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Changes in proteolytic activity during germination and early development stages of *Cucumis sativus*

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Abstract

The study constitutes an effort to investigate proteolytic activity during the juvenile stages of *Cucumis sativus* seedling development. The proteases activity evidenced in cotyledonal extracts showed one peak on the fifth day of seedling development. Changes were distinctly correlated with changes in nitrogen compound fractions of the plant organ under investigation. No similar correlations were observed in root extracts.

INTRODUCTION

Most of the investigations hitherto conducted on biochemical changes during germination and early development stages of higher plants were carried out on seedlings growing under continuous illumination or darkness conditions and limited to reserve organs of the plant (Mikola, Kolehmeinen, 1972; Yung et al., 1959; Pusztai, Duncan, 1971; Maynguy et al., 1972; Basha, Beevers, 1975; Jacobson, Varner, 1967; Chrispeels, Varner, 1967). On the other hand there is a lack of data reffering to proteolysis in other organs of the developing seedling. It is a known fact that the proteases active in reserve plant organs are engaged in the first place in the breakdown process of plant reserve proteins. It appears that determining the physiological role of proteolytic enzymes active in roots of seedlings is somewhat more difficult.

The present study was aimed at determining the changes in proteolytic activity in the cotyledons and roots of cucumber seedlings during the early stages of their development. Since proteases constitute a hydrolase group engaged in the breakdown of plant proteins, it

appeared of inerest to carry out observations on the eventual changes in the level of some nitrogen fractions on the background of the above mentioned activity.

MATERIAL AND METHODS

This investigation was carried out on cucumber seedlings (*Cucumis sativus* L., var. 'Visconsin'). The material was prepared as presented in a previous study (K ł o b u s, 1980).

The culture medium used in the experiments is optimal for cucumber growth, its composition being in accordance with I n g e s t a d (1973). Composition of the medium in mg/l: $CaCl_2 \times 6\ H_2O$ — 13.66; KNO_3 — 48.434; $NaNO_3$ — 129.046; $MgSO_4 \times 7\ H_2O$ — 25.648; KH_2PO_4 — 7.91; ferric citrate — 0.7. Microelements were added to the medium in the following amounts (mg/l): $MnSO_4 \times 7\ H_2O$ — 0.4; H_3BO_3 — 0.2; $CuSO_4 \times 5\ H_2O$ — 0.03; $ZnSO_4 \times 7\ H_2O$ — 0.3; $Na_2MoO_4 \times H_2O$ — 0.007. pH brought to 6.2.

Proteolytic activity was determinated by means of the modified B e e v e r s (1968) method. Enzymatic protein was extracted at a temperature of 0-4°C by grinding in a mortar of 1 g of fresh tissue with 10 ml 0.05 M Tris-HCl buffer with an addition of 0.005 M cysteine hydrochloride. The obtained homogenous substance was filtered through gauze and cetrifuged for 20 minutes at 12000 × g. The supernatant was used as the enzymatic extract.

The total volume of the incubation mixture was 3 ml and contained 0.5 ml enzymatic extract, 1 ml hemoglobin and 0.1 M Tris-HCl buffer, pH 6.4. After 90 minutes of incubation at a temperature of 40°C the reaction was stopped and 1 ml of cooled trichloroacetic acid added. The samples were placed in a refrigerator for 30 minutes followed by centrifuging at 15000 × g for 15 minutes and reading the supernatant extinction on a spectrophotometer at a wave lenght of 280 nm (thickness of the absorbing layer was 1 cm).

The unit of enzymatic activity was defined as absorption growth (E_{280}) of 0.1 in the conditions of the reaction.

Specific activity was expressed in units of proteolytic activity calculated per mg of protein.

Total nitrogen was determined after incinerating 30 mg of the material with 2 ml of concentrated H_2SO_4. The fraction of soluble nitrogen (including inorganic nitrogen, ammonium nitrogen, amino acids and amides, some peptides and prolamins soluble in 70% ethanol) was obtained as follows: 30 mg of ground material treated with 3 ml of 70% ethanol was shaken for one hour in a temperature of 30°C. The mixture was centrifuged for 20 minutes at 500 × g (P u r i t h, B a r c e r,

1967). Extraction was repeated three times and the liquid decanted from above the sediment, the alcohol vaporized and the sediment incinerated with 2 ml of concentrated H_2SO_4. In both cases nitrogen was determined by means of the Kjeldahl micro-method (Mejbaum-Katzenellenbogen, Mochnacka, 1969).

Protein nitrogen was determined from the difference between the value of total nitrogen and that of soluble.

All of the above presented analyses were carried out separately for the roots and the cotyledons of the cucumber seedlings.

RESULTS AND DISCUSSION

First analyses, both as concerns cotyledons as well as roots, were made after two days from the moment of soaking the seeds. Further determinations were carried out over seven successive days, separately for the roots and the cotyledons.

Fig. 1 present changes in proteolytic activity, as also in protein nitrogen fractions and in soluble nitrogen, taking place during the period of germination and seedling development. Proteolytic activity of cotyledon extracts increases distinctly up to the fifth day of cultivation. During this period there was also an acute drop in N-protein at a simultanenous increase in the soluble nitrogen fraction. After five days proteolysis intensity dropped, this being evident both in the decline of proteolytic activity as likewise in much milder changes in the fraction of the nitrogen compounds under discussion. The level of N-protein nevertheless continued to remain at a fairly high level. The decline in proteolytic activity on the sixth and seventh days of seedling cultivation, despite the continuing fairly high level of protein nitrogen, might have been caused by the amassing of large amounts of soluble nitrogen. These suggestions appear to be highly probable in the light of results obtained by Yomo and Varner (1973), who observed an inhibiting effect of accumulated amino acids on the proteolytic activity in pea cotyledons. A similar correlation between amino acids and proteolytic activity was noted also by Oalls (1965). The plant could thus already take adventage of external sources of nitrogen, in consequence of which proteolytic activity would diminish.

The discussed changes in the correlations between proteolytic activity of cotyledon extracts and the various nitrogen fractions suggest that the function of cotyledon proteases in the cucumber consists of breaking down protein reserves. Basha and Beevers (1975) in their studies on pea seedlings also report on similar conformability between growth of proteolytic activity and intensity of protein decomposition. Similar reports were given by Prisco et al. (1975) and still earlier by

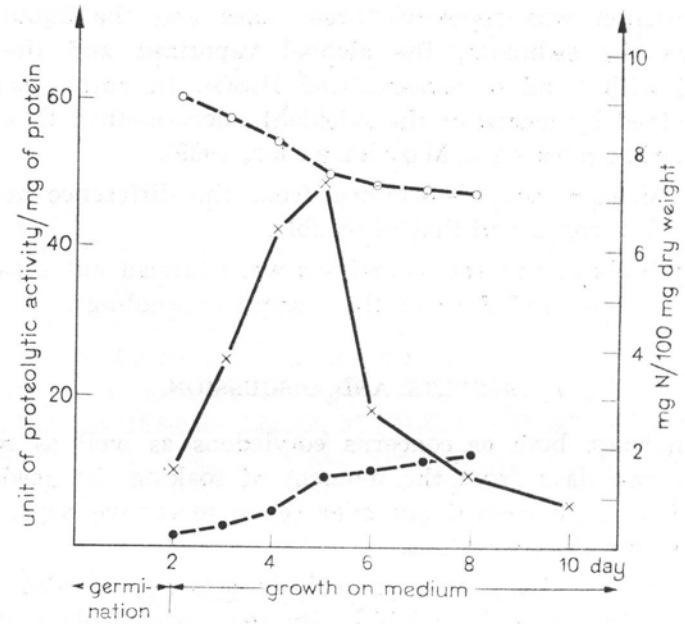

Fig. 1. Changes in proteolytic activity and nitrogen fractions in cotyledon extracts of cucumber seedlings

Mayer and Poliakoff-Mayber (1963), although most intensive proteolysis was observed by these authors somewhat earlier than in the case of the studies by the author of the present investigations. These time differences might have been caused both as a result of differences in the plant material used, as likewise by differing experimental conditions.

Similarly as was the case observed with cotyledons, root extracts (Fig. 2) showed a relatively high proteolytic activity. This was not as distinctly correlated with changes in the N-protein and N-soluble contents as was the case with cotyledons, although certain tendencies for a decline in the level of protein nitrogen and growth of soluble were observed with seedling development. The lack of sudden changes in the levels of protein and soluble nitrogen in root extracts at the moment of distinctly increasing proteolytic activity (fifth day of seedling development) could have been caused by the very rapid take up of amino acids formed as a result of protein hydrolysis in linkages indispensable in the building up of new cells. Hence the physiological function of root proteases would with more reason be connected with a reconstitution of proteins. Such a role has been attributed to numerous proteases in germinating seeds (Ryah, 1973).

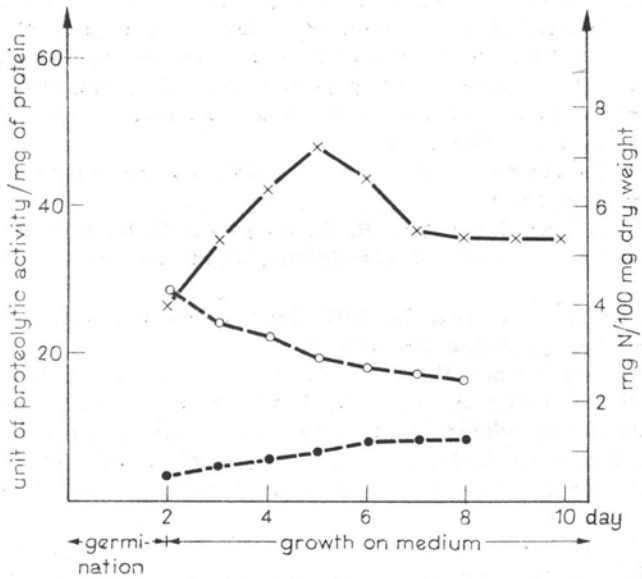

Fig. 2. Changes in proteolytic activity and nitrogen fractions in cotyledon extracts of cucumber seedlings

The very distinct correlation between proteolytic activity and changes in nitrogen fractions observed in cotyledons, and its lack in roots, constitutes a reflection of the differences in the metabolism of these plant organs. Cotyledons are the reserve sources of the developing germ for supplying simple reserve compounds to the intensively developing plant organs, hence processes of hydrolysis chiefly take place here, whereas processes of synthesis will dominate in the roots, which utilize the organic substances amassed in the reserve parts.

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Zmiany aktywności proteolitycznej podczas kiełkowania i wczesnych faz rozwoju Cucumis sativus

Streszczenie

W pracy podjęto badania nad zmianami aktywności proteolitycznej podczas juwenalnego rozwoju siewek *Cucumis sativus*. Aktywność proteaz, ujawniona w wyciągach liścieniowych, posiadała jeden szczyt 5-go dnia rozwoju siewek. Jej zmiany były wyraźnie skorelowane ze zmianami frakcji związków azotowych w badanym organie. Podobnych współzależności nie obserwowano w wyciągach korzeniowych.