

## PHYTOFERRITIN IN PLASTIDS OF THE STYLE OF *OLEA EUROPAEA* L.\*

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### SUMMARY

The presence is reported of phytoferritin inside the plastids of the cells surrounding the stylar transmitting tissue and vascular bundles of *Olea europaea* L. cv. Correggiolo. The phytoferritin particles show a crystalline arrangement, often forming finger-print like patterns. These aggregates of phytoferritin could be associated with yellowing and senescence of the style; a possible relation between phytoferritin and pollen tube is also discussed.

### 1. INTRODUCTION

Plant ferritin (phytoferritin) is a particulate protein-iron complex similar to that found in animal cells. It has been isolated from and observed in plastids of different plant cells (HYDE et al. 1963; see also WILDMAN & HUNT 1976). In addition to the normal plant cells (JENSEN 1963; CATESSON 1966; ROBARDS & HUMPHERSON 1967; DESCHAMPS 1970; PERRIN 1970; GORI 1977a), phytoferritin has been also reported to be present in degenerating chloroplasts during senescence (BARTON 1970) and in chloroplasts of virus infected plant tissue (see WILDMAN & HUNT 1976).

In the present paper we report the presence of phytoferritin in the plastids of stylar cells of *Olea europaea* cv. Correggiolo.

### 2. MATERIALS AND METHODS

Styles of *Olea europaea* L. cv. Correggiolo were gathered, one day after anthesis, from trees growing in the Botanical Garden of Siena University.

Samples were prefixed for 1 h in 2.5% glutaraldehyde in cacodylate buffer (pH 7.2) and post-fixed in 1% OsO<sub>4</sub> in the same buffer, then dehydrated in ethanol and embedded in Epon Araldite (MOLLENHAUER 1964).

Sections were cut with a diamond knife on an ultramicrotome LKB Ultratome III, stained with uranyl acetate and lead citrate and observed with a Jeol Jem 100B electron microscope.

Sections 2-3  $\mu$ m thick, fixed and embedded as described, were collected on slides and tested for proteins with the Ponceau 2R stain (GORI 1977b).

\*Research performed under CNR program "Biology of reproduction".

### 3. RESULTS

The *Olea europaea* style is of the solid type with wet stigma. In transverse section the style shows a one-layered epidermis with a thick cuticle, a main tissue formed by vacuolized cells and an inner transmitting tissue formed by only a few non-vacuolized cells. In the vacuolized tissue two vascular bundles are symmetrically arranged (*fig. 1*). The cells surrounding the transmitting tissue and the vascular bundles contain plastids with phytoferritin. The remaining cells, both in the transmitting tissue and in the vacuolized tissue, show numerous plastids none of which contains phytoferritin.

The cells of the vacuolized tissue which surround the transmitting tissue and the vascular bundles have a normal appearance (*fig. 2*); their cytoplasm forms a thin peripheral layer containing some short RER (rough endoplasmic reticulum) cisternae, few dictyosomes, plastids, mitochondria and ribosomes. The nucleus is small with one nucleolus and it can contain one or two crystals per cell section. The chromatin is not very evident. The cell walls are very thick; numerous plasmodesmata are present.

The plastids of these cells are, however, noticeable as they contain phytoferritin aggregates of different sizes and shapes. The plastids have generally clearly visible external membranes and their matrices show a higher electron-density than the cytoplasm, they contain large starch grains, some osmiophilic globules and often one or few proteinaceous roundish bodies, surrounded by only one membrane (*figs. 2, 3*); these bodies give a positive reaction after Ponceau staining. The phytoferritin aggregates have a peripheral distribution in proximity to the starch grains and show a high electron-density (*fig. 4*).

The phytoferritin particles are usually arranged in characteristic arrays of curved rows, often forming a fingerprint like pattern (*fig. 5*), giving a "crystalline" appearance. However, aggregates composed of irregular elements of phytoferritin particles are also present (*fig. 6*). A high magnification of the phytoferritin particles can be observed in *fig. 7*. The diameter of each ferritin particle is 5.2–7.0 nm and the distance from one to another is 6.5–7.0 nm. Each particle appears to have a uniform electron-density as well as a uniform structure.

### 4. DISCUSSION

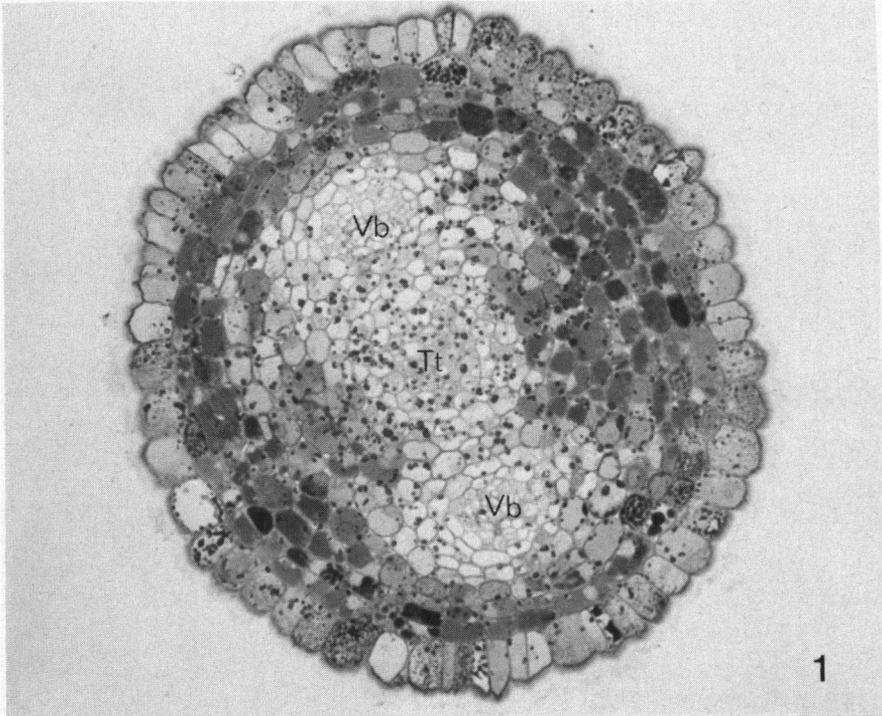
There does not seem to be a generally valid interpretation of phytoferritin aggregates in plant cells. In fact they have been found in plastids of senescing and

*Fig. 1.* Transversal section of the style of *Olea europaea*. Light micrograph.  $\times 320$ .

Vb = vascular bundles; Tt = transmitting tissue.

*Fig. 2.* Cell of vacuolized tissue in proximity of the transmitting tissue and vascular bundles. The cytoplasm forms a thin peripheral layer.  $\times 10,000$ .

N = nucleus; Nu = nucleolus; A = amyloplasts; W = wall; V = vacuole; M = mitochondria; Pb = proteinaceous bodies.



1



2

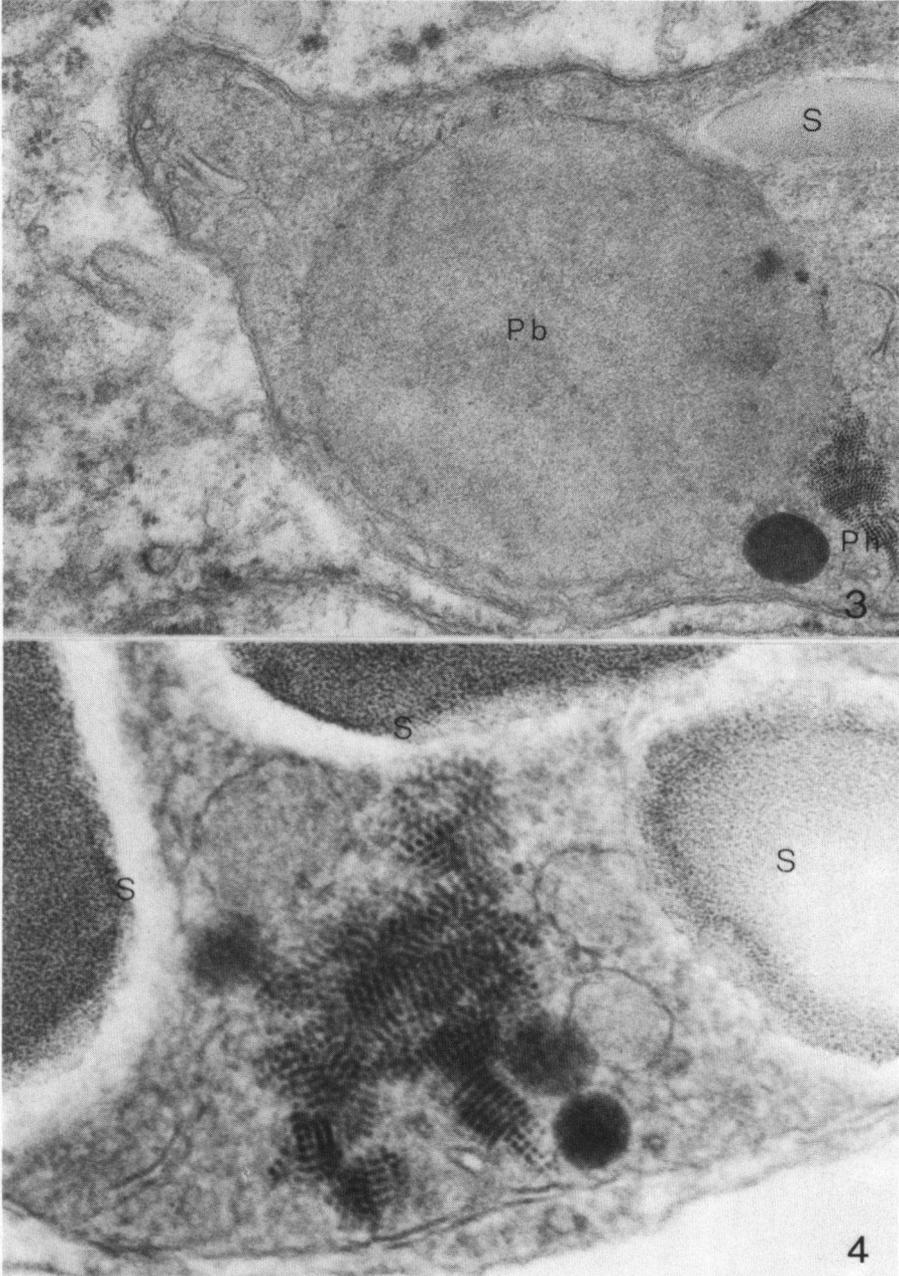


Fig. 3. Plastids with phytoferritin aggregates. A roundish proteinaceous body, surrounded by only one membrane, is visible.  $\times 51,600$ .

Ph = phytoferritin; Pb = proteinaceous body; S = starch.

Fig. 4. Plastid containing phytoferritin particles and starch grains.  $\times 121,000$ .

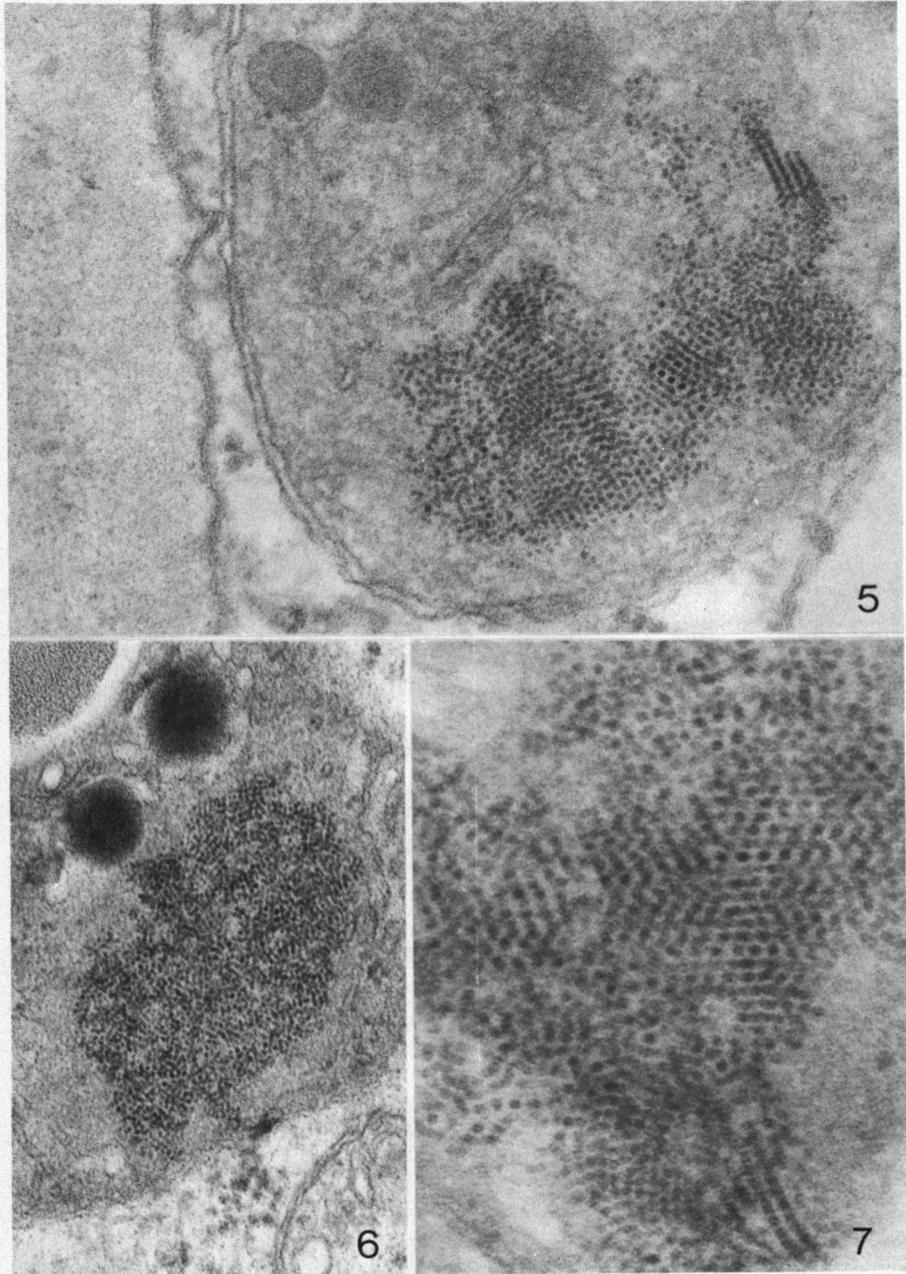


Fig. 5. Phytoferritin particles arranged in characteristic arrays of curved rows, forming a finger-print like pattern similar to a "crystalline" structure.  $\times 115,000$ .

Fig. 6. Irregular arrangement of phytoferritin aggregates inside the amyloplasts.  $\times 76,600$ .

Fig. 7. High magnification of the phytoferritin particles. The diameter of each particle is 5.2–7.0 nm and the distance from one to another is 6.5–7.0 nm.  $\times 200,000$ .

yellowing leaves (BARTON 1970; WILDMAN & HUNT 1976), in chloroplasts of virus infected plants (CHALCROFT & MATTHEWS 1966; CORBETT & GRANT 1967; CRAIG & WILLIAMSON 1969; CRONSHAW et al. 1966; HOOPER & NIBLETT 1969; LISTER et al. 1965; USHIAMA & MATTHEWS 1970), as well as in "normal" plant cells (JENSEN 1963; DESCHAMPS 1970; PERRIN 1970; GORI 1977a). The common features of ferritin containing plastids is that they are photosynthetically inactive (ROBARDS & HUMPHERSON 1967; BARTON 1970); such is the case, for instance, for the ferritin containing plastids in the integument of *Oxalis corniculata* ovules (GORI 1977a) or in various ovular tissues of *Linum usitatissimum* (DESCHAMPS 1970). What is agreed upon by most authors is that ferritin represents a non-toxic store of iron in the cell (BARTON 1970); this stored iron can represent the result of a "mopping up" operation following the dismantling of active chloroplasts (BARTON 1970), or can be kept in readiness for a growing tissue (GORI 1977b).

In *Olea europaea* the phytoferritin deposits have been only observed in the plastids of the styler cells surrounding the transmitting tissue and the vascular bundles, but not in the other vacuolized cells. The phytoferritin deposits could be connected with senescence as the styler function ceases after fertilization has occurred.

The possibility cannot, however, be excluded that phytoferritin is stored in the transmitting tissue, later to be used by the pollen tube during its growth; in fact in the plastids of the growing pollen tube phytoferritin deposits have been observed which have never been found in the ungerminated ripe pollen (PACINI et al., in preparation).

Lastly it is to be considered that the unripe pollen grains of the plants examined show a viral infection (PACINI & CRESTI 1977).

It is true that, viruses being absent both in the ripe pollen (PACINI & CRESTI 1977) and in the style, a relation between viral infection and styler phytoferritin seems unlikely. However, the distribution of the viral infection is still being investigated: should a more diffuse presence of virus in the different plant tissues of *Olea europaea* be found, the hypothesis of a relation between viral infection and phytoferritin could be re-considered.

#### ACKNOWLEDGEMENT

We are indebted to Mrs. I. Clemente for translating the text.

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