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Changes in Endogenous Level of Auxins and Cytokinins in Axillary Buds of *Pisum sativum* L. in Relation to Apical Dominance

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Abstract. Decapitation of the stem in one-week-old pea seedlings below the first node causes a rapid outgrowth of the two cotyledonary buds. One of them soon becomes dominant, while the other one is inhibited, but can be released from inhibition by cutting off the dominant bud. The level of endogenous auxins and cytokinins was determined in dominant and inhibited buds, as well as in released buds at different time intervals after deinhibition. It was found that the inhibited buds contained very little acidic, ether soluble auxins, a high level of tryptophan and also a high level of cytokinins, in comparison with dominant buds. When the inhibited buds were released from inhibition, their auxin content rose, while that of tryptophan and cytokinins decreased, reaching the level found in dominant buds within six days. Specific changes in content of two undetermined auxin-like substances were found in released buds during de-inhibition. These results are discussed in relation to the current views on the regulation of apical dominance.

Apical dominance is an interesting phenomenon of correlative inhibition, observed long before the plant hormones were discovered. Recent reviews by CHAMPAGNAT (1965), GUERN and USCIATI (1972) and PHILLIPS (1975) have discussed the still controversial state of knowledge in this field. The possible involvement of hormones in the regulation of apical dominance has been extensively studied so far, although the approach has been rather one-sided. In a great number of studies exogenous hormones were applied to intact or decapitated plants and the effects of such treatment were extrapolated to the hormonal relationship in the undisturbed system. Attempts to analyze endogenous hormones in dominant and inhibited buds have been rather infrequent. The current view of auxin — cytokinin antagonism in bud inhibition, which is deduced from hormone application experiments, does not seem to be unequivocally substantiated by the results of analytical work. Based on the fact that lateral buds can be inhibited by apical auxin application (THIMANN and SKOOG 1934), and released by cytokinins (WICKSON and THIMANN 1958), one would expect them to contain more auxins in the inhibited state, and more cytokinins when the apical dominance is disturbed. However, this had not been proved. FERMAN (1938), VAN OVERBEEK (1938)

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and THOMAS (1972) found a very low content of endogenous auxins in inhibited buds. Moreover, inhibited buds in *Xanthium* were shown to be rich in cytokinins (TUCKER and MANSFIELD 1972, 1973). Since pea seedlings have been favourite objects for the study of apical dominance, responding in a clear-cut manner to auxin and cytokinin application, we have tried to study the changes in endogenous hormones in two interdependent axillary buds during the period of deinhibition. Preliminary results have been briefly reported before (JABLANOVIĆ 1969).

Material and Methods

Pea seedlings (*Pisum sativum* L. cv. 'Alaska') were grown in vermiculite at 20—22 °C, in an artificial white light 13 h daily. For each extraction, 100 buds, 1.5—2.0 cm long, were cut off, frozen at —70 °C, homogenized and extracted with cold methanol. The methanol was evaporated off at 35 °C and the water residue was partitioned against ethyl ether at pH 3.0. The ether soluble fraction was further separated into acidic and neutral fractions using a DEAE-cellulose column (BURNETT 1963). Acidic fraction was chromatographed on silica gel H thin layers in isopropanol-methyl acetate-ammonia (35 : 45 : 20, v/v/v) (STAHL 1967) and assayed by the *Avena* first internode test (NITSCH and NITSCH 1956). The water soluble fraction was neutralized, chromatographed on Whatman 3 MM paper in *n*-propanol-water (3 : 1, v/v), the zone corresponding to tryptophan marker spot eluted with methanol and the concentration of tryptophan determined by measuring excitation/fluorescence intensity at 280/360 nm (NEŠKOVIĆ and BURNETT 1969), using an Aminco-Bowman spectrophotofluorometer.

Cytokinins were also measured in methanol extracts. The evaporated extracts were applied to columns of Dowex 50 × 4, 50—100 mesh. The column was washed with distilled water and alcohol and eluted with 5 N NH_4OH, washed subsequently with water, 0.4 N HCl and finally eluted with 6 N HCl (based on ROGOZINSKA *et al.* 1965). Cytokinin activity in the last acidic fraction was negligible. The basic fraction was chromatographed on Whatman 3 MM paper in n-butanol-NH_4OH (4 : 1, v/v) and assayed by chlorophyll retention test of barley leaf sections (KENDE 1964) and tobacco (*Nicotiana tabacum* cv. Wisconsin 38) callus assay (LINSMAIER and SKOOG 1965).

Results

The plants used for experiments were grown for one week under the conditions described, and then decapitated by cutting off the stem between the cotyledons and the first node (first decapitation). After that, two cotyledonary buds start growing rapidly. Usually one of them grows faster, takes over the function of the apical bud, and it is designated further as the dominant bud. The other one is much retarded in growth and is called the inhibited bud. After 7 days the dominant bud is cut off (second decapitation), the smaller bud starts growing faster and is subsequently called the released bud. For the determination of hormones, extracts were obtained from apical buds after the first decapitation, from the dominant and inhibited buds immediately before the second decapitation and from the released buds at different time intervals after the second decapitation.

Data obtained in the oat first internode test and by chromogenic reactions indicated that three auxin-like substances were present in the bud extracts. The water soluble fraction contained tryptophan, identified by chromatography and fluorescence spectra. The content of tryptophan was low in apical and dominant buds, but high in inhibited ones. In released buds the tryptophan content decreased within 6 days to the level found in apical and dominant buds (Fig. 1).

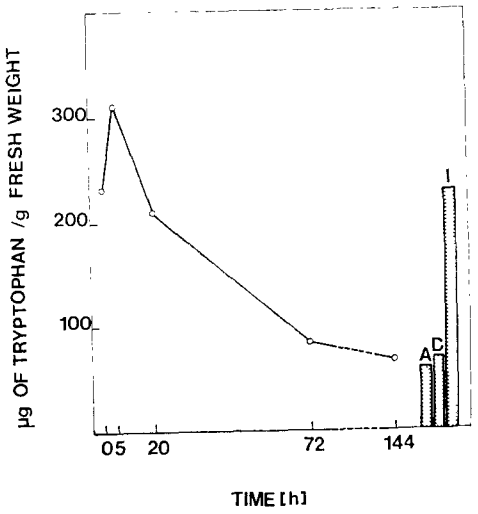

Fig. 1. Changes in tryptophan content in released lateral pea buds; columns at right show the tryptophan content in: A = apical buds of intact seedlings; D = dominant cotyledonary buds; I = inhibited cotyledonary buds.

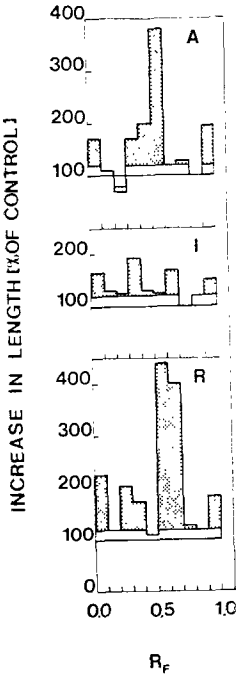

Fig. 2. Histograms of auxin-like activity in oat first internode test of the extracts of pea buds: A = apical buds of intact plants; I = inhibited buds; R = released buds.

The acidic ether soluble fraction contained two active substances in various ratios. They both had activation and fluorescence spectra at 285 and 365 nm respectively, which is characteristic of indole compounds. The total content of ether soluble acidic auxins was high in actively growing apical and lateral dominant buds, whereas the inhibited buds showed only traces of activity in the biological test. In released buds the level of auxin-like substances was increasing along with their growth (Fig. 2). Specific changes were found in two auxin-like compounds, designated substances A (Rf 0.3—0.4) and B (Rf 0.6—0.7). Substance A increased in activity during the first 15 h after bud release from apical dominance, but then decreased again, concomitantly with the rise of activity of substance B (Fig. 3). The presence of substance B is characteristic of dominant and released buds, but not of inhibited ones. In several chromatographic systems the substance B had the same Rf as the IAA marker spot.

According to the results of tobacco callus assay, the level of endogenous cytokinins was relatively high in inhibited buds, and much lower in apical

and dominant buds. These findings were confirmed by the chlorophyll retention test. In released buds the cytokinin activity decreased as a function of time after deinhibition and reached the level found in dominant buds within 6 days (Fig. 4).

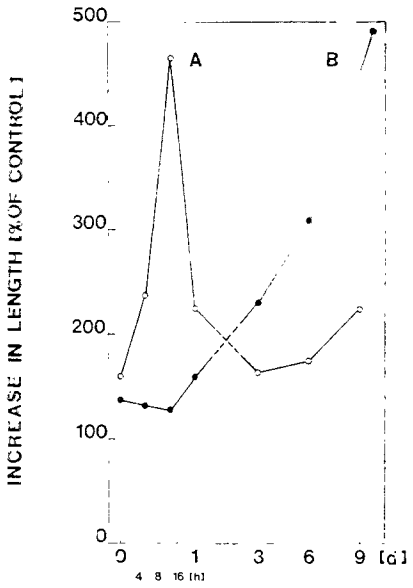

Fig. 3. The biological activity of acidic ether soluble auxins in released pea buds, measured by oat first internode test; A = substance with Rf 0.30—0.40; B = substance with Rf 0.60—0.70; abscissa: time after the second decapitation.

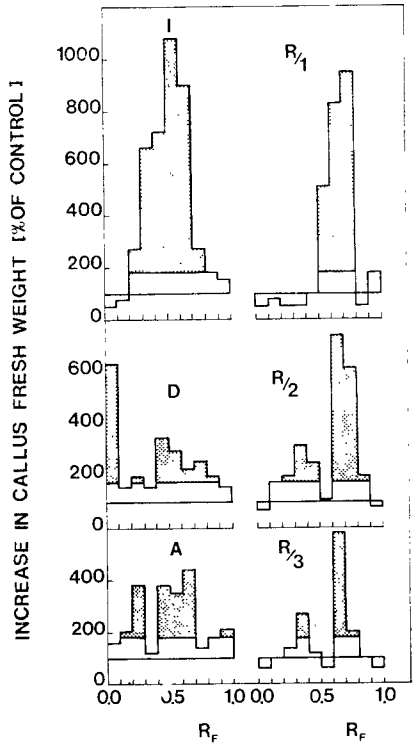

Fig. 4. Biological activity of cytokinins present in the extracts of pea buds, determined by tobacco callus assay; I = inhibited cotyledonary buds; D = dominant cotyledonary buds; A = apical buds of intact seedlings; R/1 = released buds after 1 day; R/2 = released buds after 3 days; R/3 = released buds after 6 days.

Discussion

In attempts to correlate the content of growth substances in plant organs with their growth, one should take initial or final quantitative determination with caution and kinetic study should be performed preferentially. In our experiments, the inhibited buds were shown to have very little auxins and a high content of cytokinins, which is in agreement with the data of other authors, mentioned above. The release of buds from inhibition causes their rapid outgrowth and, at the same time, considerable changes in all growth substances which were assayed. Eventually, after a few days, the released buds become similar to dominant ones, in attaining a high auxin/cytokinin ratio.

The changes in indolic substances during deinhibition seem to be rather specific. The tryptophan content, which is high in inhibited buds, rises after a short time and then gradually decreases. It was calculated from a dose-

response curve that about 250 $\mu g\ g^{-1}$ f.w. may be lost within 6 days. This may probably be explained by the incorporation of tryptophan into new proteins, elaborated in the actively growing buds. This view is supported by the determination of the level of other free aminoacids in the same tissue, which shows a similar trend in quantitative changes, *i.e.* a rise after 5 hours and a decline within 6 days (JABLANOVIĆ 1971). However, it cannot be excluded that some tryptophan may also be converted to IAA. Fig. 3 shows that the content of two ether soluble substances also changes in a way which suggests some direct relationship between them. It looks as if the substance A were the precursor of substance B, although this cannot be confirmed until their structure is known. Substance B, which is the most prominent auxin in the extracts of dominant buds, seems to be identical to IAA, whose presence in pea extracts has been shown before, on the basis of chromatographic and fluorometric data (NEŠKOVIĆ and BURNETT 1969). In all determinations of auxin-like substances, their total content was higher in growing buds than in inhibited ones. Therefore, it does not seem likely that auxins accumulate in inhibited buds and prevent the growth of their meristems. On the contrary, a physiological quantity of auxins seems to be necessary for bud growth. This is also in accordance with the findings of SACHS and THIMANN (1967).

It has recently been suggested that the synthesis of cytokinins in inhibited buds is necessary for their growth after deinhibition (KUNG-WOO *et al.* 1974). However, it follows from our experiments that inhibited buds already contain a relatively high level of cytokinins, and that their content actually decreases when the buds grow out. Since the exogenous application of cytokinins induces the growth of inhibited buds (WICKSON and THIMANN 1958), one may wonder why the endogenous substances fail to do the same. Two possible explanations may perhaps be offered. The cytokinins, which were extracted and found to be active in a tissue culture test, may not be active *in situ*, in stimulating bud growth. They may be conjugated *in vivo* and become activated only during extraction or bioassay. Alternatively, a mediator necessary for growth response may be lacking in inhibited buds. Cytokinin-binding proteins are known to occur in plant tissue (MATTHYSSE and PHILLIPS 1969). It is possible that the primary step in deinhibition is the synthesis of such receptor proteins, which enable endogenous cytokinins to be active. JABLANOVIĆ and NOODÉN (1974) have shown that the capacity for auxin binding in pea bud extracts may be correlated with the physiological state of the buds.

A possible way of interaction between auxins and cytokinins was suggested by SKOOG and ARMSTRONG (1970), who noted that cytokinins may induce the synthesis of auxins in tobacco callus tissue. It is possible that the initial high concentration of cytokinins in pea buds induces the subsequent auxin synthesis, although a direct study of the origin of auxins would be necessary as a confirmation.

Although in this work attention was centered on auxins and cytokinins, the possible interference of other growth substances was not entirely neglected. Thus it was shown that the content of gibberellins increases in released buds along with their growth (JABLANOVIĆ 1971), which is in accordance with the results of ŠEBÁNEK (1965). TUCKER and MANSFIELD (1973) have measured the content of abscisic acid in inhibited *Xanthium* buds and conclu-

ded that this substance may be the active factor of bud inhibition. We were not particularly trying to determine the content of abscisic acid. Nevertheless, the techniques used in our work would have revealed inhibitory activity in the extracts. However, we were not able to notice the significant content or the changes in inhibitory substances, which would point to their involvement in the growth of buds.

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