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ON SOME ANOMALIES DURING MEGASPOROGENESIS IN GASTERIA VERRUCOSA (LILIACEAE)

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SUMMARY

Meiotic division of the megaspore mother cell in *Gasteria* gives rise to unequal dyad cells. The next division is non-synchronous producing an intermediate triad stage. In tetrads the cell wall between the micropylar cells has an irregular shape, random orientation, and is sometimes incomplete. Normally the micropylar cell degenerates first. Rarely the second cell from below may either show characteristics of the functioning megaspore or may degenerate earlier. Degeneration is related to deficient nutrient supply and loss of turgidity of the megaspore and physical pressure of the functioning megaspore. Lower osmolarity, electron dense cytoplasm, incomplete wall synthesis are the early symptoms of degeneration. Genetical constitution, the callose deposition, the cell organell polarity, the position of plasmodesmata, contribute to the selection of one functional megaspore, but also the favourable location for the low nutrient flow to the surrounding nucellar tissue.

1. INTRODUCTION

Embryological literature is replete with examples of the occurrence of triads of megaspores (SACHAR 1956; SHARMA 1968; BEDNARA 1977; RODKIEWICZ & SNIEZKO 1978) and supernumerary megaspore formation during the course of megasporogenesis (AGARWAL 1962; JALOUZOT 1979; TILTON 1980). Such situations are known to arise in various ways: as a result of suppression or failure of division of the micropylar chalazal dyad cell or due to non-synchronous meiosis II of the dyad cells or by additional mitotic division. Failure of division as well as abortion of the upper dyad cell also lead to the origin of a row of three megaspores.

Instances are not uncommon when more than one dyad, triad or tetrad may differentiate in the nucellus (see KAPIL & AHLUWALIA 1963). Such twin structures may be formed one above the other or side by side. Although in most of the plants these situations are generally referred to as abnormalities or anomalies, there are examples where triads of megaspores are of regular occurrence as in *Isomeris* (SACHAR 1956) or are frequent as in *Parnassia nubicola* (SHARMA 1968) and any one of the megaspores may become functional. The occurrence of triads in *Gasteria* was observed and stated earlier by squash-preparations (WILLEMSE & BEDNARA 1979). The megaspore- and -gametogenesis of *Gasteria* follows the *Polygonum* type.

Some anomalies such as twin megasporocytes, and twin tetrads of megaspores were noticed by WILLEMSE & BEDNARA (1979) in *Gasteria verrucosa*. In the present communication, the process of cell formation, the formation of the cell

wall and degeneration during megasporogenesis in *Gasteria* have been studied in order to find an explanation of the causes of these processes.

2. MATERIALS AND METHODS

Buds of *Gasteria verrucosa* (Mill.) H. Duval for EM study were fixed from plants grown under controlled conditions (see WILLEMSE & KAPIL 1981) in the greenhouse of the Botanical Laboratory, Wageningen, and studied under Philips EM 301. The cytochemical and enzymatic methods used on squashed as well as sectioned materials were the same as already described by WILLEMSE & BEDNARA (1979). Callose was detected by the aniline blue technique of ESCHRICH & COURRIER (1964).

3. OBSERVATIONS

3.1. Duration of the megaspore formation

Since the position of the flowers and the length of the inflorescence axis in *Gasteria* are quite variable, the following data were calculated on the basis of mean size of the inflorescence axis of about 50 cm. The average bud size was taken from the upper part of the inflorescence.

The megaspore mother cell undergoes the first meiotic division at an average bud length of 7.4 mm. Dyads become visible within about 40 hrs, when the buds are about 8.8 mm. Triads are formed nearly 50 hrs later at a bud length of about 11.0 mm. Tetrads of megaspores are usually met with when the bud reaches the length of about 12.3 mm after approximately 30 hrs. Thus the formation of the wall and the cytokinesis after the first meiotic division takes about 40 hrs to differentiate whereas second meiotic division and cytokinesis are completed in 80 hrs.

The degeneration of non-functional megaspores sets in when the bud size is about 13.4 mm, and takes about 42 hrs. The time taken for the functioning megaspore to differentiate and develop into a 2-nucleate embryo sac is nearly 60 hrs, the bud attains a length of about 16 mm.

3.2. Dyad

The dyad consists of two unequal cells with maximal dimension of 32 $\pm$ 4 by 57 $\pm$ 5 μm. The chalazal cell is somewhat longer (33 $\pm$ 3 μm) than the micropylar (25 $\pm$ 3 μm).

In the cellular composition no striking differences are observed. The shape of the nucleus of the micropylar cell is flattened whereas that of the chalazal is round. The surrounding wall, consisting of callose, is thick, as is also the wall between the two cells. Remnants of plasmodesmatal connections are present.

Karyokinesis is initiated in the chalazal cell. The position of the spindle is generally parallel to the long axis of the cell. Cytokinesis results in the formation of a cell wall which is of the same morphology and composition as the first cell wall but is mostly somewhat smaller.

3.3. Triad

In *Gasteria* triads are mostly formed as a result of non-synchronous division in the micropylar dyad cell, only as an intermediate stage. The total length of the cell is now 62 ± 7 μm and the width 35 ± 5 μm. This stage exists for a short period of 30 hrs because the division of the micropylar cell follows when the chalazal is undergoing cytokinesis.

In the cellular composition no great differences are found. More lipid granules, however, are found in the upper dyad cell. The shape of the nucleus of the chalazal megaspore is round whereas that of the middle cell is more flattened.

The chalazal cell is no longer surrounded by a callose wall, whereas the walls of the other cells are still callosic. The micropylar dyad cell shows after fixation a tendency towards lower osmotic value when compared with the other two cells. No plasmodesmatal contacts are visible in between the cells (*fig. 1*). Results of histochemical tests on triads are the same as those of the tetrads already described by WILLEMSE & BEDNARA (1979).

3.4. Incomplete wall formation and other anomalies in tetrads

The tetrad formation begins with karyokinesis of the micropylar cell. The dimensions of the tetrad are now about 69 ± 5 by 31 ± 3 μm. The newly formed nuclei have more condensed heterochromatin and are more or less flattened. The first sign of aberration is the random position of the cell wall after the division of the micropylar cell. The cell wall formed is irregular, sinuous, sometimes incomplete and with a low callose content. Dictyosome activity in the region of the plate formation is normal, but few microtubules are observed in the vicinity of incomplete wall formation.

From this stage the callose wall begins to dissolve around the upper part of the tetrad. The cytoplasm of the micropylar cells becomes more dense. Occasionally the second cell from below obtains the characteristics of the functioning megaspore. Like a chalazal megaspore, the megaspore of the second cell shows larger size and contains lipids and amylum but is surrounded by a thicker callosic wall (*fig. 2*).

Normally one megaspore mother cell can be expected to function but occasionally two may also differentiate and form two tetrads. In their position, they may be located next to or above each other (*fig. 3*). In the latter instance a gradient of amylum in the plastids, and of the lipid droplets, can be observed. Although the chalazalmost megaspore has a higher concentration of cell organelles, the chalazal megaspore of the second, upper tetrad too seems to be functional. There is a callose wall around the cells of the twin tetrads.

3.5. Degeneration

Degeneration normally starts in the micropylar cell but the second cell from below can also show the signs of degeneration first (*fig. 4*). The undivided micropylar cell of the triad starts to degenerate almost simultaneously with its lower megaspore. The cellular dimensions in the beginning are quite the same but with the increase in the size of the functioning megaspore, they begin to be pressed.

After wall formation the dictyosomes disappear and the ground plasm becomes more electron dense. There is no increase in the number of ribosomes. The membranes of the plastids become distinct and lipid droplets disappear. The mitochondria start to swell. The nucleus is electron dense and there is more aggregation of heterochromatin.

The ground plasm becomes still more electron dense and in the plastids and mitochondria electron dense granules are formed. In the cell vesicles and small vacuoles appear containing fibrillar material and lipid granules. No particular change is noticed in the membranes which do not constitute as well developed system. Thereafter the cell contents become osmophilic. This is accompanied by loss of turgidity in the degenerating cells. The cell volume is further reduced due to pressure from the functioning megaspore. The degeneration process of the three megaspores follows a similar pattern.

4. DISCUSSION

The megaspore mother cell almost always gave rise to daughter cells of unequal size (NEWCOMB 1973; RUSSELL 1979; WILLEMSE & BEDNARA 1979). The micropylar derivative is not only smaller than the chalazal but also does not stretch more. Besides, the next division is often non-synchronous. The chalazal dyad cell divides earlier so that a row of three cells is produced instead of four with a possibly larger complement of cell organelles, without a callose wall and situated near the nutrient flow for the ovule, the chalazal cell has better possibilities of further growth and seems to divide earlier. The round shape of the nucleus of the future functioning megaspore seems to be a result of the larger dimensions of this cell or is a sign of its future function.

According to BEDNARA (1977) triads are produced in *Epilobium palustre* as a result of failure of cytokinesis in the binucleate chalazal cell of the dyad accompanied by low activity of dictyosomes in the zone of cell plate formation. In *Gasteria*, however, dictyosome activity seems to be normal in this region.

The direction of cell plate formation in the chalazal cell of *Gasteria* is almost always horizontal and the wall is straight. In the micropylar, besides being horizontal, it may be vertical and sinuous, irregularities in shape and thickness are not infrequent. The orientation of the wall in the upper dyad cell depends probably also on the cellular dimensions. The two nuclei take their position along the longest axis of the cell which can result in an other direction of the cell plate. This phenomenon is comparable with the findings of DE BOER-DE JEU (1978) in *Lilium*.

It is often difficult to detect the incipient or incomplete wall which follows karyokinesis in the upper dyad cell. In this context DE BOER-DE JEU's (1978) observations on *Allium* are interesting. An ephemeral cell wall is present in the two-nucleate megaspore as well as partially formed cross walls in the micropylar dyad cell before its degeneration. This phenomenon was indicative of some relationship between the monosporic and bisporic types of development, and the derivation of the latter type from the former or vice-versa. In *Gasteria* too such

incomplete wall formation has been observed in the micropylar dyad cell which supports the viewpoint of DE BOER-DE JEU. The incomplete cell wall development in *Gasteria* is probably due to the loss of viability of the cell. The few microtubules, the irregular thickness and an absence of parts of the cell wall indicate a stop of this process and a lack of control.

In a tetrad instead of the chalazal cell the second cell from below shows the characteristics of a functional megaspore. It means that a megaspore develops surrounded by a callosic wall. The first stages of development are probably independent of a special flow of nutrients, or the chalazal cell permits the transport of nutrients in this case. In general this means that the chalazal cell retards the development of the other megaspores which degenerate normally.

Although the first symptoms of degeneration are difficult to state, the low cellular activity of the two to three micropylar megaspores seems to initiate the process by showing more electron dense cytoplasm and lower osmolarity and the stop of normal wall formation. In *Gasteria* ordinarily the degeneration of the micropylar megaspores is simultaneous and somewhat faster than the adjacent megaspore. Simultaneously, the functioning megaspore develops vacuoles and takes up water from the adjacent nucellar tissue and increases considerably in volume. So that degeneration of the non-functional megaspores in *Gasteria* appears to be mainly due to a lack of nutrients, a loss of turgidity and a result of pressure of the growing chalazal megaspore.

According to DE BOER-DE JEU (1978) a transfer of the material from the degenerating megaspores to the functional megaspore is possible. The cross walls of the degenerating megaspores during and after the dissolution of callose become more permeable and do not offer any obstruction in the movement of their contents to the developing megaspore.

However, the movement in the reverse direction of certain products of the functional megaspore to induce the degeneration of the megaspores cannot be ruled out although in a monosporic type the product has to cross two callose walls.

The degeneration pattern of the megaspores is not always the same. The compartmentalized areas of concentric lamellar RER near the chalazal walls of the non-functional megaspores as observed by RUSSELL (1979) in *Zea mays* have not been noticed in *Gasteria*. Also the sequence of the degeneration pattern can be altered. This indicates that degeneration depends not only on a genetic load or on the cellular individuality but also on other influences. These include also an effect of the chalazal nucellar tissue. Although it is still finishing its differentiation (WILLEMSE & DE BOER-DE JEU 1981) it provides in a low nutrient supply. The polar position and the absence of callosic thickening around the functional megaspore are the mean factors for the degeneration of the other megaspores. So the degeneration is partly also a result of the differentiation of one megaspore.

Ordinarily a single megaspore mother cell differentiates in the young nucellus of *Gasteria* but sometimes two separate cells may develop simultaneously giving rise to two tetrads which may be situated either side by side or one above the other. Although two embryo sacs were not observed, it appears that both the

tetrads one above the other have an elaborated chalazal cell. This is rather more an expression of their genetical constitution. Nevertheless, other influences are involved too such as cell polarity, probably localized absence of callose, presence of plasmodesmata, and the decreasing gradient to the micropylar part of storage products of lipid and starch granules demonstrates very well the inequality between both tetrads. Here also the chalazal tetrad contains more storage products.

5. CONCLUSIONS

The triad stage is caused by a non-synchronous II meiotic division. Comparing the normal development to one functional megaspore, including the degeneration, with the abnormal patterns and the formation of the twin tetrads one above another, some conclusions can be given about the polar position of the functional megaspore.

The polarity in the tetrad is based on genetical constitution. In *Gasteria* the functional megaspore will develop on the chalazal side, the other megaspores will degenerate. But also external factors become manifest during the selection. The chalazal part of the nucellar tissue is situated very near to the nutrient flow necessary for ovular development. During the tetrad stage the nucellar tissue is still in development; besides, near the chalazal part also a hypostasis-like tissue differentiates in this region. These processess need a constant nutrient flow. The special nutrition which provides in the formation of the embryo sac starts after the selection of the functional megaspore. The breakdown of the callose wall by the nucellar tissue (WILLEMSE & BEDNARA 1979) can be considered as a starting point. However, some influence of the nutrients during the period of the selection of the functional megaspore is manifest, as well as during the formation of the callose wall and the appearence of storage products. Besides, the local absence of the callose wall as well as the presence of plasmodesmata on the chalazal side favour a supply to the chalazal megaspore. The degeneration of the other megaspores is related to the selection of the functional megaspore and a lack of nutrient supply. When the influence of nutrients is absent, too low or retarded, aberrations in the normal pattern of polarity can occur and other developmental patterns can be found.

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LEGENDS

The line on the photographs represents 1 μm.

Fig. 1. Triad showing difference in schrinkage and density of cytoplasm of micropylar cell (MC), Ca = callose wall. × 2700.

Fig. 2. Tetrad with incomplete cell wall of micropylar cell (arrow). Second chalazal cell shows characteristics of functional megaspore (MS). × 3200.

Fig. 3. Two tetrads arranged in line; notice the gradient in amyloplast (A) and lipid droplets (L). × 2500.

Fig. 4. Tetrad with degenerating second cell from below. × 2400.

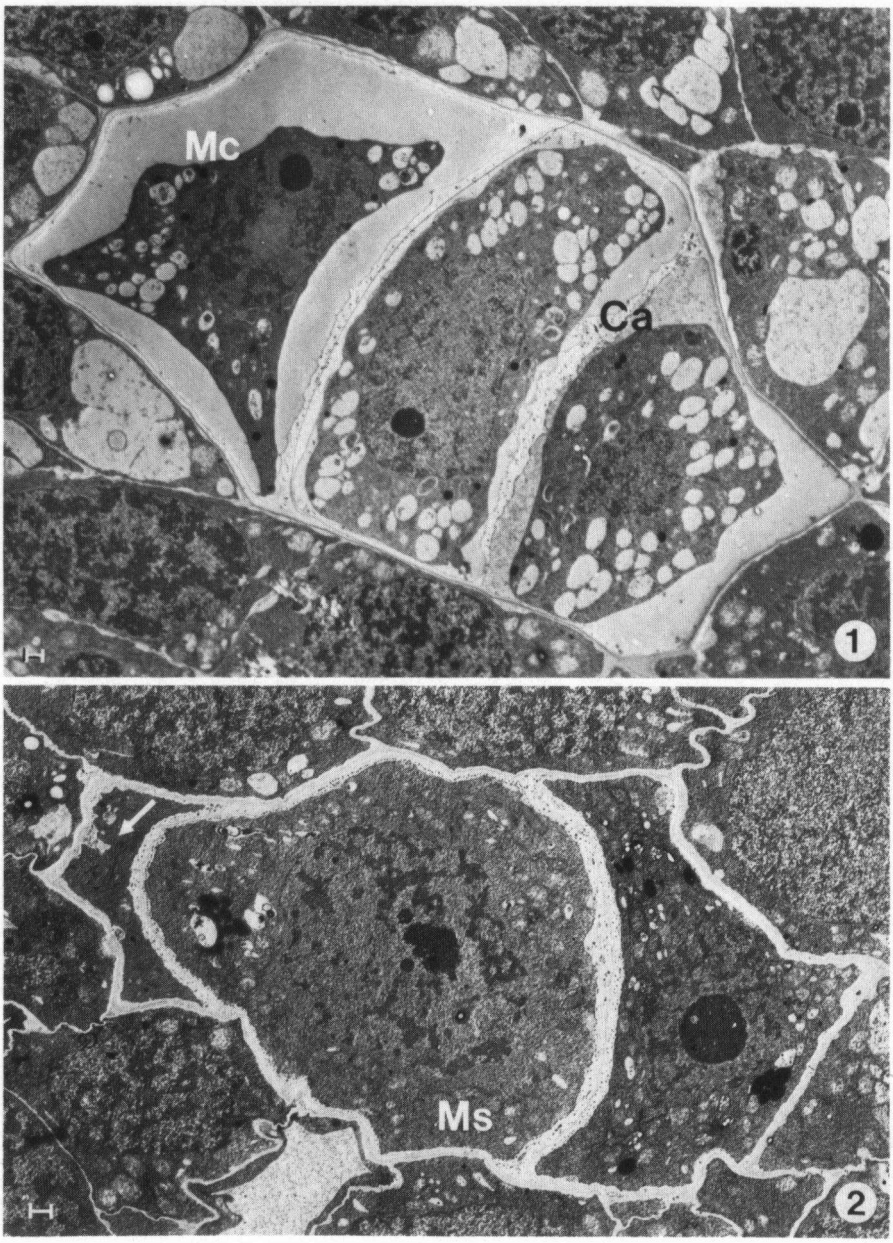

Plate 1.

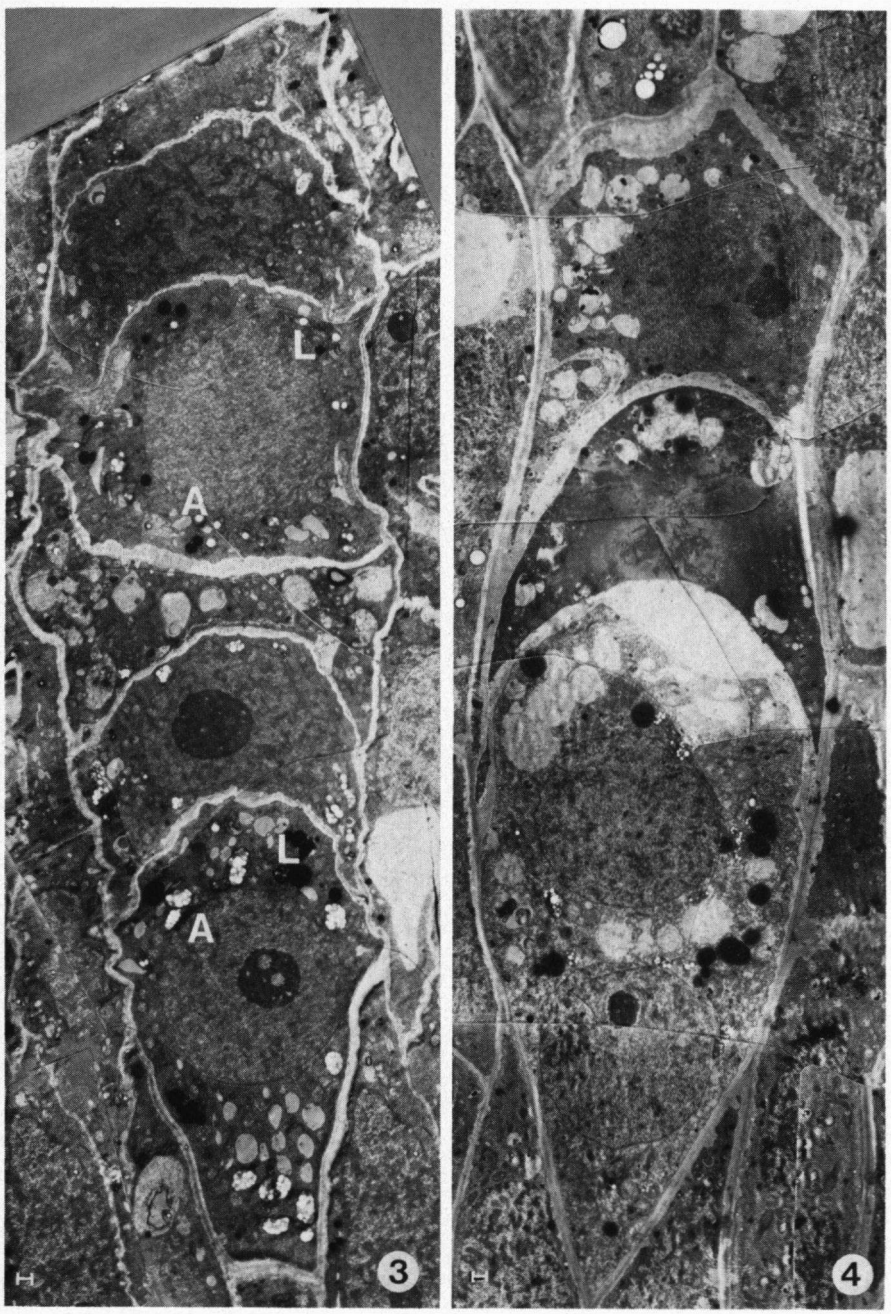

Plate 2.