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The Interaction of Endogenous Gibberellins in Correlation with Cotyledons and Axillary Buds in the Pea (*Pisum sativum* L.)

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Abstract: After the decapitation and amputation of one cotyledon in germinating pea seedlings, the axillary bud of the amputated cotyledon always grows and the growth of the axillary bud of the remaining cotyledon is inhibited. Before morphological differences appear between the axillary bud of the amputated and preserved cotyledon, a higher endogenous gibberellin content can be demonstrated chromatographically in the axillary bud of the amputated cotyledon. This indicates that the increased growth of the axillary bud of the amputated cotyledon is in connection with an earlier increase in the activation of endogenous gibberellins.

Germinating pea seedlings with their hypogeous cotyledons richly supplied with nutrients are a good experimental and morphological object for the study of correlations. The most noteworthy is the correlation between the cotyledon and its axillary bud. If the epicotyl is amputated in germinating pea seedlings cultivated in the dark, both axillary buds usually grow equally, but if one cotyledon is amputated together with the epicotyl, the axillary bud of this cotyledon always grows more rapidly after a certain time (DOSTÁL 1908). Dostál, with great foresight reported this correlation in the paper quoted at the beginning of this century, in connection with the probable existence of inhibitory substances in the cotyledon. The purpose of the present work was to discover if there is a relationship between the inhibitory effect of the cotyledon and the endogenous gibberellin level in the axillary bud of the cotyledon.

Material and Methods

The experiments were carried out with the pea, *Pisum sativum* L. variety Raman. The seeds were soaked in water for 24 hours and then placed radical downwards in moist sawdust for 4 days at laboratory temperature in the dark. They were then transferred to glass flasks filled with mains water and covered

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with a perforated lid through which the roots were placed, and cultivated further in the dark. 5—6 days after soaking the seeds the epicotyl and one cotyledon were amputated. 48—72 hours later the activity of endogenous gibberillins was determined in the axillary bud of the amputated cotyledon and that of the remaining cotyledon, separately. 20 plants in which the axillary buds were still of equal size were selected for the analysis. The axillary buds were carefully excised using a lens and razor blade and then thoroughly homogenized with sand in a mortar and extracted for 24 hours in 25 ml. methanol at 5° C. The extract was then filtered and evaporated to dryness using a water pump at 40° C. The residue was dissolved in 0·5 ml. ethylacetate and separated by thin layer chromatography. Glass plates of 8 × 14 cm. were used. A thin layer of Silikagel G was placed on the plate using the method of Stahl, so that 2 g. of Silikagel mixed with 5 ml. of distilled water was used for one plate. 0·3 ml. extract was placed on each plate at the start in the form of a narrow band 3 cm. long. A drop of the Polish preparation Gibreskol, produced by Polfa in Kutna, production series No. 630511, dissolved in ethanol was used as standard and placed at the edge of the plate. It contains gibberellin A_3 with a small admixture of A_1. The front of the chromatogram was always denoted 10 cm. above the start by a transverse groove. A mixture of chloroform, ethylacetate and glacial acetic acid 80 : 20 : 5 (SEMBDNER et al. 1962) was used as solvent. They were developed in a tightly closed tank for about 15 minutes. The plates were then dried at laboratory temperature in a steam of air for 12 hours. Then the part of the plates corresponding to the extract was divided from the start to the front into 10 bands 1 cm. wide in accordance with the Rf values. These parts of the chromatogram were evaluated biologically in germinating lettuce plants, variety Stupický Kamenáč (FRANKLAND and WAREING 1961 using the modification of KREKULE and TELTSCHEROVÁ). The silikagel was scrapped off from each cm-wide band of the plate using a razor blade, into a small Petri dish with 3 ml. of distilled water. The Silikagel was carefully but thoroughly mixed with the water and then 12 equally sized germinating lettuce seeds were placed in the dish on filter paper. The lettuce were cultivated for 72 hours with continuous fluorescent lamp illumination at laboratory temperature, after which the length of the hypocotyl was measured. Every experiment was repeated a number of times. The part of the chromatogram corresponding to the standard Gibreskol was detected chemically by spraying with a mixture of ethanol and concentrated sulphuric acid 8 : 2 and after drying determining the position of the spot in ultraviolet light. The spot always appeared at Rf 0·1—0·2. Later, preparations of pure gibberellins were used to confirm that the spot corresponding to Gibreskol is concordant with gibberellin A_1 and A_3.

Results

Fig. 1 gives a typical example of the chromatograms obtained. The endogenous gibberellin content in the axillary bud 48 hours after amputation of the cotyledon can be seen in the figure, sub A, 1) for the axillary buds of the remaining cotyledon and sub 1, 2) for the axillary bud of the amputated

cotyledon. The analysis was always repeated 3—4 times. The curves of Fig. 1, however, are typical examples of results obtained and not averages from a number of experiments. This is because a fluctuation between Rf 0·3—1·0 developed during repeated analysis for which no regular trend could be determined. However, the strongly stimulating action of extracts from the

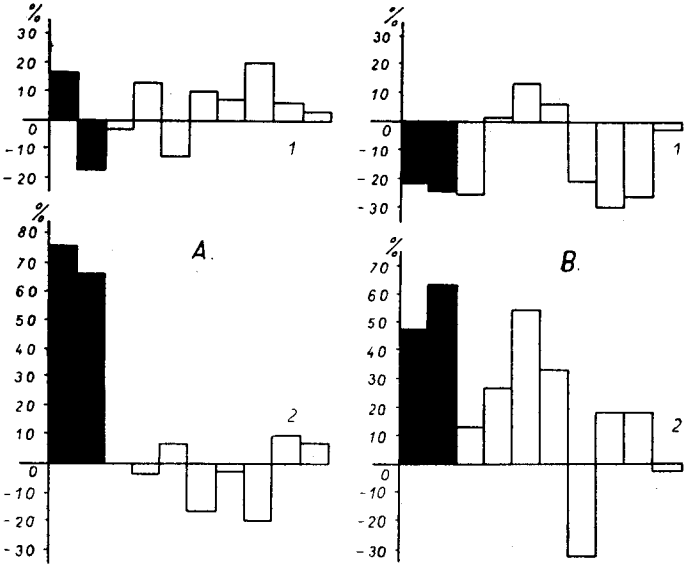

Fig. 1. Examples of separation of endogenous gibberellins from the axillary buds of the cotyledon of the pea on chromatograms. x Rf, y increment in length of hypocotyls of lettuce in %. Control denoted by horizontal line.
A. Analysis of axillary buds 48 hours after amputation of one cotyledon.
B. Analysis of axillary buds 72 hours after amputation of one cotyledon.
1. Bud from axil of remaining cotyledon.
2. Bud from axil of amputated cotyledon.

axillary buds of the amputated cotyledons appearing between Rf 0·1—0·2, i.e. Rf corresponding to gibberellin A_1 and A_3 was always displayed. The extract from the axillary bud of the remaining cotyledon did not show this stimulating effect. The above differences were even greater when the analysis was made 72 hours after the amputation of the cotyledon and shown on Fig. 1. sub B. In this case too striking differences between Rf 0·1—0·2 occurred on repetition. Fluctuation appeared on repetition here between Rf 0·3—1·0 but there was in general a typical increasing trend of inhibition under the influence of the remaining cotyledon.

Discussion

The inhibitory effect of the pea cotyledon on the growth of the axillary bud was first reported in relation to growth substances, when after decapitation of the seedlings both cotyledons were decreased in size to a half by a perpen-

dicular cut and a source of natural auxin, such as synthetic indolylacetic acid placed on the cut surface of one cotyledon (WANKA 1929, PLCH 1936, DOSTÁL 1939). In this case inhibition of the axillary bud of the cotyledon on which indolylacetic acid was placed always occurred. According to this, that growth substance, whose relation to inhibition is indisputable, can imitate the inhibitory effect of the cotyledon. On the other hand, gibberellin applied to the plant externally can weaken this inhibitory effect of the cotyledon. This is suggested by an experiment in which the epicotyl and one cotyledon were amputated and gibberellin paste then painted directly on the petiole of the remaining cotyledon. In this case the inhibitory effect of the cotyledon is weakened, so that in most plants the axillary bud grows in the axil of the remaining cotyledon as well as in that of the amputated cotyledon (DOSTÁL 1960). It is evident that the growth-substance character of the correlation between cotyledons and axillary buds has previously been studied by the exogenous application of growth substances. The results presented in this paper are the first attempt to explain this correlation from the aspect of the endogenous level of growth substances. The author is of the opinion that the determination of this level may contribute to explaining the cause of the correlation between cotyledons and their axillary buds. He starts from the fact that at the time of the analysis of growth substances both axillary buds (of the amputated cotyledon and the remaining cotyledon) are still morphologically the same, since too small a time period had elapsed from the amputation of the cotyledon to the analysis for differences in the cotyledons to be displayed morphologically. However, biochemical processes are taking place in both cotyledons which later always determine the inhibition of one of them. The author is trying to explain these processes. The present work clearly shows that 48 hours after amputation of the cotyledon there is a marked increase in the gibberellin level in the tissue of the axillary bud of the amputated cotyledon.

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J. ŠEBÁNEK, Katedra fyziologie rostlin Vysoké školy zemědělské v Brně: **Interakce endogenních giberelinů v korelaci mezi dělohami a kotyláry u hrachu (*Pisum sativum* L.).** Biol. Plant. **7** : 194—198, 1965.

Po dekapitaci a amputaci jedné dělohy na klíčních rostlinách hrachu vyrůstá vždy axilár amputované dělohy a zadrží růst axiláru dělohy ponechané. Ještě než se objeví morfologický rozdíl mezi kotylárem v úžlabí dělohy ponechané a amputované, již se dá chromatograficky dokázat vyšší obsah endogenních (nativních) giberelinů v kotyláru z úžlabí dělohy amputované. Z toho vysvítá, že silnější růst kotyláru v úžlabí amputované dělohy souvisí s předcházející silnější aktivací nativních giberelinů.

И. ШЕБАНЕК, Кафедра физиологии растений Высшей сельскохозяйственной школы, Брно: **Взаимодействие эндогенных гиббереллинов в корреляции между семядолями и котиледонами у гороха (*Pisum sativum* L.)** — Biol. Plant. **7**: 194—198, 1965.

После декапитации и ампутации одной семядоли на прорастающих растениях гороха всегда прорастает аксиляр ампутированной семядоли и тормозится рост аксиляра оставленной семядоли. Еще до того, как проявится морфологическое различие между аксиляром в пазухе оставленной и ампутированной семядоли, возможно хроматографически доказать повышенное содержание эндогенных (нативных) гиббереллинов в почке из пазухи ампутированной семядоли. Из этого вытекает, что более интенсивный рост почки в пазухе ампутированной семядоли связан с предшествующей более сильной активацией нативных гиббереллинов.