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ANTIPODALS OF GASTERIA VERRUCOSA (LILIACEAE) –AN ULTRASTRUCTURAL STUDY

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SUMMARY

The formation and degeneration of the antipodals show some ultrastructural changes in the cell. These are related to their ephemeral nature as well as to their transport function. On the contrary, the egg apparatus and the central cell continue to differentiate subcellularly. There is probably little functional relationship between the antipodals and other constituent cells of the embryo sac.

1. INTRODUCTION

The antipodals have received comparatively much less attention of the investigators than the egg apparatus because of the dominant role played by the latter in the life of a plant. Besides, for a long time these have been considered to be inert structures situated in the chalazal region of the gametophyte performing no important role of much consequence. However, recent researches indicate their involvement in the secretion, absorption and transport of nutrients to the developing egg apparatus-central cell complex before and after fertilization (see KAPIL & BHATNAGAR 1978). In this study the antipodal cell development is investigated in relation to their function, from their inception until degeneration.

2. MATERIALS AND METHODS

Plants of *Gasteria verrucosa* (Mill.) H. Duval were grown in a greenhouse within a temperature range from 18–23 °C and relative humidity of about 60% and a long day regime of 16 hrs light and 8 hrs darkness. Thick sections of the young ovary until bud length of 18 mm were fixed in 0.1 M phosphate buffer pH 7.2 with 2.5% glutaraldehyde for 20 hrs. Those from older ovaries (18–26 mm) were fixed in 0.01 M phosphate buffer pH 7.2 with 2.5% glutaraldehyde for 20 hrs. Subsequently the specimens were rinsed with the same buffer and fixed for 1 hr in 1% 0.01 M buffered OsO_4. After dehydration with alcohol they were embedded in Epon and counterstained with lead citrate. The sections were examined and photographed on Philips EM 301 at 60 kV.

3. RESULTS

The transformation of the young embryo sac with 4 nuclei to the 7-nucleate undifferentiated stage takes minimal 6 hrs and maximal 30 hrs depending upon

the position of the flower on the inflorescence axis and the length of the axis. The period of differentiation of young antipodal cells, present at a flower length of about 15 mm, until it opens, has a duration of about 140 hrs. In the present investigation flowers of size 14–16 mm, 18–20 mm, and 23 mm were examined. The time taken for this differentiation is about 45 hrs for 14–16 mm, 45 hrs for 16–18 mm, 26 hrs for 18–20 mm, and 50 hrs for 20–23 mm. The flower opens when it is about 22 mm long and degeneration of the antipodals sets in from this time onwards. Although the antipodals appear at about the same time as the egg apparatus, the differentiation of the latter rather takes longer time. In the mature embryo sac the area occupied by the antipodals is less as compared to the egg apparatus. The elongation of the gametophyte is mostly in the middle region.

3.1. The chalazal part of the four-nucleate embryo sac

In the cytoplasm at the chalazal end (*fig. 1*), two round nuclei of unequal size are situated above each other. The heterochromatin is nearly equally distributed and a large nucleolus bordered with granules is present. In the dense ground plasm many ribosomes, mainly polysomes are observed. The endoplasmic reticulum (ER) is not abundant and consists mostly of RER and some SER. Dictyosomes are few and dictyosome vesicles fewer. The mitochondria are round and have few cristae. Plastids contain amylum grains, dilated thylakoids and some plastoglobuli. The amylum in the plastid is diminished as compared to the two-nucleate stage. The lipid droplets are large and common. The plasma membrane shows some undulations and has open plasmodesmatal contact with the nucellar cells at the chalazal side. The cell wall of the young embryo sac is regular and thin whereas that of the bordering nucellar cells is thick and has irregular thickenings in which the plasmodesmata are visible.

3.2. The young antipodal cell

At bud length of 14–16 mm the juvenile antipodals have a conical form, and are situated with one end near the chalazal side of the embryo sac. The shape of the nuclei is similar but somewhat smaller than those of the nucellar or the central cells. The round or oval nucleus has a regular heterochromatin pattern and one or two large nucleoli. The dense ground plasm now mostly contains ribosomes whereas RER and SER are scanty and dictyosomes are not seen. Some small vacuoles with fibrillar contents are, however, visible.

The mitochondria have less cristae and the plastids contain flat thylakoids. Amylum as wel as lipid droplets are almost absent. Between the cells thin walls with many plasmodesmata are observed. The wall facing the central cell is thin and undulated with plenty of plasmodesmata whereas along the chalazal region it is smooth and thin. Adjacent nucellar cell walls are, however, thick and irregular. There are open plasmodesmatal connections at this stage between antipodal and nucellar cells.

In comparison with the cytoplasm of the antipodal cells the central cell has a larger nucleus as well as nucleolus, more RER and SER, and highly vacuolated cytoplasm with smaller vacuoles in less dense ground plasm.

3.3. The mature antipodal cells

At bud length of 18–20 mm, the nucleus has more condensed heterochromatin, and a small nucleolus. The nucellar membrane is in close contact with the SER. The dense ground plasm contains ribosomes as well as polysomes. Mostly RER but also SER are observed. Dictyosomes are not seen. In the cytoplasm more vesicles are present and more vacuoles too. These vacuoles contain membranes and fine fibrillar material.

The mitochondria begin to swell up and have few cristae. Also the plastids become more round (WILLEMSE & FRANSSEN-VERHEIJEN 1978) and have some dilated thylakoids and more plastoglobuli. Locally the plasma membrane is undulating. Between the antipodal cells the plasmodesmata persist and in the now stretched cell wall near the central cell many plasmodesmata are visible. The thin walls of the antipodal cells develop irregular thickenings. All plasmodesmatal contacts continue to be present (*fig. 2*). At maturity the antipodal cells develop vacuoles in the centre of the cell below the nucleus. Near the chalazal end, the cytoplasm has scanty organelles and contains mostly RER and ribosomes.

At this stage, the central cell has a large nucleus and nucleolus. The ground plasm is more dense, has many vacuoles as well as lipid droplets. The mitochondria and plastids still have a normal structure (*fig. 3*).

3.4. The degenerating antipodal cells

The antipodal cells begin to degenerate when the bud opens (length 22–23 mm). The nucleus is either round or has an irregular shape. The heterochromatin becomes condensed, and the nucleolus too is irregular (*fig. 4*). The contact between the nuclear membrane and the RER persists. The now electron transparent ground plasm shows some ribosomes (*fig. 5*).

The RER and some SER lie in the cytoplasm. Many vesicles and vacuoles with mostly fine fibrillar material are also present.

The mitochondria are dilating and contain condensed material. The plastids are round and have dilated thylakoids and many plastoglobuli. Some lipid droplets are also present.

The three antipodal cells do not seem to degenerate simultaneously, the one closer to the central cell longer remains healthy. The walls of the degenerating cells still have many plasmodesmata and those towards the nucellar cell side are thick.

By this time the central cell stretches considerably, although the area occupied by antipodals remains more or less the same. It has a large nucleolus, and the ground plasm is dense with many ribosomes. More ER, some dictyosomes and small vesicles are present. Mitochondria have a normal shape and amylum develops in the plastids (*fig. 4*).

4. DISCUSSION AND CONCLUSIONS

The micropylar and chalazal parts of the embryo sac at the 4-nucleate stage show differences in their cytoplasmic organelles especially in the presence of higher numbers of dictyosomes in the micropylar part. At the inception of cell formation the RER also is more in the developing synergids. The cytoplasmic differences of the antipodals with the central cell are, however, more pronounced. This is in contrast with *Lilium* which has tetrasporic ontogeny (MIKULSKA & RODKIEWICZ 1967).

When compared with the 4-nucleate stage, the young antipodal cells as well as the mature ones show a nucleolus and a ribosome-polysome pattern that can be attributed to a moment of synthetic activity. Besides, the presence of RER indicates a possible synthesis of, for example, proteins. The appearance of an irregular wall denotes the addition of material such as carbohydrates to the wall. This type of deposition of wall material is characteristic of the embryo sac and nucellar cells around it and the deposit can be considered to be storage product. It is utilized for nutrition, as is evident from changes occuring in it, leading to its degeneration. However, the low profile of mitochondrial cristae, the decrease and loss of amylum in the plastids, the disappearance of lipid material and the formation of vacuoles point to a low metabolic activity in the cell. Presence of membranes in the vacuoles can probably be related with tonoplast formation but there is no increase in the volume of vacuoles. The tendency to large-sized vacuole formation is, therefore, not being realized by these cells.

The antipodal cells start to degenerate just before, or at maturity of the embryo sac. In general, the cellular changes, low metabolic activity, the short time of their presence, and early degeneration, indicate a very unimportant role of the antipodal cells in *Gasteria*. They do not seem to be involved even in the storage of any metabolites. But the embryo sac with a still enlarging vacuole of the central cell and a differentiating egg apparatus needs a supply of nutrients. The possibility that some products of the antipodal cells support their development cannot, therefore, be totally ruled out. Their function, however, seems to be mainly transport of nutrients. The open plasmodesmata connections between chalazal nucellar tissue and the antipodes, between the antipodals themselves, and between the antipodal and central cells show the direction of the translocation of nutrients. The location of the antipodal cells over a file of nucellar cells, the polarity in the distribution of organelles in the chalazal region of the antipodals seem to be oriented to partly achieve this objective, so also the prominent cell protuberances present on their walls as well as those of the nucellus traversed by plasmodesmata. The presence of protuberances mostly around plasmodesmata can be considered the result of deposition of nutrients along the route. Such wall projections in *Gasteria* are not confined to the chalazal part of the antipodals as in *Zea mays* (DIBOLL & LARSON 1966) but are prominent towards the central cell side as well.

The rate of transport activity is high in the beginning but gradually slows down during the degeneration process of the antipodals and eventually becomes pas-

sive after their degeneration when no control is exercised by these cells over the nutrient supply. The appearance of storage material in the central cell supports the viewpoint that the degenerated antipodals do not block the movement of metabolites.

The onset of degeneration of antipodals seems to be associated with the enhanced cellular activity of the central cell which by this time develops its own machinery for the synthesis and storage of nutrients for the developing egg apparatus so that antipodals play a short-lived role in their differentiation.

Although the exact impact of pollination or fertilization on antipodals in *Gasteria* has not been investigated, their degeneration prior to double-fertilization rules out its influence on them. Moreover, their presence is not essential for endosperm development as observed by MORRISON (1955) and KALTSIKES (1973) in wheat and triticale, respectively.

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The line on the photograph represents a length of 1 μm. For symbols see *fig. 1.*

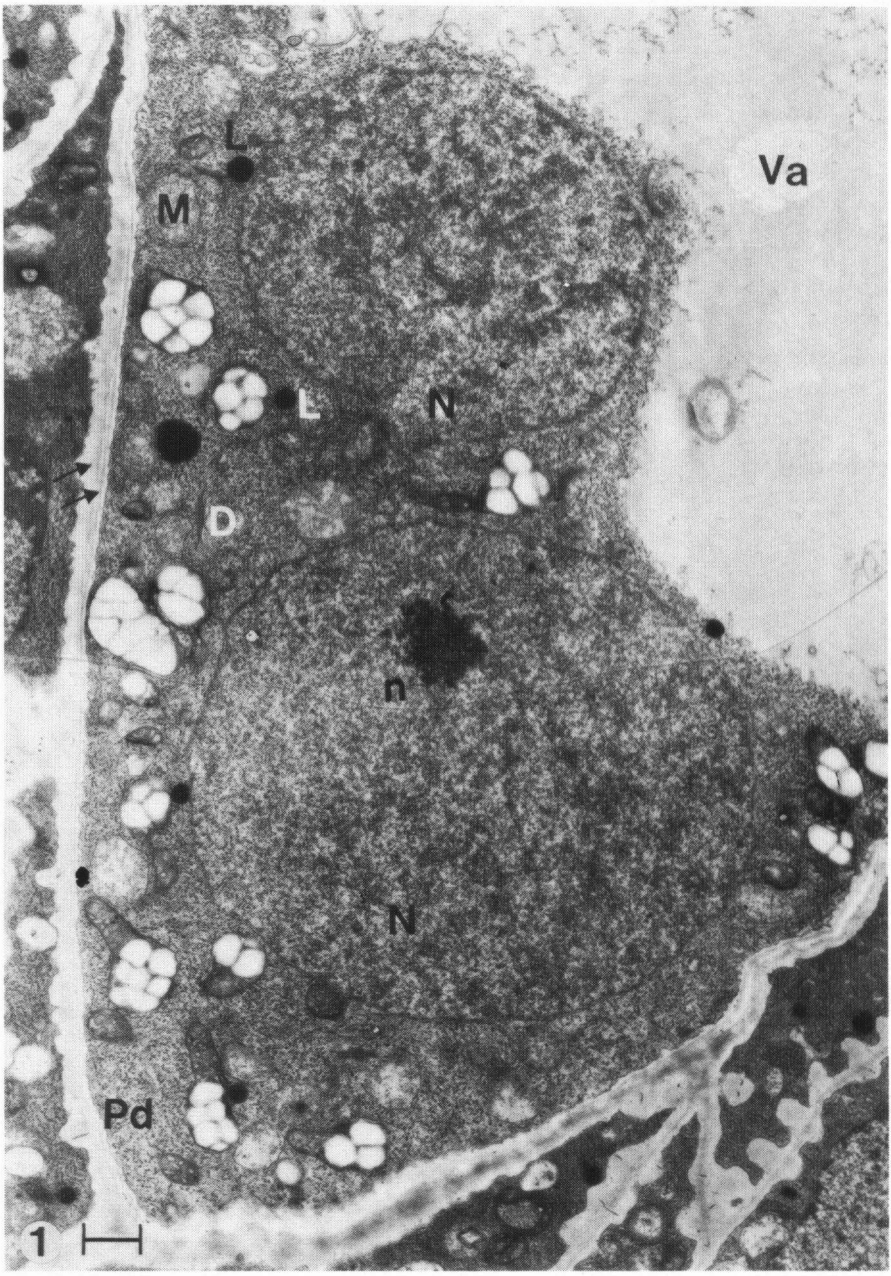

Fig. 1. Chalazal part of four-nucleate embryo sac. D = dictyosome, L = lipid droplet, M = mitochondria, N = nucleus, n = nucleolus, P = plastid, Pd = plasmodesmata, Va = vacuole. The embryo sac wall is thin whereas the nucellar cell wall is thick and irregular (see arrows).

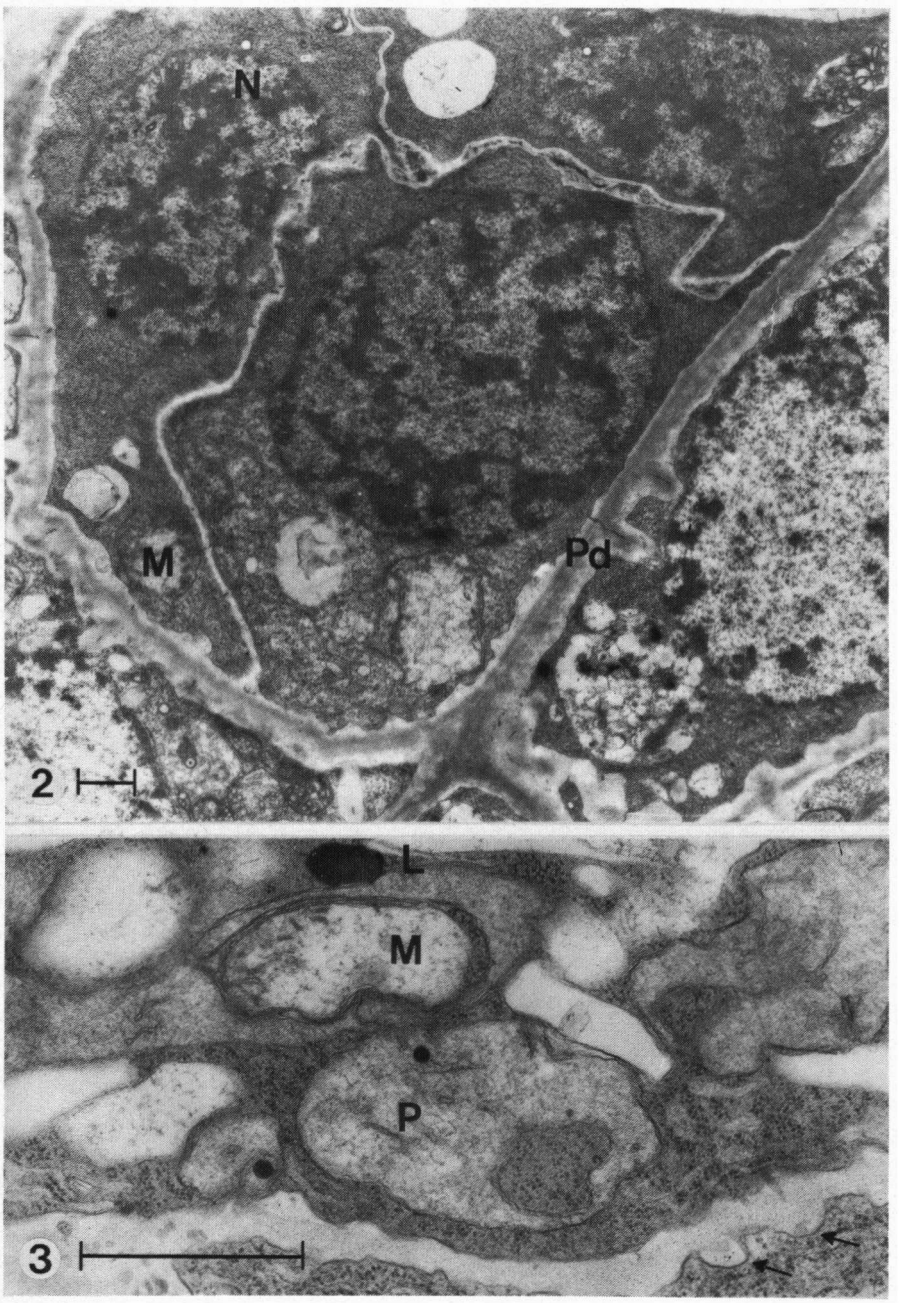

Fig. 2. Mature antipodals.

Fig. 3. Cytoplasm of central cell.
Notice the difference in density of ground plasm and thickenings of antipodal wall (arrow).

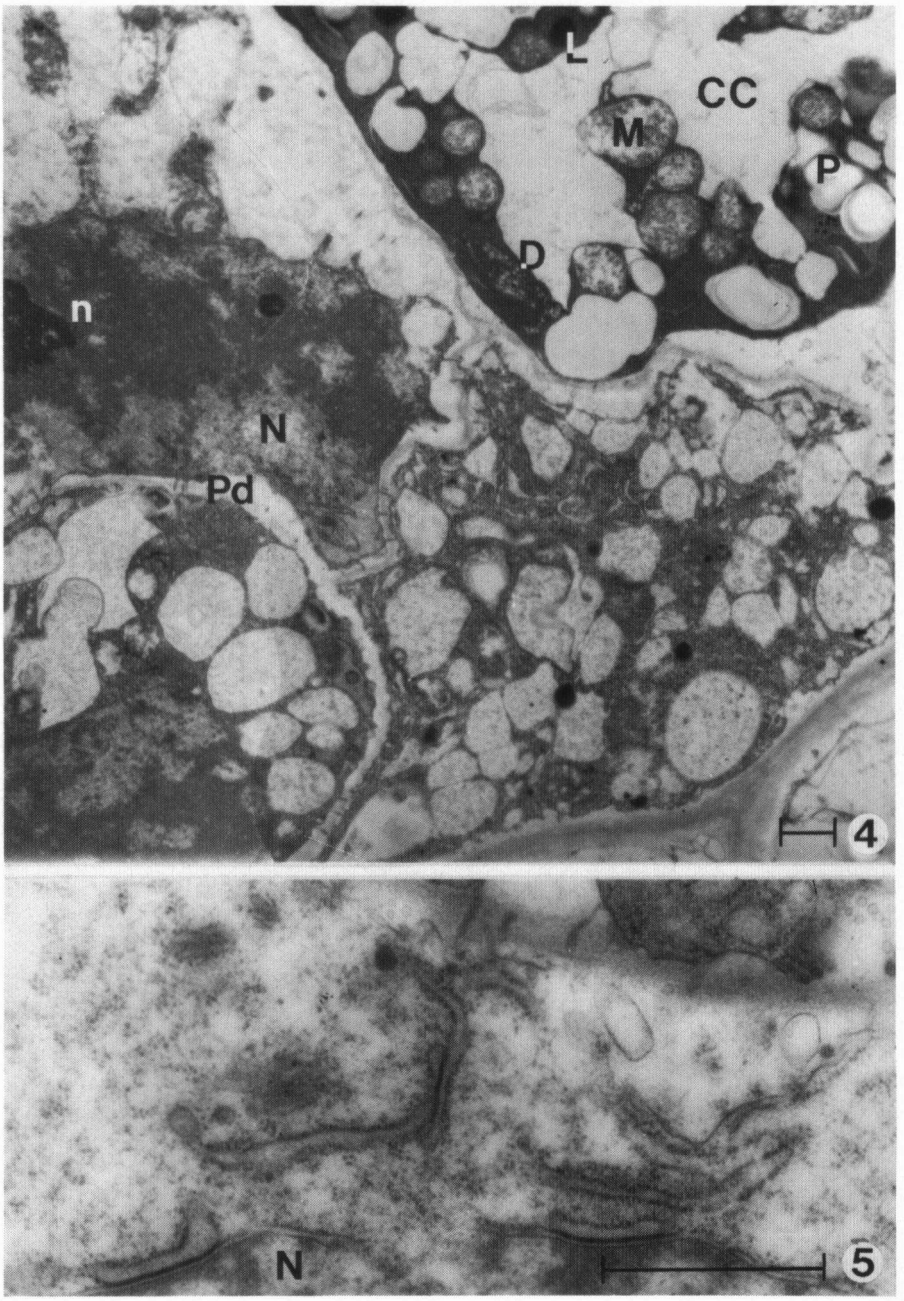

Fig. 4. Degenerating antipodals and adjacent part of central cell (CC).

Fig. 5. Detail of degenerating antipodal cell. ER's contact with nucleus persists.