Estimation of Substances with the Cytokinin Activity in Studies on the Correlation between the Cotyledon and Its Axillary Bud in Pea (Pisum sativum L.)

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Abstract. The content of substances showing the cytokinin activity was estimated on decapitated and one-cotyledon-deprived pea plants during that period when the promoting effect of the cotyledon excision had not yet been manifested. Results of the bioassay showed that after the excision an increase in the level of substances with cytokinin activity occurred only in cotylars growing in axillas of these excised cotyledons. These results coincide with earlier data about the content of gibberellins and auxins in the same object.

Additional index word: cotylars.

After decapitation of one-week-old pea plants grown in darkness a simultaneous growth of both cotylars may be observed; later, however, one of these cotylars grows faster and takes over the apical function while the other is correlatively retarded in its growth.

In decapitated pea plants the amputation of one cotyledon causes that after an initial, approximately 20-hour’s simultaneous growth (Šebánek and Hradilík 1974), a more intensive growth of the cotyledon situated in the axilla of excised cotyledon may be observed (Dostál 1908).

Several studies tried to contribute to the explanation of endogenous mechanism of this correlation; for example Šebánek (1965) estimated the level of endogenous gibberellins in cotylars growing in axillas of excised and remaining cotyledons and Šebánek and Hradilík (1974) studied the content of endogenous auxins. In this paper results of experiments investigating the content of native cytokinins in the same object are presented.

Pea seeds (Pisum sativum, cv. ‘Raman’) were imbibed for 12 h; then they were placed into damp sawdust and incubated in a dark thermostat at 20 °C for five days.

After the incubation the pea seedlings were decapitated and established in special vessels as a water culture without mineral nutrition in darkness at 20 °C. 72 h after decapitation one
cotyledon was excised and 12 h later cotylar buds were taken off from axillas of both the excised and remaining cotyledons. Immediately after the separation buds (1 000 pieces, 4 mg each) were placed into methylalcohol and homogenized in a piston homogenizer.

The disintegrated tissue was extracted in a refrigerator at +2 °C for 24 h. After filtration the extract was evaporated in a rotating vacuum evaporator (40 °C) and the evaporation residue was purified according to a modified (Dowex 50 H⁺ was used) method described by Pereira et al. (1972). The fraction with the expected presence of substances showing a cytokinin activity was, after concentration, spotted on the start of a chromatogram (TLC MN 300) and separated by means of a mixture n-butanol : ammonia (4 : 1). For one determination the extract of 1 000 cotylars growing in axillas of remaining cotyledons was used.

The distance between the start and the front of chromatogram was divided into 10 zones according to the Rf value and each of them was suspended in 60 ml of hot nutrient medium MS 62 (Murashige and Skoog 1962) with 1 mg of 2-naphtylacetic acid in one litre of medium. After a thorough shaking in a shaker the medium was divided into three 100 ml Erlenmeyer flasks (20 ml of medium each), closed, and sterilized at 1.5 bar for 15 min.

The content of cytokinins in medium was tested by means of a soya bioassay (Miller 1963) on cotyledon segments of the Acme cultivar (3 explants in one flask).

The explants were weighted after six weeks of incubation in darkness at 27 °C and relative humidity of 75 ± 5 %. Results of this bioassay are presented in Fig. 1. The experiment was replicated three times and in all cases similar results were obtained.

At the beginning the decapitation of young pea plants induces the growth of both lateraly established cotylar buds. The excision of one cotyledon results in decapitated plants in an expressed growth determination causing a more intensive growth of the bud in the axilla of excised cotyledon (Dostál 1908). This growth determination, however, does not occur immediately after the amputation; it may be observed approximately 20 h later (Šebánek and Hradilík 1974).

This 20-h interval is therefore important for the explanation of hormonal mechanisms of this correlation. Within this interval (which precedes the period of intensive growth of cotylar in the axilla of excised cotyledon) the