ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 50, nr 1—2: 89—97
1981

Cytophysiological and ultrastructural modifications induced by cold in the microsporocytes and tapetum of *Rhoeo discolor* Hance

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Abstract

The exposition of *Rhoeo discolor* to cold induces an alteration of the microsporocytes (PMC) and tapetum ultrastructure. In the young cooled PMC, the mitochondria present short and vesiculate cristae, the stroma of proplasts is clearer and the polyribosomes are deteriorated. During the phase tetrads-microspores, the alterations are more important: the chromatin coagulates, the nucleus swells while the nuclear membrane is modified; some large vesicules appear outside of the plasmalemma. In the cooled periplasmodium we can observe many groups of vesicules, mitochondria with dilated cristae, rough endoplasmic reticulum without their ribosomes and a breaking up of nucleoli. Our observations are in correlation with the previous results obtained by autoradiography and photometry, and are discussed with the bibliographical results.

INTRODUCTION

We have undertaken the study of the effect of low positive (1° C to 4° C on the overage) temperatures on the pollen mother cells (PMC) and the tapetum of *Rhoeo discolor* Hance, using photometric, autoradiographic and cytomorphologic (light microscopy) methods. Part of the results have been published (Souvré, 1971, 1974; Souvré, Albertini, 1974, 1978; Souvré et al., 1978) so we shall present here only the most significant facts.

The photometric study (DNA-Feulgen) of the anthers and ovules of cooled plants (1° C to 3° C) whole and in flower pots shows that: 1) an exposition to cold during 4 days does not significantly modify the amount of nuclear DNA in the PMC at synizesis nor of the tapetal nuclei at G_1 post-mitotic phase (Souvré, Albertini, 1974); 2) the long (10 days) exposition hardly affects the amount of DNA in the nuclei of the nucellus of the *Rhoe discolor* ovule (Souvré et al., 1978).

As concerns the nucleic acids and the proteins, we have obtained by autoradiography the following results:

1) DNA (precursor: ^{3}H-thymidine, 20 μCi/ml); a) when the application (at 2° C) of the precursor on the excised anthers is preceded by an exposition of the plants in pots to cold (2° C) for 3 days, the incorporation of ^{3}H-thymidine by the PMC and the tapetum is reduced considerably (Souvré, 1974); b) if the excised anthers are incubated during 2 hours in the temperature scale: 0°C, 2°C, 4°C, 8°C, 14°C or 24°C, before the application of precursor at the same temperature, the synthesis of nuclear DNA on the PMC and tapetum which are very reduced but not null at 0° C, seems to recover a level comparable to that of the controls when the temperature is higher than 8° C (not published); c) the cold (2° C to 4° C during 4 days) applied to the whole plant after the precursor incorporation (application of ^{3}H-thymidine at the level of the inflorescence axis) hardly modifies the labelling of the DNA synthetised before the action of cold (Souvré, 1971).

2) RNA (precursors: ^{3}H-uridine and ^{3}H-orotic acid, 10 to 20 μCi/ml); a) the amount of incorporation of ^{3}H-uridine by the different structures (nucleus, nucleolus, cytoplasm) of the PMC, and tapetum on the excised anthers of the cooled plants in flower pots (2° C during 3 days), proves that the cold blocks the most part of RNA synthesis activity (Souvré, 1974). These results are confirmed by a study on the incorporation of ^{3}H-orotic acid by the cooled (1 h at 4°C) excised anthers before the application of precursor (2 h to 14 h at 4° C) (Souvré, Albertini, 1978).

The reincubation at room temperature of the plants in flower pots (Souvré, 1874), or of the cooled excised anthers (not published), quickly reestablishes the level of the nucleic acid (RNA and DNA) in the staminal tissues; the incorporation of a precursor of RNA (^{3}H-uridine), more affected by cold than that of the DNA precursor (^{3}H-thymidine), is the one which more quickly recovers its normal level (Souvré, 1974).

3) Proteins (precursor: ^{3}H-leucine, 10 μCi/ml); the level of nuclear protein synthesis, reduced but not negligible, measured on anther put for 1 hour at 4° C before the application of the precursor (2 h to 14 h at 4° C), suggests that the cold acts preferentially on the enzymatic reactions necessary to the protein synthesis; the RNA messengers present in the cell at the moment of cold action are still able to induce moderate nuclear proteosynthesis, while the synthesis of nuclear RNA is null (Souvré, Albertini, 1978).

We studied the ultrastructural modifications which could be induced by cold in the PMC and tapetum of *Rhoeo discolor*, and correlated these observations with the cytophysiological data.

MATERIAL AND METHODS

The control and cooled (4° C to 5° C during 4 days) anthers of *Rhoeo discolor* are excised and fixed in paraformaldehyde at 4% in phosphate buffer, post-fixed by osmium tetroxyde in a solution at 1% in the same buffer and embedded in Epon. The secfions are contrasted by uranyl acetate and lead citrate, according to the usual techniques.

RESULTS AND DISCUSSION

In his study on the "chilling-stress", Levitt (1972) considers that the plant of subtropical origin such as *Tradescantia spp.* (and *Rhoeo discolor*), are very sensitive to the cooling, even if it does not induce the freezing of cell material. Our experiments on *Rhoeo discolor* seem to prove that the lowering of temperature to 4° C for 4 days is not sufficient to induce an important, irreversible, alteration of the structure of meiocytes, and above all of the tapetum. The observed morphological alterations of low amplitude can be correlated with the fact that the PMC and the tapetum of cooled plants can synthetise the nucleic acids and the proteins even after a short reincubation time (Souvré, 1974).

We will describe the ultrastructural modifications induced by cold on PMC and tapetum taken at different stages of microsporogenesis.

Young pollen mother cells

In normal conditions, at the premeiotic rest stage, the PMC of *Rhoeo discolor* actively synthetise nucleic acids and proteins (Albertini, 1971a). During this period the very dense cytoplasm of the meiocytes contains many organelles: polyribosomes, proplasts (some containing a little polysaccharide granule), mitochondria, dictyosomes, rough endoplasmic reticulum (RER) with many ribosomes.

After the application of cold the structure of the mitochondria is modified, the cristae becoming short and vesicular. This type of alteration of the mitochondria observed by Geneves (1970) in the meristematic cells of the roots of *Allium cepa* and by Kwiatkowska (1970) at the epidermic level on *Agropyrum glaucum*. In the sweet-potato this alteration is induced by a structural modification of the phospholipidic and proteic fractions in the mitochondria membranes (Yamaki, Uritani, 1974). Morever, the stroma of the proplasts, usually dense at this early stage possesses electron-clear areas; Niki et al. (1978) as well as Kimball and Salisbury (1973) had also established the proplast sensitivity towards the action of cold. The structure of polyribosomes also seems perturbated.

Plate I

Anthers of *Rhoeo discolor*

Fixation: paraformaldehyde-osmium. Treatment on slides: uranyl acetate and lead citrate.

Treatment by cold — 4 to 5° C for 4 days.

c — callose; e — exine; m — mitochondria; N — nucleus; n — nucleolus; p — proplast; REF — rough endoplasmic reticulum; t — tapetum; v — vacuole; ve — vesicule.

Fig. 1. PMC in meiosis. The nuclear chromatine is conglomerated. The nucleus develops an expansion at the level of which the two sheets of the nuclear membrane move away from each other. (× 10 000)

Fig. 2. Tetrad stage. Alteration of plasmalemma and formation of large vesicules between the plasmalemma and the special callose wall. (× 10 000)

Fig. 3. Tetrad stage. Each tetraspore undergoes an alteration different from the others. (× 6 000)

We think that the ultrastructure alteration observed in mitochondria, proplastids and polyribosomes are to be correlated with our autoradiographic study (Souvré, Albertini, 1978) which proves that cold for a short time considerably lowers the proteic synthesis in PMC.

PMC during meiosis — young tetrads

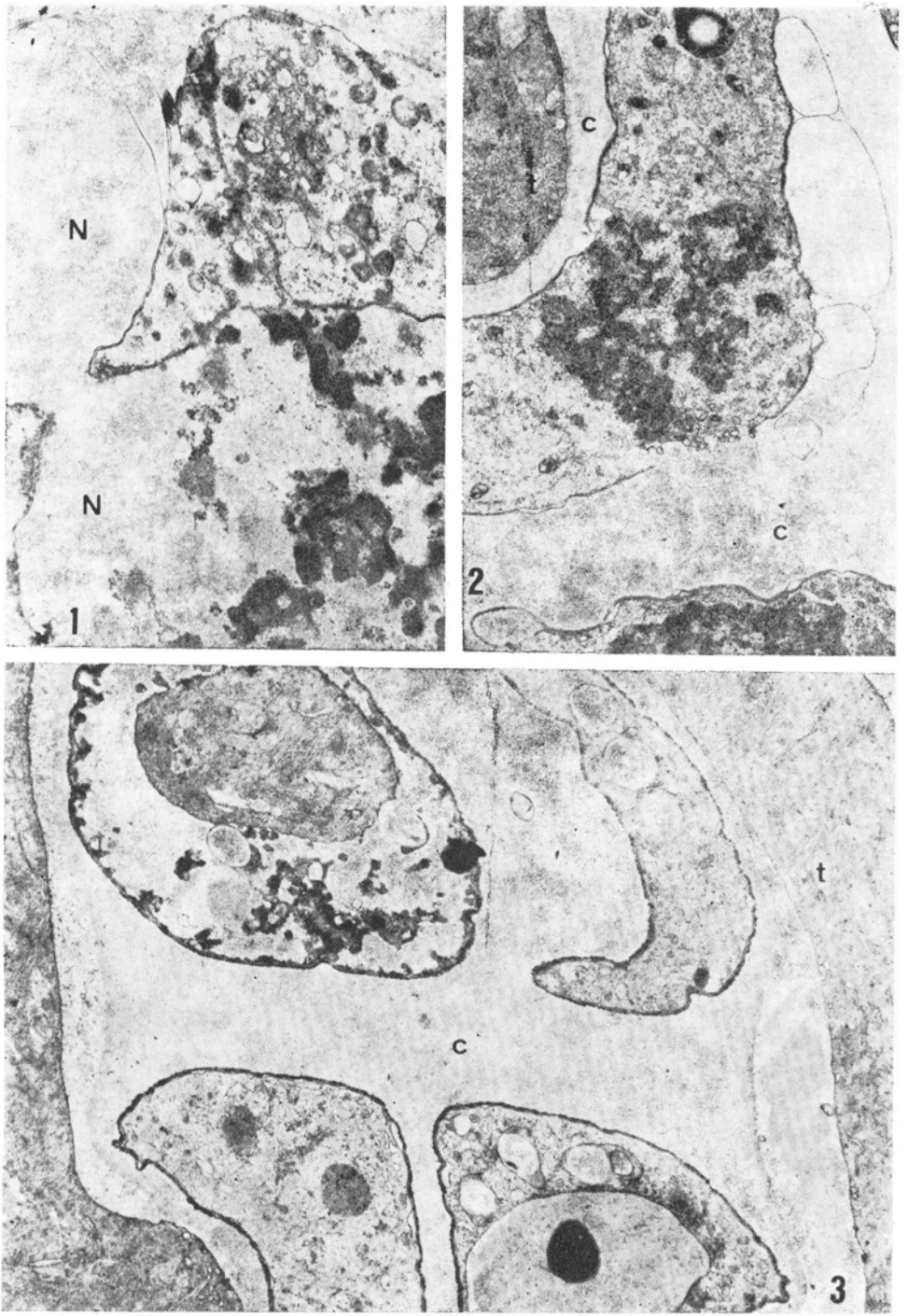
According to Albertini (1971a), from the beginning of meiosis to the young tetrads, the cytoplasm of PMC does not synthetise RNA and the level of the incorporation of protein precursors (^{3}H-leucine and ^{3}H-arginine) is considerably reduced after the premeiotic rest.

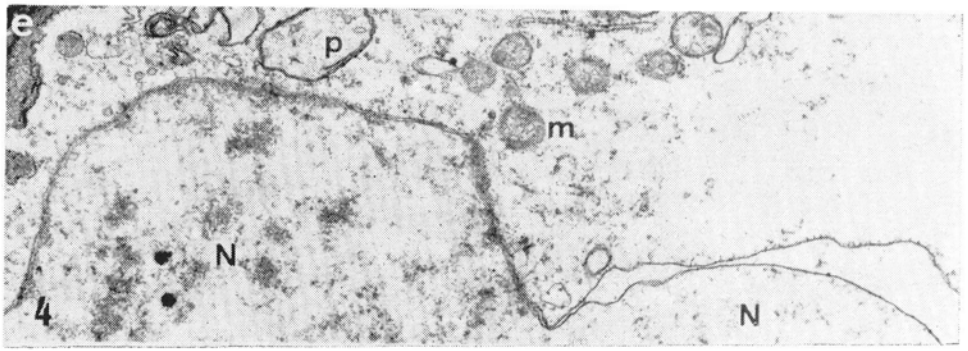
Our EM observations show that during this period in normal conditions the cytoplasm of meiocytes is not so dense; this cytoplasm which has no polyribosomes shows small mitochondria, few RER, whereas the polysaccharide granule increases in volume in the proplasts. During this phase the special callosic wall, which will isolate the tetrad elements, is built.

The deterioration of the cooled meiocytes is more important after the apparition of callose and, above all, at the tetrad stage. The modifications of the nuclear structure observed in light microscopy (Souvré, 1974) are confirmed (Fig. 1): Chromatin agglutination gives the nuclei a "pseudo-prophasic" aspect of the same type as that in the cooled meristematic cells of *Allium ursinum* observed by Delay and Linder (1969). In some cases (Fig. 1), the nucleus of the meiocytes develops an expansion, while the sheets of the nuclear envelope move away from each other.

At the tetrad stage, we noticed (Fig. 2) the presence of large electron-clear vesicules, between the plasmalemma and the callosic wall,

PLATE I

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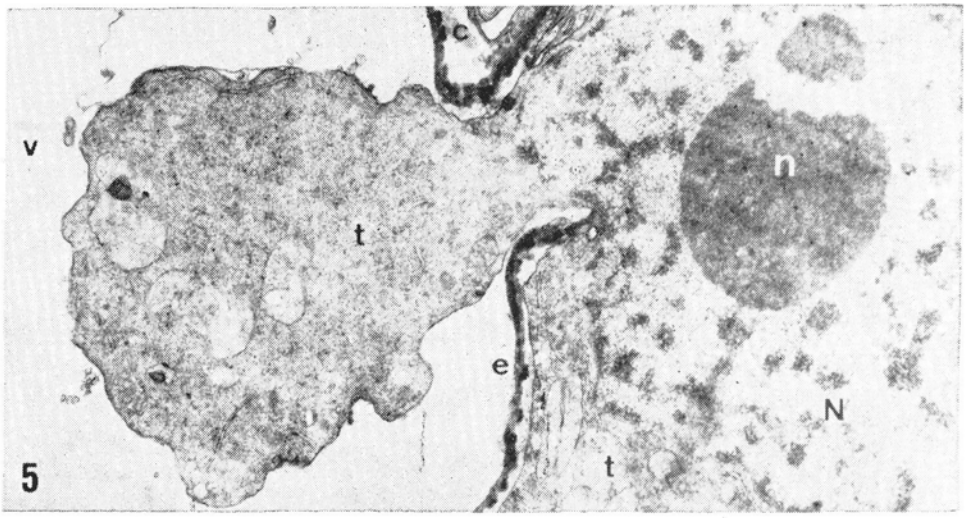
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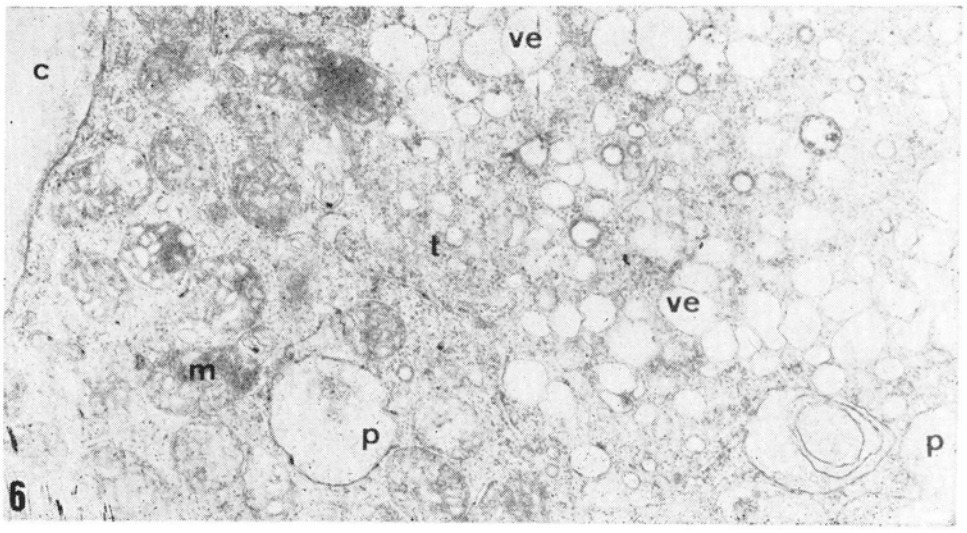
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Plate II

Anthers of *Rhoeo discolor*

Explanations as on Plate I

Fig. 4. Old microspore stage. Notice the expansion of the nucleus of microspore and the space which is formed between the sheets of the nuclear membrane. (× 15 000)

Fig. 5. Young microspore stage. After the break of the sporoderm the periplasmodium enters the microspore. The nucleolar structure is altered by cold (fragmented). (× 10 000)

Fig. 6. Diakinesis-metaphase I. Notice, in the periplasmodium, the presence of numerous vesicules and mitochondria with dilated cristae and grouped. (× 20 000)

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which seem to come from the abnormal functioning of plasmalemma. Kupila-Ahvenniemi et al. (1978) observed identical vesicules on sporogen cells of Scotch pine undergoing winter cold, without giving their origin.

Experimentation on the anilin blue callose fluorescence does not prove that the cold, as it is applied on *Rhoeo discolor*, modifies the thickness and the nature of the callose of the meiocytes (Grenet-Auberger, not published), in opposition to what sometimes happens after traumatism (Ilker et al., 1976). This observation agrees with that of Nishiyama (1970) on anthers of rice.

Old tetraspores-microspores

In *Rhoeo discolor*, the greater part of the exine is formed while the tetrad is in its callosic matrix. After the callose dissolution, the microspore liberated in the pollinic loculus increases in volume while a large vacuole is formed in the microspores. According to Albertini (1971a) only 20% of the microspores will be fertile.

Ito's (1976) ultrastructural study on moderatly cooled anthers of rice suggested that on the microsporocytes the phase of the microsporogenesis most sensitive to the action of cold is that of tetrads-young microspores. Figs 3, 4 and 5 present different aspects of nuclear or cytoplasmic alterations. The asymetrical dilatation of the microsporal nucleus (Fig. 4) comes with the separation of the nuclear envelope sheets and the lobule so formed well corresponds to the observations made by Souvré (1971) in light microscopy. Fig. 5 shows a broken sporoderm at the apertural level: this break has allowed the periplasmodium to penetrate inside the microspore. Attention must also be drawn to the frequent pictures of pseudo-plasmolysis in the young microspores, the plasmalemma moving away from the sporoderm and keeping only some points of contact with the latter. Ilker et al. (1976) who have

Plate III
Anthers of *Rhoeo discolor*
Explanations as on Plate I.

Fig. 7. Young microspore stage. Aspect of a periplasmodium little altered by cold. The arrow indicates the invagination in the membrane of a proplast, presence of numerous vacuoles and vesicules. The RER is dilated. (×20 000)

Fig. 8. Old microspore stage. Notice, in the periplasmodium, the well developed cytomembranes (RER without ribosomes; the arrows indicate the dilatation of RER). (× 40 000)

noted an identical phenomenon in the cells of cooled cotyledon of tomato, estimate that a modification of the plasmalemma permeability is at the origin of this plasmolysis.

Young tapetum (before establishment of periplasmodium)

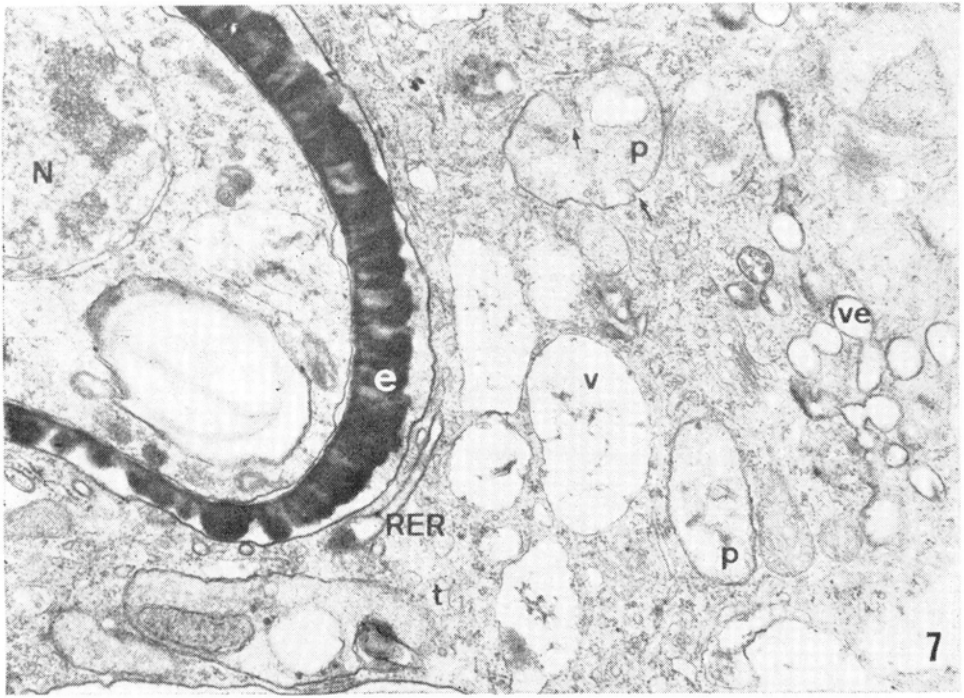
In *Rhoeo discolor* during the synizesis, invariably a tangential synchronous division of the tapetal nuclei takes place, which is not accompanied by cytokinesis. The tapetum is then constituted by one layer of binucleate cells which lose their walls. At the end of synizesis or at zygotene stage, the tapetum forms a syncitium, the periplasmodium, which begins to slip in between the PMC (Albertini, 1970).

Our observations of the control plants by EM prove that before this division the ultrastructure of the tapetal cells is very similar to that of the PMC, the only tangible difference being the absence in the tapetal cells of polysaccharide granule in the proplasts.

At this premitotic stage the cooling of the plant does not greatly modify the fine structure of tapetal cells but we find again in the mitochondria and proplasts some alterations similar to those observed in the PMC at the same stage. The presence of many electron clear vesicules is to be underlined in the cells of this young tapetum. These vesicules are made more voluminous by the lowering of temperature and they seem to correspond to the vesicules of dictyosomal origin observed by Mepham and Lane (1969) in the tapetum of *Tradescantia bracteata*.

Periplasmodium

In normal conditions, from synizesis to stage 4 of microspores, the periplasmodium nuclei and cytoplasm actively synthetise the RNA and proteins, with optimum activity in the tetrad-stage 2 of microspore phase (Albertini, 1971a). Our ultrastructural observations prove that the tapetum during this period is well provided with organelles (big mitochondria, numerous proplasts, RER with parallel sheets, polyribosomes, dictyosomes).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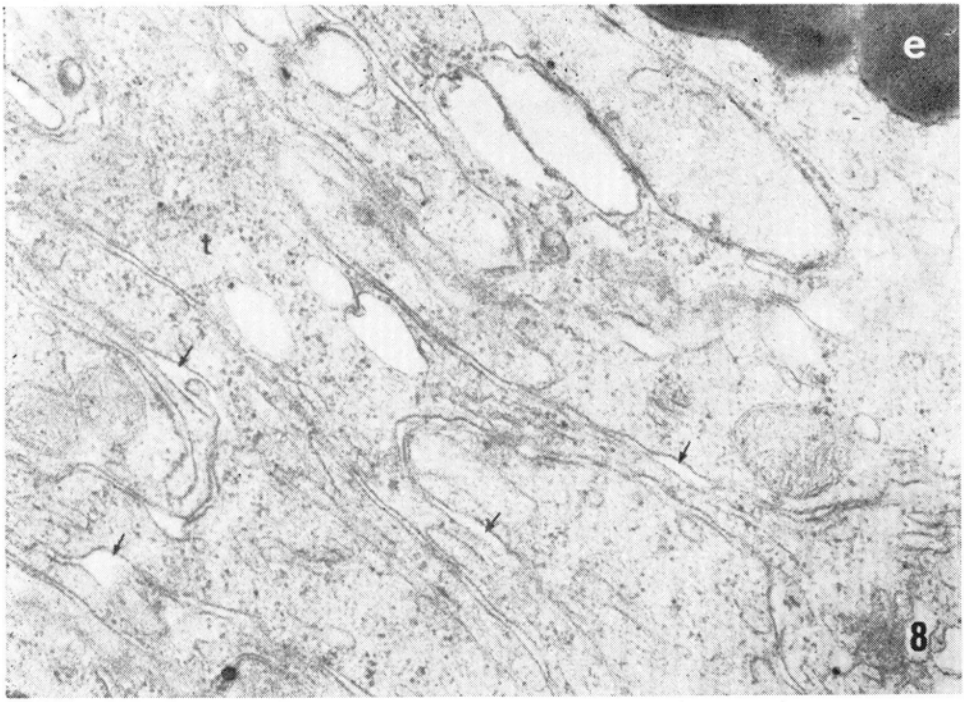
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These results confirm the fact already established by Albertini (1965) and precised by Mepham and Lane (1969) that the tapetum at a periplasmodium state is an organized and functional structure.

Inside the cytoplasm of periplasmodium (Figs 6, 7 and 8) the cold induces the formation of numerous vesicules limited either by a tonoplaste or by a double membrane. According to some authors (Niki et al. 1978; Ilker et al., 1976; Rochat, Therrien, 1975; Kupila-Ahvenniemi et al., 1978) these vesicules with autolytic functions have their origin in the dictyosomes or the dilated RER which has lost its ribosomes. Geneves (1965) certifies in endives cultivated at about 0° C that the vacuolisation which varies with the time of treatment is a reversible phenomenon.

The cristae of the mitochondria in the periplasmodium are dilated (Fig. 6) by the cold. The vesicules and the mitochondria, structure of which is modified, are grouped (Fig. 6) giving the tapetum a heterogeneous aspect. On the cooled coleoptyle of *Agropyrum glaucum* Kwiatkowska (1970) has observed the formation of globulous mitochondria aggregates giving birth (by fusion) to elongated mitochondria. This would reduce the mitochondrial biochemical activity reducing the contact surface cytoplasm-mitochondria. The changes in cytoplasmic viscosity would be at the origin of this aggregation. Our observation corresponds perhaps to the first phase of a mitochondrial transformation of the same type, also induced by cold.

Under the action of cold beyond the tetrad stage, the membrane of the proplasts of periplasmodium frequently invaginates itself (Fig. 7), and we note the presence of long, flexuous, dilated in some places cytomembraneous sheets without ribosomes (Fig. 8), which seem to be altered RER, as described by Niki et al. (1978) and Rochat and Therrien (1975).

In the conditions of our experiments, unlike to what occurs in the PMC (Figs 1, 4 and 5), we observed no significant alteration of the tapetal nucleus envelope. On the other hand, the nucleolus of these nuclei (Fig. 5) seems to split up into several fragments, a clear areas occurring between the dark areas (fragments). Villiers (1972) has observed modification in the nucleolar volume and structure in the root meristem of *Fraxinus excelsior* exposed to 5°C and an increase in the nucleolar RNA synthesis. The reversible segregation of cooled nucleolus materials is identical to that observed after inhibition of the RNA synthesis in the prophase nucleolus of *Allium cepa* meiocytes (Stockert et al., 1971). In *Rhoeo discolor* the cold completely blocks the nucleolus synthesis of the RNA in the tapetum (Souvré, Albertini, 1978). The influence of cold on the tapetal nucleolus reminds of the action of actinomycine on the same organelle (Albertini, 1971b).

CONCLUSIONS

In *Rhoeo discolor* treated by moderate cold during a medium period, the greater part of the observed alterations of ultrastructure of meiocytes and tapetum correspond to the modifications established by the different authors. As for the lipid inclusions and the starch granules we think, similarly as Kupila-Ahvenniemi et al. (1978), that the variations observed are not sufficiently marked to be significant.

The changes of structure in the cooled organelles are perhaps, as estimated by Kwiatkowska (1970), the reflection of an adaptation to cold by the cell, the alterations becoming irreversible only if the tonoplast on the lytic vesicules is damaged (Niki et al., 1978). The possibility of the cooled meiocytes and tapetum of *Rhoeo discolor* to synthetise nucleic acids and proteins after reincubation (Souvré, Albertini, 1978) is a strong argument in favour of this reversibility of the alteration of organelles, quoted also by Geneves (1965), as long as the nuclear structures remain complete or almost intact.

If we link the cytochemical aspects (reduction or blockage of synthesis) to the ultrastructural modifications observed (alterations of mitochondria, proplastids, polyribosomes, tapetal nucleolus), we can think, similarly as Lazzarini and Johnsson (1973) that, as far as RNA synthesis is concerned the integrity of structural and enzymatic compounds is necessary to maintain the cellular synthesis.

The alteration more readily noticed in the PMC of *Rhoeo* as regards the tapetum corresponds perhaps to an inhibition of the nutrient transfers which take place from tapetum to microspores (migrations of RNA and proteins (Albertini, 1971a) and simple glucides (Albertini, Souvré, 1978)). Indeed it is known that cold is a factor slowing down or stopping the migration of chemical compounds (Olszewska, Rodkiewicz, 1963). This possible inhibition of transfer can be linked to that observed by Nishiyama (1970).

Acknowledgments

This research was supported by the C. N. R. S., France (RCP n° 574).

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