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Developmental differences in acid phosphatase and agglutination activity in roots of *Cucurbita ficifolia* seedlings. Purification and some properties of lectin*

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Acid phosphatases (AcPases) form a widely distributed group of nonspecific enzymes which are involved in many physiological processes but their biological functions are still obscure. Most of the enzymes isolated from plants are glycoproteins [1–4] containing mainly mannose, glucose, *N*-acetylglucosamine and fucose. The presence of sugar in the molecules of AcPases makes possible their interaction with other proteins, especially with lectins.

Lectins are proteins or glycoproteins which posses the ability to bind reversibly free saccharides or glycosylated macromolecules in soluble or membrane bound form [5, 6].

In the previous study we have demonstrated that acid phosphatases activity and lectins content in cotyledons of germinating seeds and developing seedlings undergo developmental changes and that the lectins isolated from seedlings bound to endogenous AcPases stimulate their activity [7].

This paper reports the results of studies on lectin and AcPase of roots from germinating seedlings of *Cucurbita ficifolia*.

We found that AcPase activity and lectin content of roots were like in cotyledons of *C. ficifolia* [7], rather low at the onset of germination, but increased in seedlings especially on the 6th day; then decreased again (Fig. 1).

Affinity electrophoresis with free Con A [8] (Fig. 2) showed that in roots at all the developmental stages of seedlings, two forms of glycoprotein acid phosphatases occur which differ in binding to Con A. This could point to differences in the sugar component of these enzymes.

To purify AcPases and lectins, the protein from 6-day old seedlings were extracted with 0.1 M acetate buffer, pH 5.1, precipitated with ethanol, and subjected to affinity chromatography on Con A-sepharose [7].

Proteins were eluted from the column with 15 mM α-methyl-D-mannopyranoside (fraction Ba) and then with 300 mM solution of this sugar (fraction Bb).

Fraction Ba was applied once again to Con A-Sepharose and the proteins were eluted with 2.5 mM, 5 mM and 15 mM α-methyl-D-mannopyranoside. All fractions contained only AcPase activity (AcPase Ba1-3), while proteins with agglutination activity were not bound to the column and were present in the effluent.

Lectin was further purified by ion-exchange chromatography on SP-Sephadex C-50 and released from the column with 0.2 M NaCl. The purified lectin (RLABa) did not precipitate with free lectins: Con A, LcA, PHA or WGA; this could suggest that this lectin has no sugar component or the sugar units are not recognised by the lectins. In this respect root lectin differs from the cotyledon lectins of *C. ficifolia* which are glycoproteins and interact with free Con A.

We have also shown that the root lectin RLABa, cotyledon lectins and Con A are antigenically related (Fig. 3). The lectin from roots

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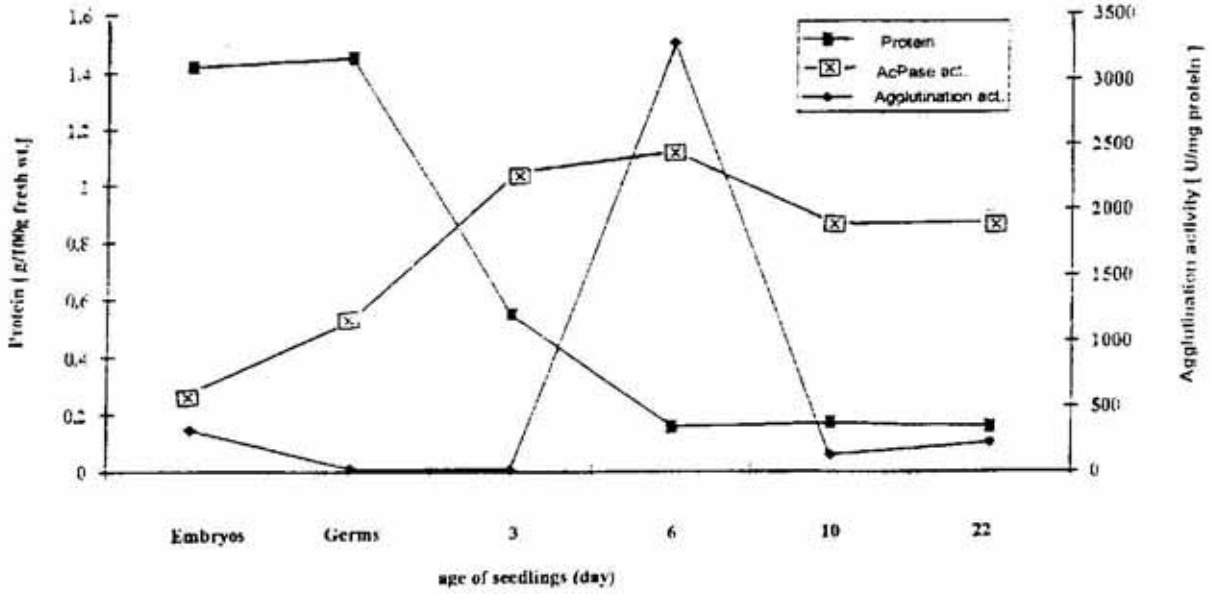

Fig. 1. Changes in acid phosphatase activity, agglutination and protein content in roots of germinating seeds and seedlings of Cucurbita ficifolia at different developmental stages.
Embryos of dry seeds, germs of imbibed seeds, and roots of seedlings were used.

agglutinated both human erythrocytes of group 0 and sheep erythrocytes. RLABa showed very weak activity towards red cell of group A and B differing in this respect from lectins of cotyledons [7].

To determine the specificity of RLABa lectin we have examined the inhibition of lectin activity by typical saccharides. Among the monosaccharides tested as inhibitors of agglutination L- and D-Ara were most active, while Glu, Gal, GLcNAc and GalNAc were less effective inhibitors (not shown).

The purified lectin formed an affinity precipitate with β1-SP1-glycoprotein from human serum and bound to endogenous AcPases changing their activity. In the presence of RLABa the activity of root AcPases decreased by 30%–40%. It has been found earlier that the activity of some plant glycoprotein acid phosphatases was altered in the presence of lectins,

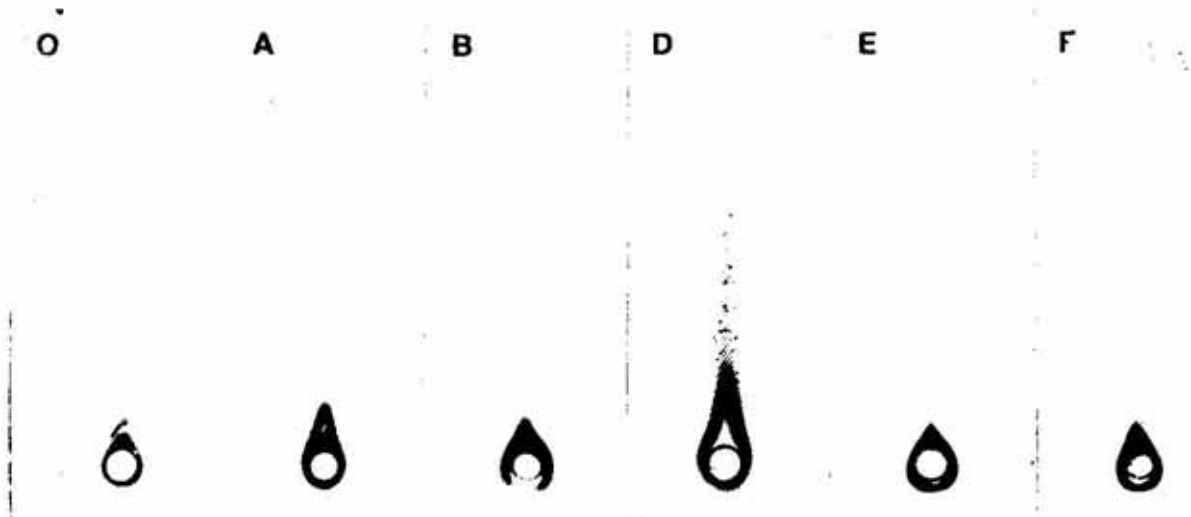

Fig. 2. Rocket affinity electrophoresis of root AcPases from Cucurbita ficifolia seedlings into 1% agarose gel containing Con A (25 μg/cm²).
After electrophoresis the plates were stained for acid phosphatase activity as described in [11]. AcPase from: O, embryos; A, germs of imbibed seeds; B, 3 d-; D, 6 d-; E, 10 d- and F, 22-day old seedlings.

Fig. 3. Ouchterlony immunodiffusion of RLABa lectin from roots against cotyledon lectin of C. ficifolia and against Con A antibodies.
Lectin RLABa was applied in the central well (a); the peripheral wells were filled with antibodies raised against cotyledon lectin from *C. ficifolia* (1, 2) and against Con A (3, 4).

most of the AcPase being activated [10–14]. RLABa represents a lectin that inhibits enzyme activity.

The ability of root lectin-RLABa to bind to endogenous AcPases suggests that both proteins exist in a complex from which they are released during seed germination and seedling development. This could explain the increase in the enzyme and agglutination activities observed on the 6th day of germination.

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