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# Immunocytochemical localization of lectin in cotyledons of developing seedlings of *Cucurbita ficifolia**

Irena Lorenc-Kubis and Paola Bonfante[a]

*Institute of Biochemistry, University of Wrocław, Tamka 2, 50–137 Wrocław, Poland*
[a]*Dipartimento di Biologia Vegetale dell' Universita Torino, Italy*

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Lectins are usually defined as proteins or glycoproteins of nonimmune origin, which interact with free saccharides or glycosylated macromolecules in soluble or membrane-bound form.

In some plant families, such as *Leguminoseae* or *Gramineae*, lectins are of widespread occurrence, whereas in others, such as *Euphorbiaceae* they have been found in a few species only [1].

Lectins are present in different plant organs: they usually reach the highest concentration in seeds [2, 3]. However, in some members of *Cucurbitaceae* (*Cucurbita maxima, Cucumis sativus* and *Cucumis melo*) lectins were not detected in seeds or in seedlings during early stages of development [4].

In previous studies on lectins from *Cucurbita ficifolia* we found a low agglutination activity in cotyledons of resting seeds and in seedlings at the onset of germination. The activity increased during the development of seedlings [5].

The aim of this investigation was to localize lectins in the cotyledons of germinating seeds of *C. ficifolia* by using immunogold techniques. An antibody raised against the cotyledon lectin [5] and purified from rabbit antiserum according to Harboe & Ingild [6] was used as a specific probe for the localization experiments.

Previous studies on the agglutination activity of the proteins extracted from cotyledons of seeds and seedlings (Fig. 1) have shown that the lectin activity of extracts of seeds and 3-day old seedlings was very low, but increased on the 6-day of germination and then sharply decreased [5]. For this reason, only cotyledons from 3- and 6-day-old seedlings were choosen for studying the intracellular localization of lectin. The cotyledons were fixed in 2.5% glutaraldehyde in 10 mM Na-phosphate buffer (pH 7.2) for 2 h at room temperature. Then they were rinsed with the same buffer, postfixed in 1% $OsO_4$ in distilled water for 1 h, washed three times with distilled water and dehydrated in ethanol series at 30, 50, 70, 90 and 100% concentrations, (10 min each step) at room temperature. The cotyledon segments were infiltrated in 2:1 (v/v) ethanol/LR White resin for 1 h, 1:2 (v/v) ethanol/RL White resin for 2 h and 100% LR White resin overnight at 4°C according to Moore *et al.* [7].

Semi-thin sections were stained with 1% toluidine blue for morphological observation. For electron microscopy, thin sections were incubated in the antibody raised against the *C. ficifolia* lectin and then treated with the secondary antibody, which was bound to 15 nm gold particles (Bio Cell, Cardiff, U.K.) according to Bonfante *et al.* [8].

A light microscope section of a cotyledon from a 3-day-old seedling showed that cortical cells were filled with protein bodies containing prominent crystalloids. When seen at ultrastructural level and after the treatment with the antibody raised against the lectin, the crystal-

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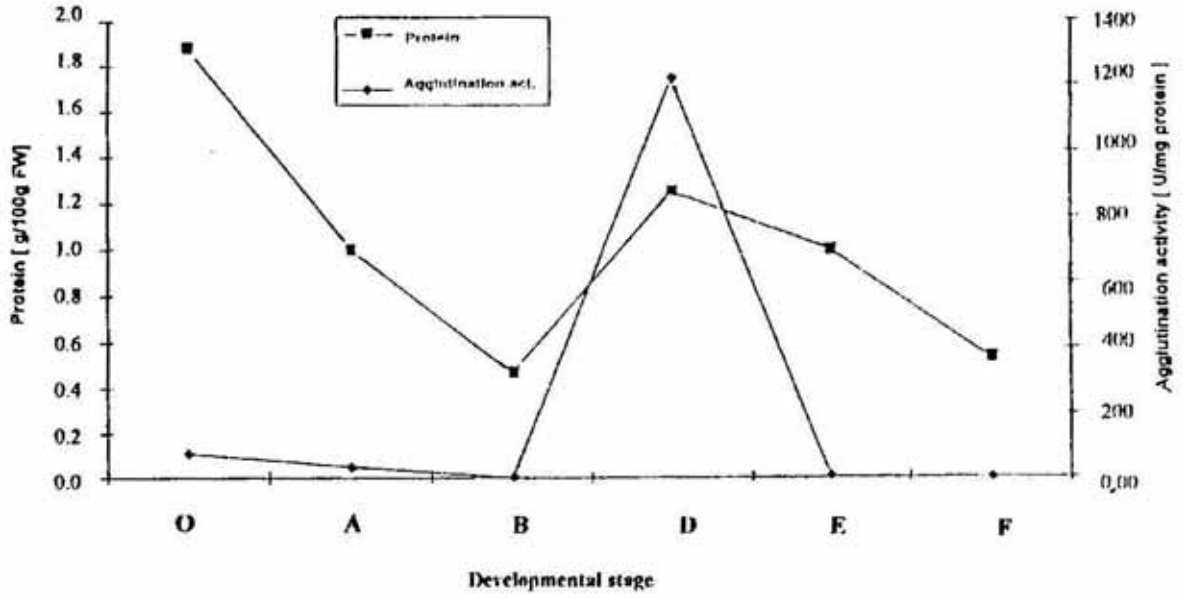


*Fig. 1. Change in protein content and agglutination activity in cotyledons of seeds and developing seedlings of C. ficifolia.*
0, Dry seeds; A, imbibed seeds; B, 3-d-; D, 6-d-; E, 10-d-; F, 22-day old seedlings.

loids were heavily labelled by the gold granules (Fig. 2a). Only a few granules were present in the matrix of protein bodies. No labelling was found in the epidermal cells or in vascular elements. Protein bodies were not labelled in the control sections, without the primary antibody.

The crystalloids from 6-day-old seedlings were less prominent, while protein bodies showed either internal or peripheral degradation. This process led to formation of a central vacuole(s) in the storage cells, in agreement with the description by Weber & Neuman [9].

After immunological experiment, gold granules were still found over the crystalloids (Fig. 2b) but their density was changed depending on the degradation step and on the protein body structure. When the crystalloid was reduced in size and the matrix became loose, only rare gold granules were found.

The results presented suggest that the lectin of *C. ficifolia* is first accumulated in the protein bodies inside the crystalloids. Even if quantification of the gold granules has not been performed, a decrease in the labelling of the

*Fig. 2. Immunolocalization of lectin from C. ficifolia in cotyledons cell of: a, 3-day; b, 6-day old seedlings.*
Electron micrograph of protein bodies. a, Gold granules (15 nm diam.) are found in the crystalloid part of protein bodies; b, only few gold granules are found in the degraded protein bodies. (× 52000).

protein bodies was observed in older seedlings (6-day-old). Interestingly, this decrease corresponds to the peak of agglutination activity [5]. This may suggest that the lectin is first involved in cross-linking of some proteins [10], and therefore it can be localized in the protein bodies. Later, it is released from the protein complexes and becomes soluble. This may be one of the reasons of the increased lectin activity in the cotyledons on the 6th day of germination and of decreased gold labelling at that time.

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