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Ultrastructural changes in chloroplasts of the hemiparasite *Rhinanthus minor* L. correlated with the removal of the host.

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Summary

Plants of the hemiparasite *Rhinanthus minor* L. grown without a host are always stunted and chlorotic: their chloroplasts show a reduced grana-fretwork system, starch grains and sometimes a strikingly high number of osmiophilic globuli. On the contrary, plants of the parasite attached to roots of *Medicago sativa* L. are healthy and have well developed chloroplasts very poorly provided with starch grains. Nevertheless, when the aerial parts of the host are removed, the leaves of the parasite become chlorotic beginning with the youngest ones and, at the ultrastructural level, a regression of the grana-fretwork system takes place; frequently an increase of osmiophilic globuli can be noted at the same time. The chlorotic aspect either of attached plants deprived of the aerial parts of the host or of unattached plants is ascribed to a reduced availability of mineral elements; the requirement of carbohydrates from the host is considered inconsistent. A close relation between the system of thylakoids and osmiophilic globuli is hypothisized.

INTRODUCTION.

It was demonstrated that *Rhinanthus minor* L. (Scrophulariaceae) is incapable of growing to maturity without being attached to a host plant (MELCHIONNA, 1983); indeed, in absence of the benefit of the host its growth is limited and its leaves appear chlorotic.

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It was also shown that vigorous and healthy plants of the parasite, growing attached to a host, become stunted and their young leaves turn yellow rapidly if the aerial parts of the host are completely removed (MELCHIONNA, 1983). This means that both physiological changes and some remarkable modifications occur at ultrastructural level.

The aim of the present paper is to find out what happens inside the chloroplasts in the above-mentioned experimental situation in order to get indirect indications concerning the correlated physiological changes.

MATERIALS AND METHOD.

Seeds of *Rhinanthus minor* L. whose dormancy was broken by storage for 3 months at about 4°C. were sown in pots. Some plants grew in the presence of *Medicago sativa* L. as a host plant, others in absence of a host.

The whole aerial part of the host plants was removed 30 days after the attachment of the parasite whose young leaves, therefore, rapidly became chlorotic.

For the electron microscopy the following material was used:

— young green leaves from vigorous, healthy plants of *Rhinanthus minor* attached to plants of *Medicago sativa;*

— young chlorotic leaves from plants of *Rhinanthus minor* grown without a host;

— young leaves of the parasite that became chlorotic after removal of the aerial parts of the host plants.

The specimens were fixed for 1 hr at room temperature in 6% glutaraldehyde buffered with 0.2M sodium phosphate pH 7.2, rinsed in the same buffer for 5 hrs and post-fixed for 1 hr in 1% buffered OsO4.

After dehydration in graded ethanol series and treatment with propylene oxide the materials were embedded in an Epon-Araldite mixture (MOLLENHAUER, 1964).

The sections, obtained with a LKB Ultrotome III, were stained with uranyle acetate and lead citrate (REYNOLDS, 1963) and viewed with a Philips EM300 electron microscope at 80 kV.

OBSERVATIONS.

Plants of *Rhinanthus minor* attached to the host have green and thickened leaves. Palisade parenchima cells are highly vacuolated and show numerous chloroplasts, along the anticlinal walls, usually disposed in a row in the peripheric layer of the cytoplasm. Mature chloroplasts are lens-shaped in profile view; they have well developed grana but are very poorly provided with or completely devoid of starch. They also have a large stromatic area in the region of the chloroplast closely adjacent to the cell wall which lacks the grana-fretwork system (Tab. I, Fig. 1). The nuclei of these cells are often rich in unusual bodies (Tab. I, Fig. 2) probably related to the crystalline formations already described in some Rhinanthoideae (HESSE, 1974; PONZI & MELCHIONNA, 1979). Such bodies are particularly evident and frequent in the epidermic cell nuclei. Chloroplasts of the cells near the vascular bundles are slightly richer in starch and sometimes have few if any grana. Wherever present, starch grains stain rather lightly. It seems significant that plastoglobuli are nearly absent in chloroplasts lacking in starch and that they are always present when starch grains are present.

The leaves from unattached plants of *Rhinanthus minor* are chlorotic and never reach the size of the mature leaves of plants attached to the roots of *Medicago sativa*. The chloroplasts of these chlorotic leaves (Tab. 1, Fig. 3) have nearly the same size and shape as the chloroplasts from leaves of attached plants, but clearly differ from these in having a reduced grana-fretwork system; they contain a striking amount of starch grains which in electron images appear intensely stained with the internal region more electron translucent. Plastoglobuli are always present and their number is sometimes strikingly high. The unusual intranuclear bodies are present either in the mesophyll or, more often, in the epidermic cells.

Chloroplasts from leaves of attached plants that became chlorotic after having the aerial parts of the host removed are very similar to the chloroplasts belonging to the parasite grown without a host. The wideness of the grana-fretwork system appears manifestly reduced (compare in Tab. 1, Fig. 4 with Fig. 1) and starch grains are remarkably increased in number; they stain as intensely as the ones from the chloroplasts of the unattached plants. Plastoglobuli scattered in the stroma are always evident and frequently their number is very high. Intranuclear bodies persist either in the mesophyll or in the epidermic cells.

Discussion and conclusions.

Limiting factors cause plants of *Rhinanthus minor* grown without a host to remain small and stunted compared to the well developed attached plants. In *Rhinanthus serotinus,* studies on physiological changes which occur before and after attachment (Klaren & Janssen, 1978) demonstrated that a great stimulation to the growth of the attached plants was correlated with a strong increase in the content on N, P, K, Na and Mg; it was also found that N and P are rapidly incorporated into essential organic compounds (nucleic acids and proteins). It can be postulated that *Rhinanthus minor* behaves in the same way; if so, the described normal growth of the attached plants and the well developed grana-fretwork system in their chloroplasts, might be related to a greater availability of mineral elements. It is likely that when the aerial parts of the host plant are removed, the flow of such mineral elements from the host to the parasite ceases; chlorosis symptoms rapidly appear in the young leaves and a regression of the grana-fretwork system takes place in the chloroplasts, whose structure consequently comes to be similar to that of chloroplasts from unattached plants.

The results of the present observations do not indicate which of the mineral elements causing chlorosis is most responsible for the ultrastructure shown by the chloroplasts of *Rhinanthus minor* suffering from nutritional deficiencies. In fact, thylakoids can be reduced in number or in length or can be altered in their organization by the lack of different single elements such as N, Fe, Mn, Mg, P, K. Nevertheless, the present observations, together with the results obtained for *Rhinanthus serotinus* suggest that in attached plants of *Rhinanthus minor* normal growth occurs because mineral elements are absorbed from the host.

The fact that starch content, in the chloroplasts from plants living without a host, is higher than in chloroplasts from attached plants, indicates that the parasite is not subject to the host for satisfying its requirement for carbohydrates.

In tobacco plants GEROLA & DASSU (1960) found that during the first stages of experimental induced whitering, an attenuation of the starch demolition process occurs; they also found that during chloroplast regression, induced by nutritional deficiency, starch opacity to electrons increases. The above-mentioned higher starch content and its increased opacity, in leaves of *Rhinanthus minor* showing symptoms of chlorosis, suggest that the metabolism of this polysaccharide is affected by nutritional deficiencies.

Finally, the high number of osmiophilic globuli frequently found in chloroplasts from plants of *Rhinanthus minor* living without a host is probably due to the storage of lipidic material not utilized for the formation of thylakoids; analogously, in the case of chloroplasts from plants that became chlorotic after having the aerial parts of the host removed, the frequently high number of these osmiophilic globuli might be due to a store of material released by the breakdown of lipoprotein membranes of thylakoids.

RIASSUNTO

Le piante dell'emiparassita *Rhinanthus minor* L. crescono sempre stentatamente ed appaiono clorotiche se non sono legate ad un ospite: i cloroplasti mostrano un sistema lamellare scarsamente sviluppato, grossi granuli di amido ed un elevato numero di globuli osmiofili.

Le piante parassite su radici di *Medicago sativa* L. sono sane, con cloroplasti ben sviluppati ma con piccoli e rari granuli di amido. Tuttavia, quando vengono eliminate le parti aeree dell'ospite, le foglie del parassita diventano clorotiche, a cominciare da quelle più giovani, ed a livello ultrastrutturale i cloroplasti mostrano una regressione del sistema tilacoidale ed un incremento nel numero dei globuli osmiofili.

L'aspetto clorotico, riscontrato nelle piante cresciute sempre senza ospite e nelle piante private dell'ospite, viene attribuito ad una deficienza di elementi minerali; si ritiene invece inconsistente la richiesta in carboidrati.

Viene ipotizzata una stretta correlazione tra lo sviluppo del sistema tilacoidale ed il numero dei globuli osmiofili.

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Fig. 1. - Mature chloroplast with a well developed grana-fretwork system and a large stromatic area in a leaf of *Rhinanthus minor* attached to roots of *Medicago sativa.* CW = Cell Wall; S = Starch. x 20,000.

Fig. 2. - Nucleus (N) with a proteic crystalline inclusion. x 18,000.

Fig. 3. - Chloroplast, from a chlorotic leaf of unattached *Rhinanthus minor,* showing a reduced grana-fretwork system and numerous plastoglobuli (p). S = Starch. x 35,000

Fig. 4 - Chloroplast with large starch grains in a chlorotic leaf of *Rhinanthus minor* after removal of the host. CW = Cell Wall; S = Starch. x 25,000.

PONZI R. and M. MELCHIONNA: Ultrastructural changes in chloroplasts of the hemiparasite *Rhinanthus minor* L. correlated with the removal of the host.

TAV. I