Fig. 4F). Presence of starch grains in the ovule inner integument cells in both *V.cordata* and *V.nigra* is similar to the data obtained by Johansson and Walles (1993a) who noticed starch grains in ovule

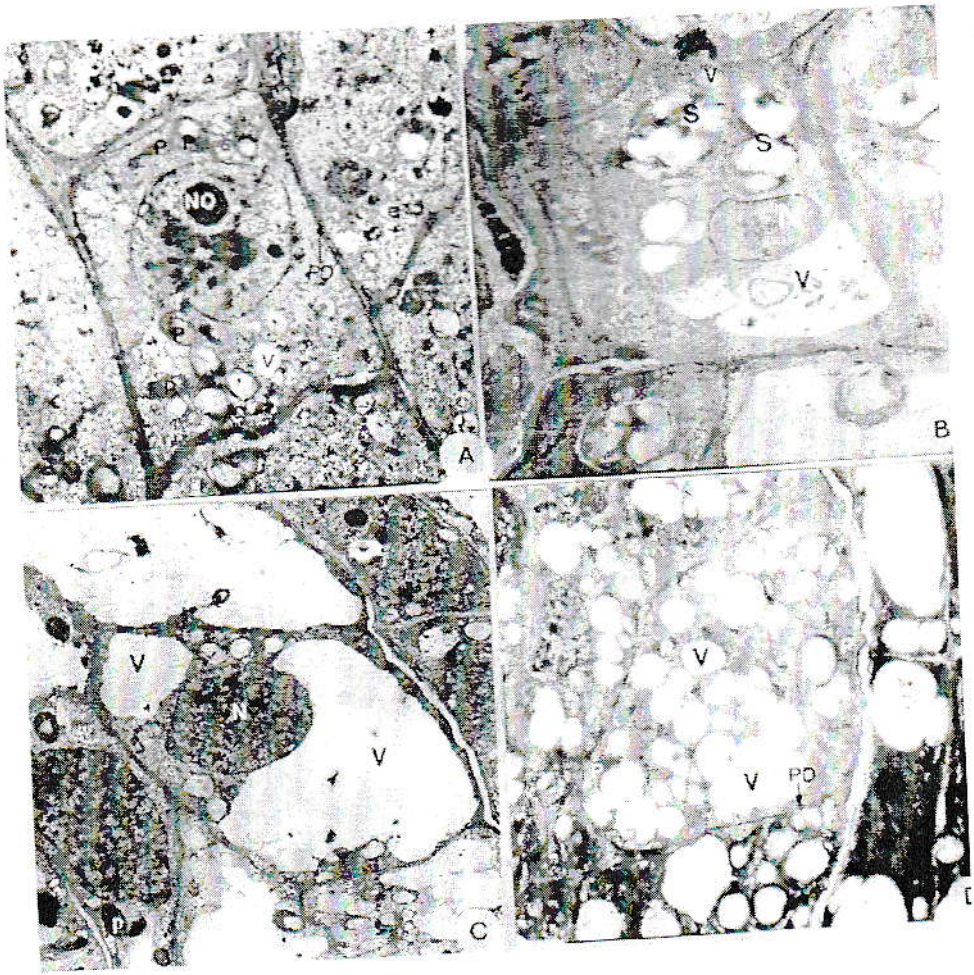

Figure (4):- Transmission electron micrographs of the mesoparenchyma cell of ovule inner integument of:-

A- *V.cinerea* (x 8700)
B- *V.cordata* (x 8700)
C- *V.faba* cv.Giza 2 (x 2700)
D- *V.monantha* (x 5800)

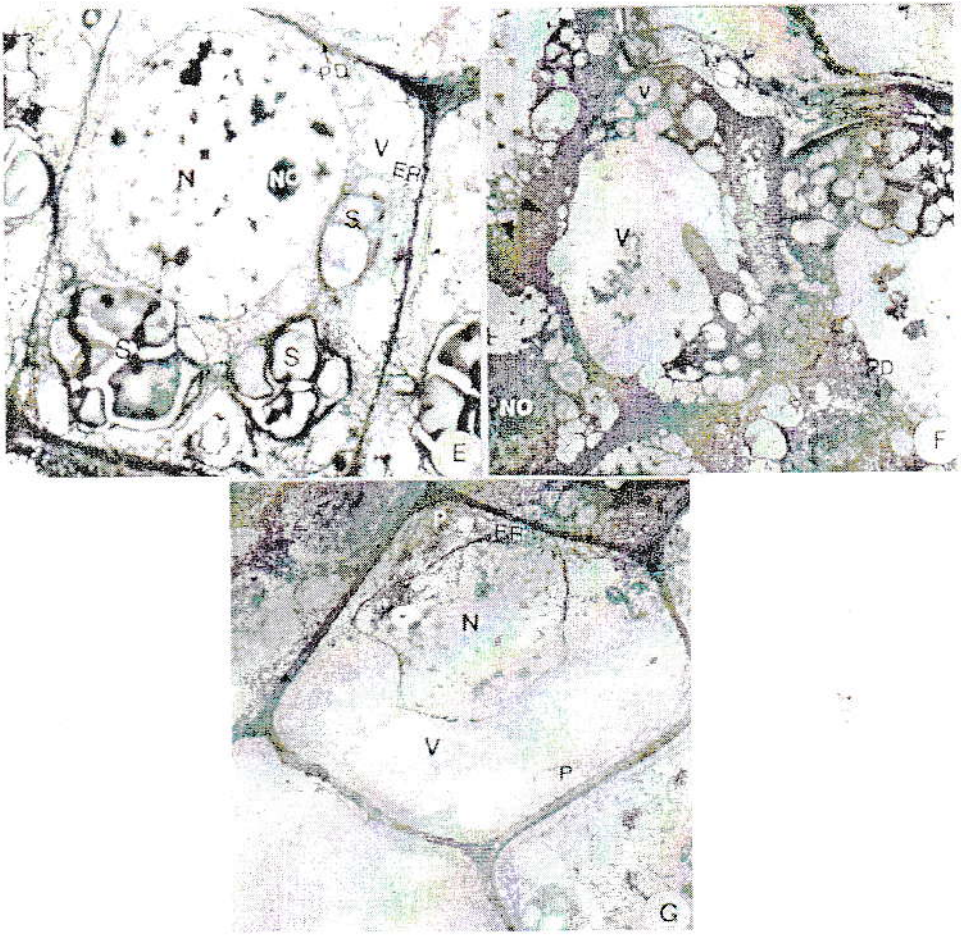

Figure (4):- Cont.

E- *V.nigra*	(x 8700)
F- *V.peregrina*	(x 3870)
G- *V.sativa*	(x 6860)

inner integument cells of *V.faba*. Moreover, absence of plastids in the ovule inner integument cells of *V.cordata*, *V.faba* cv.Giza2, *V.nigra* and *V.peregrina* agrees with Johansson and Walles (1993b) who noticed that plastids are not seen anywhere else in the ovule. In addition, presence of osmiophilic bodies in ovule inner integument cells of *V.peregrina* is in accordance with data obtained by Johansson and Walles (1993b) who observed them at the plasmalemma and in the walls at the micropylar end of the *Vicia faba* inner integument. On the other hand, absence of some cellular components of mesoparenchyma cells of integuments for example, nucleous, plastids is a result of the very thin ovule transverse sections.

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فحص اغلفة البويضة لبعض الفئات التصنيفية لجنس الفول بواسطة المجهر الالكترونى النافذ عند تفتح الازهار

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ملخص

درست تحضيرات لبويضات بعض التراكيب الوراثية لجنس الفول (*Vicia cinerea* و *V.cordata* و *V.faba* cv.Giza2 و *V.monantha* و *V.nigra* و *V.peregrina* و *V.sativa*) بواسطة الميكروسكوب الالكترونى النافذ عند بدء تفتح الازهار. اختلف شكل الكيوتيكل ، متوسط سمك الكيوتيكل + جدار الخلية (بالنانوميتر) ، وجود الفراغ بين الغلاف الخارجى والداخلى للبويضة ، شكل كل من الخلايا البارنشيمية الوسطية للاغلفة الخارجية والداخلية ، اقصى اتساع (بالنانوميتر) للفراغ بينهما، الشكل العام للخلية، الفجوات ، حبيبات النشا، البلاستيدات لكل من الخلايا البارنشيمية الوسطية لاغلفة البويضة الخارجية والداخلية على حسب التركيب الوراثى المدروس.

يعتبر الكيوتيكل المستوى و الحواف المسننة للاغلفة الخارجية والداخلية والشكل العام المعين – غير المنتظم للخلية البارنشيمية الوسطية للغلاف الداخلى للبويضة خصائص مميزة للنوع *V.cordata* . ويعتبر غياب الفراغ بين الاغلفة الداخلية والخارجية مميزا للنوع *V.nigra* ، بينما الكيوتيكل المنشارى والشكل البيضى المنقلب للخلية البارنشيمية الوسطية للغلاف الداخلى تشكل دلائل تصنيفية للنوع *V.sativa* . فى نفس الوقت فان شكل خلايا البارنشيما الوسطى للغلاف الداخلى المثلثى و الكلوى ووجود الاجسام الـ Osmiophilic تكون مميزة للانواع *V.monantha* و *V.peregrina* على التوالى.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٥٣) العدد الأول (يناير ٢٠٠٢): ٣١-٤٦.