

Embryonal Growth-correlative Effects in Pea Seedlings as Reflected in the Level of Endogenous Gibberellin-like and Cytokinin-like Substances

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Abstract. The level of endogenous gibberellin-like and cytokinin-like substances was estimated in the plumule and the root of 2- to 3-day-old intact pea seedlings, in the plumule of seedlings from which the radicle was cut off, and in the root of seedlings from which the plumule was removed.

An increase in the level of gibberellin-like substances and a decrease in the level of cytokinin-like substances in the plumule as a consequence of radicle amputation were observed within 48 h. Plumule amputation resulted in a decreased level of both gibberellin-like and cytokinin-like substances in the root in the same period.

Growth-correlative relationships and effects between the root and the stem of pea seedlings in relation to the level of endogenous auxin were first studied by DOSTÁL (1941) and later by TAN *et al.* (1984) and ŠEBÁNEK *et al.* (1984). A positive influence of the root on both IAA level and the level of enzymes involved in auxin biosynthesis was recorded in 10-day-old pea seedlings. ŠEBÁNEK (1965) reported a positive influence of the root on the level of endogenous gibberellin-like substances in the apical part of the epicotyl in 7- to 14-day-old pea seedlings. This paper deals with the growth-correlative effects between the plumule and the root in 2- to 3-day-old pea seedlings, that is at the very beginning of their germination. The embryonal growth-correlative effects have been studied in relation to the level of endogenous gibberellin-like and cytokinin-like substances both from the viewpoint of the growth-correlative effect of the root on the plumule and *vice versa*, *i.e.* of the plumule on the root.

MATERIAL AND METHODS

Pisum sativum L. served as experimental object in our study. Pea seedlings were grown during the entire experiment in the dark at $20 \pm 3^\circ\text{C}$. Seeds were soaked for 20 h in water and then placed with the radicle downwards into wet sawdust. Seedlings were taken out from the sawdust after 48 h, their testae were removed, and then the seedlings were divided into three

experimental groups: the first group included intact plants cultivated in vessels containing water and covered with nets through which seedling roots were inserted; the second group was cultivated in the same way but the plumule was cut off from the seedling; in the third group, there were seedlings with cut-off roots and cultivated in wet cotton-wool. Plumule and root samples, 2 grams in mass, were taken from each seedling group at 0 time, and 24 h and 48 h after plumule or radicle amputation, and compared with plumule and root samples taken from intact plants of the same age.

For the estimation of the level of endogenous gibberellin-like substances, plant material was homogenized, and extracted twice with 50 ml of ethanol, in both cases for 24 h at 5 °C. The homogenate was filtrated after the extraction and the filtrate evaporated. The rest was dissolved in 1 ml of ethylacetate and applied onto the chromatographic plate Silufol R. Chromatographic separation was carried out in the mixture chloroform : ethylacetate : acetic acid (60 : 40 : 5) (SEMBDNER *et al.* 1962). Lettuce seedlings (*Lactuca sativa* L. cv. Pražan) were used in the bioassays (FRANKLAND and WAREING 1961, HRADILÍK 1977). The level of endogenous gibberellin-like substances was estimated on the basis of measured length of lettuce seedling hypocotyls.

For the estimation of the level of endogenous cytokinin-like substances, plant material was homogenized, heated to boiling point, and then extracted twice with 50 ml of 70 % ethanol for 24 h at 20 ± 3 °C. Combined filtrates were evaporated on a water bath (LETHAM 1974). The rest was dissolved in 20 ml of 70 % ethanol and shaken with petrolether. The ethanolic fraction was acidified to pH 2 and passed through a column of Dowex 50, H⁺. The purified solution was evaporated on a water bath. The remainder was dissolved in 0.5 ml of ethylacetate and applied onto the chromatographic plate Silufol. The solvent mixture was *n*-butanol : ammonia (4 : 1). Particular R_f regions of the chromatograms were transferred to Petri dishes containing 2 ml of 0.2 M Na, K, phosphate buffer (pH 6.3). The *Amaranthus*-betacyanine bioassay (KÖHLER and CONRAD 1966, BIDDINGTON and THOMAS 1973) was employed for the estimation of cytokinin-like substances. *Amaranthus caudatus* seeds were germinated in a dish on filter paper soaked with distilled water. Seed germination was always carried out in the dark at 25 °C. After 72 h, 10 seedlings from which roots were removed were transferred onto Petri dishes containing buffer and the scraped-away layer from individual R_f regions of the chromatogram. The dishes were left in darkness at 25 °C for 24 h, during which the positive response to cytokinins manifested itself in stronger activation of betacyanine. After 24 h of such cultivation, parts of *Amaranthus* plants used in the bioassay were transferred to a test tube containing 3 ml of distilled water, frozen in a freezer to disrupt the cell walls and cell membranes and enable the diffusion of betacyanine into ambient water, and thawed at 25 °C. The freeze—thaw cycle was repeated twice. Colour density was measured with a photocolormeter. Betacyanine content was estimated by calculating the difference between the absorbance at 542 nm and at 620 nm against the control lacking cytokinins (expressed in percentage).

RESULTS AND DISCUSSION

Fig. 1 shows the level of endogenous gibberellin-like substances in the roots of intact seedlings (A,B,C) and in the roots of seedlings from which the plumule was removed (E,F); Fig. 2 shows the level of endogenous gibberellin-like

substances in the plumule of intact seedlings (A,B,C) and seedlings from which the root was removed (E,F); Fig. 3 shows the content of endogenous cytokinins in the roots of intact seedlings (A,B,C) and roots of seedlings from which the plumule was removed (E,F); Fig. 4 the level of endogenous cytokinin-like substances in the plumule of intact seedlings (A,B,C) and of seedlings from which the root was removed (E,F).

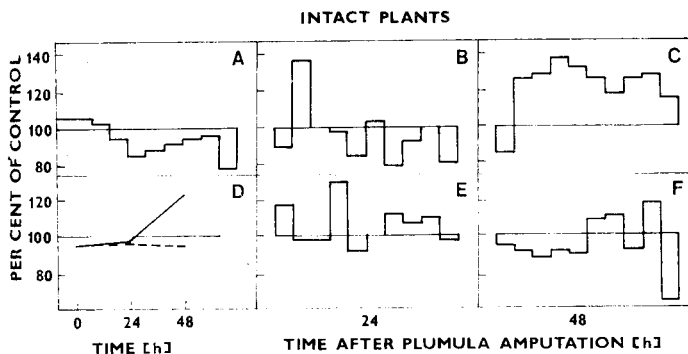


Fig. 1. The level of endogenous gibberellin-like substances in the roots of intact seedlings (A,B,C) and seedlings from which the plumule was amputated (E,F). — D: mean values of individual samples. — Full line: intact plants; dashed line: operated plants

Young root still exerts a regulatory-inhibitory influence on plumule growth in 2- to 7-day-old pea seedlings (DOSTÁL 1941). For this reason, root amputation even in 7-day-old seedlings results in an increase in the level of endogenous gibberellin-like substances in the epicotyl (ŠEBÁNEK 1965). The same effect was also recorded in 2- to 3-day-old seedlings in our experiments. Young root obviously has a high level of inhibitory substances at its disposal (KUNDU and AUDUS 1974, RIVIER *et al.* 1977, KEFELI and KISLIN 1982); thus, after its amputation, the antagonistic influence of these substances, especially of ABA, on endogenous gibberellins stops to operate (NAGY and HODUR 1982). As concerns the endogenous cytokinin-like substances, a de-

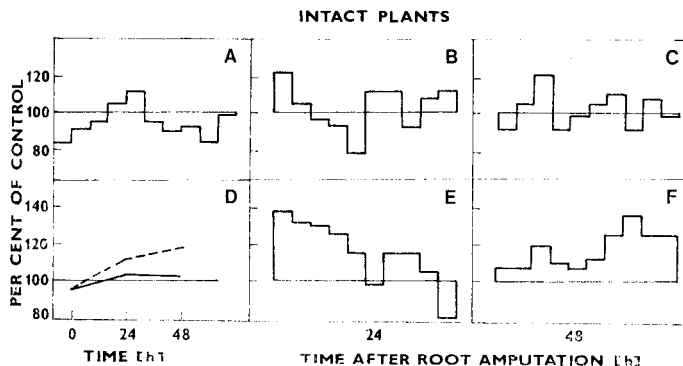


Fig. 2. The level of endogenous gibberellin-like substances in the plumules of intact seedlings (A,B,C) and seedlings from which the root was amputated (E,F). — D: see Fig. 1.

crease in their level within 48 h after root amputation was observed in the plumule in our experiments. The decrease is presumably connected with the fact that the root is from the earliest developmental stages a significant site of cytokinin biosynthesis (WEISS and WAADIA 1965, YOSHIDE *et al.* 1971). A decrease in the level of endogenous cytokinin-like substances was also

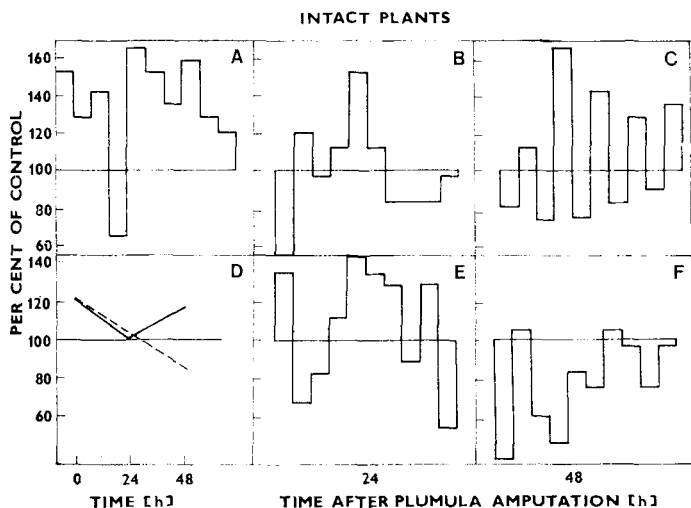


Fig. 3. The level of endogenous cytokinin-like substances in the roots of intact seedlings (A,B,C) and seedlings from which the plumule was amputated (E,F). — D: see Fig. 1.

found in the cotyledons of flax seedlings within 48 h following root amputation (TAN and ŠEBÁNEK 1981).

The growth-correlative effect of the epicotyl on pea root system was first studied in more mature pea plants by DOSTÁL (1941), who recorded disturbances in lateral root initiation when the stem had been removed; these disturbances were prevented by IAA application. Growth-regulatory effects on the initiation and development of lateral roots have recently been studied in 3-day-old pea seedlings especially by WIGHTMAN and THIMANN (1980) and WIGHTMAN *et al.* (1980). They confirmed the inhibitory effect of cytokinins and the stimulatory effect of auxins on the initiation of lateral roots. They also demonstrated that epicotyl amputation resulted in a mild stimulation of primary root elongation growth in 3-day-old pea seedlings. With respect to the inhibitory effect of cytokinins on root growth, this stimulatory effect of epicotyl amputation on root growth might be compared with the positive effect of the plumule on the level of endogenous cytokinins in the root, recorded in our experiments. Our experiments have also shown that plumule amputation from 2- to 3-day-old pea seedlings results not only in a decrease in the level of endogenous cytokinin-like substances but also in a decrease in the level of gibberellin-like substances in the root within 48 h. It is likely that auxin or other active substances necessary for the synthesis of gibberellins in the root which are normally translocated from the stem apex to the root (KIRK and JACOBS 1968, SCOTT 1972, ELLIOT and GREENWOOD 1974, MARTIN *et al.* 1978) are lacking when the plumule is absent.

The decrease in the level of endogenous gibberellins in the root caused by stem amputation could be prevented by IAA application in 10- to 14-day-old pea seedlings (ŠEBÁNEK 1966). It seems likely that auxin from the plumule similarly affects favourably cytokinin biosynthesis in the root. However, the plumule is not the only and exclusive source of IAA involved in growth-

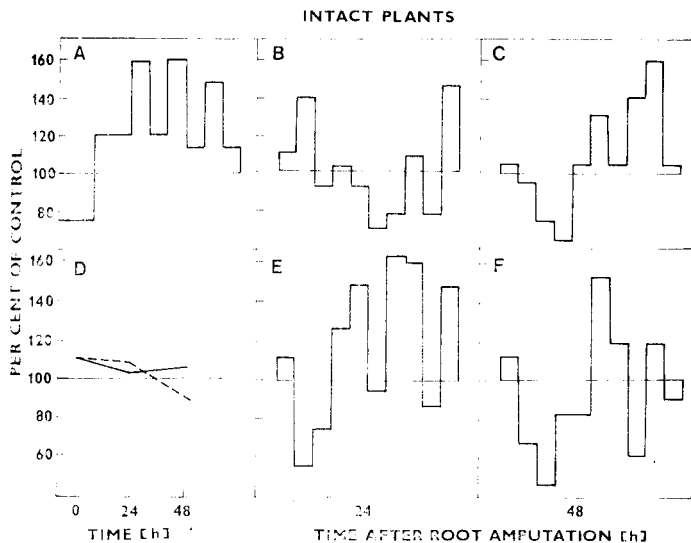


Fig. 4. The level of endogenous cytokinin-like substances in the plumules of intact seedlings (A,B,C) and seedlings from which the root was amputated (E,F). — D: see Fig. 1.

correlative phenomena in pea seedlings, because IAA and other regulatory substances originating in the cotyledons can also take part in these processes; the role of the cotyledons in growth-correlative phenomena was investigated in our earlier experiments (TAN *et al.* 1979, ŠEBÁNEK *et al.* 1982).

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