The Role of Endogenous Cytokinins in Correlation between Cotyledon and its Axillary Bud and in Hypocotyl Regeneration of Flax

J. Šebánek, Hoang Minh Tan and Jarmila Blažková

Department of Botany and Plant Physiology, Agricultural University, Brno*

Abstract. When flax seedlings are decapitated above cotyledons and three days later one of the two cotyledons is removed then the remaining cotyledon stimulates in four to five days growth of its axillary bud. It has been found that content of endogenous cytokinins was higher in the stimulated bud as compared with the other one already 12 h after the cotyledon removal.

Flax seedlings decapitated under cotyledons regenerate adventitious buds on the hypocotyl stump during 5—6 days. The endogenous phytohormonal preparation of this regeneration was investigated in the 20 mm apical part of the hypocotyl stump. Decrease in auxin and increase in gibberellins was already found during the first day after decapitation while the level of cytokinins increased as late as three days after the apex removal.

When the flax seedling is deprived of the hypocotyl and one of the two epigeic cotyledons then the remaining cotyledon exhibits a stimulating effect on the growth of its axillary bud (Košínek 1922, Komárek 1930). The mechanism of this stimulation has been investigated only with respect to endogenous gibberellins (Tan et al. 1979a). The aim of this communication is the investigation of this growth correlation in relation to the endogenous cytokinins' role which is supposed to be of fundamental importance in this process.

Flax seedlings are also a very suitable model for investigation of growth-regulating mechanisms of stem regeneration. When seedlings are decapitated under the cotyledons adventitious buds are regenerated on the hypocotyl stumps (Dosťál 1967). The growth-regulating nature of this regeneration has been so far investigated only in relation to endogenous auxins and gibberellins (Šebánek et al. 1979). The second aim of this communication is therefore an explanation of the nature of the hypocotyl regeneration of flax seedlings in relation to the endogenous cytokinins.

MATERIAL AND METHODS

Flax seedlings (Linum usitatissimum L., cv. Věra) were grown in garden soil in pots under continuous fluorescent light (3000 lx) at 20—23 °C.
Growth correlation between the cotyledon and its axillary bud was studied on 11 day old seedlings which were decapitated just above the cotyledons. One of the two cotyledons was removed 72 h after decapitation when both axillary buds had elongated equally (1–2 mm). Twelve hours after the cotyledon removal the endogenous cytokinins were estimated separately in axillary buds adjacent to the remaining and to the excised cotyledon.

The hypocotyl regeneration was investigated on seedlings which were decapitated on their 11th day of growth just under the cotyledons. The endogenous cytokinins were estimated in a 20 mm apical part of the hypocotyl stump at the time of decapitation and 48 h and 72 h later.

For estimation of cytokinins 10 g samples fresh weight were homogenized in 50 ml of 70% (v/v) ethanol. The homogenate was heated to boiling temperature and twice extracted with the same volume of 70% (v/v) ethanol at room temperature for 24 h. Filtrated extracts were combined and evaporated over a water bath. The residuum was dissolved in 20 ml of 70% ethanol (v/v) and partitioned against petroleum ether. The ethanolic phase was adjusted to pH 2 and further purified on DOWEX 50 H+. The eluate was evaporated to dryness over the water bath and the solid residuum was dissolved in 0.5 ml of ethylacetate and applied on chromatographic “Sulifol” plates. Chromatograms were developed using n-butanol: ammonium hydroxide (4:1, v/v) mixture. Individual Rf regions were extracted in Petri dishes in 2 ml of 0.2 N Na-K-phosphate buffer (pH 6.3). Cytokinin activities were tested in Amaranthus-betacyanin bioassay (KÖHLER and CONRAD 1966, BIDDINGTON and THOMAS 1973). Seeds of Amaranthus caudatus were germinated on filter paper saturated with distilled water in darkness at 25 °C. After 72 h 10 seedlings deprived of roots were transferred to a Petri dishes which contained buffer with solids removed from tested Rf region of the chromatogram. The dishes were then incubated in darkness at 25 °C for 24 h. During this period the cytokinins induce betacyanin pigment formation. After incubation the seedlings were transferred to test tubes containing 3 ml of distilled water and betacyanin was extracted by a twice repeated freezing and thawing procedure during which the cell walls were disrupted.