

MORPHOGENETIC RESPONSE, TRANSLOCATION, AND METABOLISM OF ROOT-APPLIED AUXIN IN PEA SEEDLINGS

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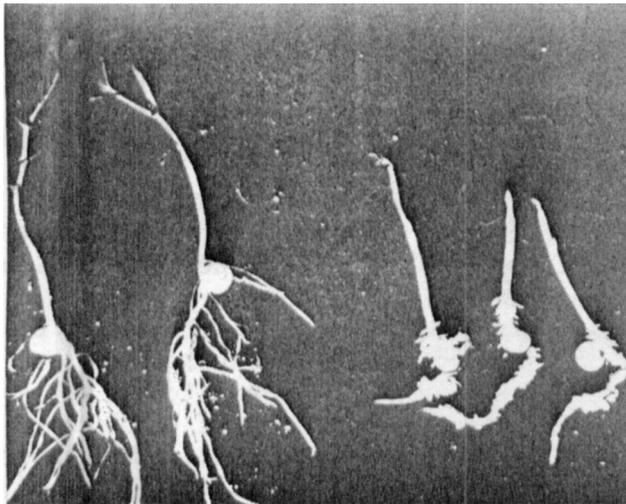
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Gene-dependent expression of phenotypic characters in plants seems to be mediated by the various phytohormones. Since we have only a crude understanding of phytohormone action, model systems have been developed for evaluating their activity in addition to their effects on morphology. For short-term effects, cereal coleoptile served as a model tissue, while the study of long-term biochemical effects was made possible mainly by using soybean hypocotyl or pea epicotyl. At higher concentrations there seems to be no culture system permitting comprehensive studies of the effects of different auxins at the morphological, physiological, and biochemical level. In previous studies we tried to investigate auxin action using *in vitro* systems (1), but for a number of reasons we judged these systems to be unsuitable. So we developed a system using intact pea seedlings and studied the different effects of root-applied auxins (2,4-D, NAA, IAA). In our *in vivo* system we found different morphogenetic responses detectable within one week, providing us with sufficient amounts of tissue for investigation of auxin-protein interaction. It has been suggested that different modes of auxin action in pea are connected with differences in translocation and metabolism of the respective auxin (2). The present paper reports the effects of exogenously applied auxins with respect to their uptake, transport, and metabolism in intact pea seedlings. Concurrently, studies were carried out to detect changes in protein level and to prove the existence of specific auxin-binding proteins (3). Furthermore, the system may allow us to correlate possible different auxin-sensitivities of pea mutants during germination with their genetic background, thus obtaining some information, on the physiological level, about the particular mutational event.

One-week-old etiolated pea seedlings grown on moist vermiculite were carefully washed and placed on plastic grids in glass vessels. This arrangement allows controlled uptake of the auxins by roots of intact seedlings (10 ml/seedling of  $10^{-4}$ M water solution of unlabeled auxin with  $3 \times 10^5$  dpm/ml labeled 2,4-D and NAA, and about  $1 \times 10^5$  dpm/ml labeled IAA). After 24 h -pulse of auxin in the dark, treated seedlings were placed on moist vermiculite under a light/dark cycle of 16/8 h (2500 lux) for one week. Uptake of the auxins was recorded by measuring loss of radioactivity (i.e. dpm or decompositions per minute) in the medium. The distribution of the auxins in the seedling was determined after differential extraction from organs or segments and by autoradiography of the dried seedlings with X-ray film. Ethanol soluble extracts were analyzed by paper chromatography and on cellulose thin layer aluminium sheets using different solvent systems.

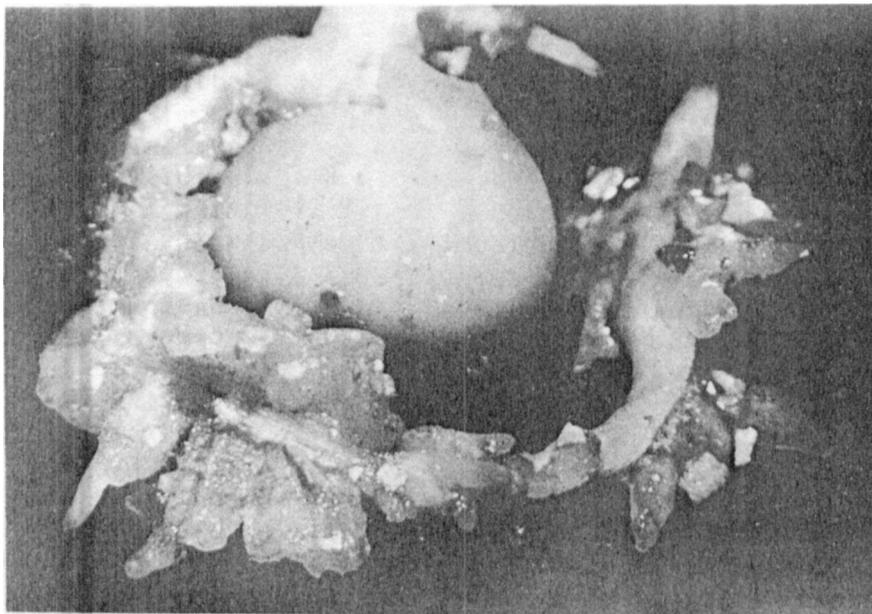
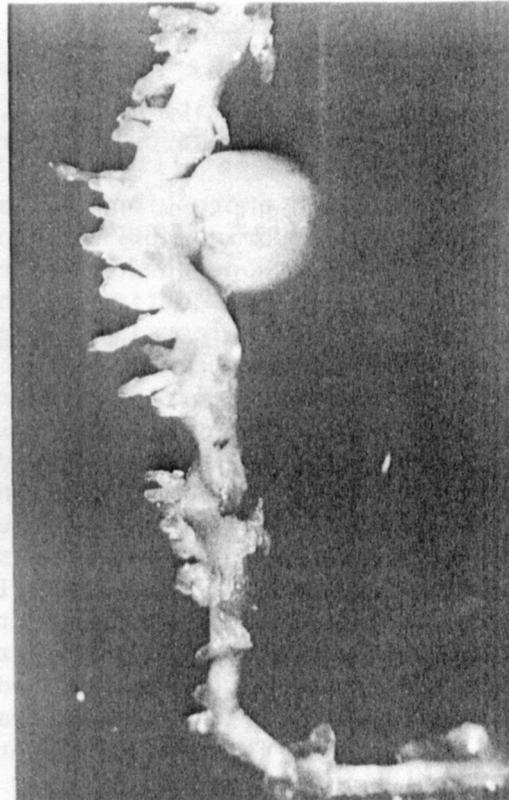
Morphogenetic response: 2,4-D ( $10^{-4}$ M) inhibited natural differentiation of pea seedlings treated with a 24 h -pulse by inducing abnormal growth in root and shoot within one week (Figs. 1-4). NAA and IAA did not inhibit natural differentiation of the seedlings under the same conditions ( $10^{-4}$ M) and the seedlings are comparable to the untreated control although a slight stimulation of normal root formation was observed. This raises the question: What are the physiological reasons for these different morphogenetic responses?

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1. Effect of root-applied 2,4-D on pea seedlings : Inhibition of natural differentiation within one week (untreated seedlings on the left).

2. Induction of abnormal adventitious roots and lateral roots after 2,4-D treatment (on the right).



3. Induction of abnormal lateral root formation and callus-like structures after 2,4-D-treatment (left).



4. Effect of root-applied 2,4-D: radial expansion of stem tissue.

The physiological results obtained in this system are summarized in Table 1. In contrast to NAA and IAA, 2,4-D is characterized by a high mobility and a low degradation in the intact plant. Uptake of 2,4-D by the roots was about 6% after the 24 h-pulse, for NAA about 10%, and for IAA about 70% as measured by loss of activity in the medium. In the case of 2,4-D, following the pulse, radioactivity was accumulated in the root and during the following culture period was transported into the shoot by the transpiration stream as shown by differential extraction of segments and by autoradiographs. The  $^{14}\text{C}$ -activity of 2,4-D seems to be polar, having accumulated in the tip regions of the lateral and adventitious roots, but being diffusely distributed in stem and primary root tissue of the seedlings. There is no clear evidence for degradation of 2,4-D. On the other hand NAA and IAA were characterized by low mobility and high metabolic susceptibility of their molecules. After one week most of the  $^{14}\text{C}$ -activity from IAA or NAA still is fixed in the primary root. Degradation or amino acid conjugation were detectable already after the 24 h-pulse. The amount of free IAA is highly reduced by oxidation and amino acid conjugation, while NAA seems to be mainly immobilized by conjugation. These results confirm earlier investigations (2, 4) and indicate that the herbicidal effects of 2,4-D in pea depend on the endogenously available amount of free unmetabolized auxin.

Table 1. General aspects to translocation and metabolism of root-applied auxin ( $10^{-4}\text{M}$ ) in pea seedlings.

Auxin (root-applied $10^{-4}\text{M}$ )	Uptake	Metabolism			Mobility
		Oxidation	Conjugation	Free auxin	
2,4-D	low	+	-	+++	high
IAA	very high	+++	+	±	low
NAA	low	-	+	+	low

The data clearly demonstrate the advantages of the 2,4-D system used in our investigations for studies of auxin-protein interactions in the higher concentration range, 1) because of its pronounced morphogenetic response, and 2) because of its less complex metabolic susceptibility.

Moreover, there are other methodological reasons favoring this culture system: 1) The root-application of auxins has some advantages compared with foliar-application often used. It allows easy control of uptake and calculation of accumulated auxin in the plants, and 2) last but not least this culture system permits auxin action to be studied on the intact plant level, thereby providing advantages over in vitro systems. Therefore, we think that the 2,4-D system is suitable for biochemical studies of auxin effects in the herbicidal range. It was useful in investigating levels of response of cytoplasmatic and nuclear proteins and auxin-binding proteins. We assume that auxin-mediating proteins could play an important role in long-term morphogenetic effects as demonstrated in the 2,4-D system.

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