

MORPHOGENETIC EFFECTS. AUXIN UPTAKE AND METABOLISM OF SOME PEA VARIETIES AFTER ROOT APPLICATION OF AUXINS

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Previously we proposed a system to test the effects of root applied auxins on morphogenesis, auxin uptake, and metabolism of young pea seedlings (1,2). The present paper summarizes the results of experiments with six varieties of peas using the described test system (Table 1). The varieties differed in seed color and weight and in epicotyl length under uniform cultural conditions. Our hope was to find some pea varieties that showed differences in response to root application of auxins.

One-week-old pea seedlings were treated with the auxins 2,4-D, 2,4,5-T, NAA, and IAA (10^{-4} M, 14 C-labeled) for 24 hours via the roots (10 ml medium per seedling). The loss of radioactivity in the medium was measured and ethanolic extracts from the root tissue were analyzed by thin layer chromatography (TLC). These experiments showed no significant differences in uptake behavior, auxin metabolism, or morphogenetic response among the varieties tested. All varieties showed the following uptake behavior: The 2,4-D and 2,4,5-T uptake was very low (about 10%), the uptake of NAA and IAA was very great (more than 30, 40%, respectively) under these conditions. The TLC-analysis of ethanolic root extracts by autoradiography and the liquid scintillation count data were in accord with the well-known explanation for this uptake behavior: The 2,4-D and the 2,1,5-T radioactivity is only represented by the spot of the free auxin. However, because the NAA and IAA form conjugation products with aspartic acid the radioactivity appears in two spots, viz. the free auxin and amino acid-conjugation products (NAA). Little difference in this reaction pattern was found among the varieties.

The morphogenetic response among varieties was also similar: The 2,4-D- and 2,4,5-T-treated seedlings were strongly inhibited in their light dependent morphogenesis (tissue swelling, irregular lateral roots, callus structures), while the NAA- or IAA-treated seedlings were only slightly affected due to the metabolic inactivation of high amounts of the free auxin. The results are summarized in Table 1.

These preliminary investigations confirm the view that the genes which are involved in regulation of the endogenous auxin content (for example for conjugating enzymes) seem to be very conservative within the species. Further tests with other Pisum stocks are necessary to find auxin-sensitive mutants, which differ in their response to auxins.

Table 1. Responses of different genotypes to root applied auxins

Lines tested	Auxin effects		
	Uptake	Metabolism	Morphogenesis
var. <u>pseudoroeseum</u> 'Kleine Rheinlanderin'	2,4-D >2,4,5-T	Free auxin	Inhibited, abnormal
'Dippes Gelbe Viktoria' var. <u>nigro-violaceum</u>	>NAA >IAA		
var. <u>hibernieum</u> ssp. <u>arvense</u>		Amino acid conjugates	Similar to control

1. Ingensiep, H. W., M. Hertl, and H. L. Jacobsen. 1981. PNL 13:21-23.
2. Ingensiep, H. W. 1982. PNL 14:19-20.

A TEST SYSTEM FOR SHOOT APPLICATION OF AUXINS COMBINED WITH PHOSPHOLIPIDS USING SEEDLINGS OF PISUM SATIVUM

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A test system is described which allows us to characterize the auxin-sensitivity and effects of phospholipids in auxin solutions following application to the shoots of young pea seedlings. Co-applied phospholipids may be useful to reduce the amount of herbicides as has been demonstrated previously in field experiments by Maas (1). Simple test systems as introduced here may be a practical means of screening for substances which reduce the amount of auxin-herbicides or of identifying mutants of Pisum with abnormal auxin-sensitivity.

The test system is schematically summarized in Fig. 1. One-week-old etiolated seedlings cultivated on moist vermiculite were further cultivated for two days under a light/dark rhythm of 16 hr/8 hr to induce light dependent morphogenesis. Then the auxins were applied to the shoots by dipping the top of the epicotyl in aqueous auxin solutions (10^{-4} M) with and without phospholipids (0.02%). As typical auxin-herbicides we used 14 C-labelled 2,4-D, 2,4,5-T, and MCPA. After this treatment the seedlings were again cultivated on moist vermiculite and the uptake or radioactivity was measured during the following 24 hours. At each measuring point the seedlings were dipped in ethanol to remove the surface-associated auxin and after that the shoot tissue was extracted by an ethanol procedure. Auxin-radioactivity on the shoot and in the shoot were obtained and measured by a liquid scintillation counter. The distribution of radioactivity one week after the shoot application was also measured by autoradiography of the whole seedlings on X-ray films.

These experiments showed that the presence of phospholipids in the aqueous auxin solutions enhanced the detectable auxin-radioactivity on the shoot surface up to three times and in the shoot tissue up to two times compared with solutions containing only the auxins. Moreover, the analysis by autoradiography showed broader spots of radioactivity on the leaves if phospholipids were present in the solutions. Also the typical