A SIMPLE ASSAY FOR GENOTYPE-DEPENDENT AUXIN-SENSITIVITY WITH YOUNG SEEDLINGS OF PISUM SATIVUM

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Based on earlier investigations and considerations about auxin-dependent morphogenetic response of pea seedlings (1, 2), a simple assay has been developed for pre-screening auxin-sensitive genotypes in the young seedling stage using 2,4-D, NAA, and IAA. The idea was to try to correlate morphogenetic differences (from the initial line) with some alteration(s) in the endogeneous auxin control system of the plants. The main regulation points for the latter may be on the level of auxin synthesis, auxin reception or auxin inactivation. The irreversible degradation of auxin by oxidation and the possible reversible conjugation to amino acids seem especially important to establish endogenous gradients of the free auxin controlling shoot eleongation or axillary bud elongation. Therefore seedlings of the initial line (IL), 'Dippes Gelbe Viktoria', were compared with a recombinant (R 650 A) which contains genes for long internodes, afila, fasciation, and extremely late flowering in an assay system which may give some hint if these deviations are due to some alteration in genes involved in the system of auxin inactivation.

Seedlings of IL and R 650 A grown in moist vermiculite for about one week (light/dark=16/8 hr, 25C, 50% RH) were decapitated 1 cm above the second node and prepared pieces of chromatography paper,dipped in ethanolic solutions of the auxins up to 10^{-2}M, were inserted at the top. After 24 hr the pieces were removed and the seedlings were observed during the following period of cultivation under the same conditions.

Fig. 1 shows the typical morphogenetic reaction pattern of IL seedlings in this assay after apical application of 2,4-1) with increasing amount. 1) Apical shoot tissue swelled and showed callus structures with increasing concentrations of 2,4-1) up to 10^{-2} M. 2) Axillary buds at the second node were inhibited in elongation and often swollen with increasing amounts of 2,4-D which is in contrast to the control where these buds elongated and were the dominant regenerating shoots, **3)** Axillary buds at the first node showed a maximum elongation at about 10^{-3} M, but were inhibited at higher concentrations. The IL control normally shows no bud elongation growth at the first node. At the concentration of 10^{-2} M 2,4-D a characteristic morphogenetic reaction pattern was evident which is similar to the well known "herbicida1" morphogenetic response.

Now, the other auxins, NAA and LAA, were compared to 2,4-D and the control at these higher concentrations (10^{-2} M) using seedlings of IL (Fig. 2, below). 1) The NAA- and IAA-treated seedlings did not show the drastic tissue proliferation in the apical region that 2,4-D did. 2) Axillary buds at the second node were more inhibited in elongation compared to the control, but less inhibited compared to the 2,4-D seedlings. Lowest inhibition was observable after IAA-treatment, 3**)** Axillary bud elongation in the first node was inhibited similar to the control in the case of IAA and a stimulation of bud elongation was observed after NAA—treatment as in the case of 2,4—D.

[1/] This paper is dedicated to Prof. Dr. W. Gottschalk, who kindly supported my scientific work over the past years at our Institute.

This mode of different: morphogenetic response of IL shoots to the three auxins seems to depend on the different ways of inactivation of each auxin as has been shown for roots earlier (1): IAA is mainly inactivated by oxidation (irreversible) and conjugation to aspartic acid (possibly reversible), NAA by conjugation to amino acids, and 2,4-D is only minimally affected by both, i.e. it is very stable in its free active form. More interesting is the morphogenetic reaction pattern of the recombinant R 650 A, where two main points deserve emphasis (Fig. 2, upper part): 1) The control showed stimulated elongation of both axillary buds. 2) IAA-treated seedlings showed a strict inhibition of both axillary buds within the first week after decapitation and auxin applications. Although these are the main differences in the morphogenetic reaction pattern between IL and R 650 A, the 2,4-D and the NAA-effects seem also to be stronger than in the IL.

Interpretation of these results is very difficult. Obviously this recombinant is more sensitive to exogenous auxin than the initial line. One explanation could be that it is less able to inactivate the exogenous auxin, but it cannot clearly be decided it genes governing the oxidation or the conjugation system are involved. In the case of a negative mutation in a gene controlling the oxidation system (for instance, for the degrading enzyme or enzymes that produce Inhibitor substances) the resulting effect of a higher amount of free active auxin within the shoots could lead to enhanced bud inhibition as found here for the recombinant. On the other hand, the 2,4-D and especially the NAA-treated recombinants show more inhibition of bud growth compared to the initial line. This would imply mutations in genes which have something to do with the conjugating system.

Another point is that in these early seedling stages the recombinant shows two remarkable morphogenetic effects which may have something to do with their endogenous auxin level: the double bud stimulation after decapitation and the long internodes (up to 3 times longer than in the IL). The double bud stimulation points to a deviation in the endogenous auxin gradient. While normally the buds at the first and the second node can distinguish between different levels of the auxin along the axis, which leads to a dominance of the second node bud, the recombinant is obviously not able to distinguish clearly between the two node buds. This suggests that in systems normally establishing these endogenous auxin gradient something is altered. Also the long internodes of the recombinant could be explainable by a defect in the endogenous auxin inactivation system, leading to higher amounts of endogenous auxin and therefore to a striking stimulation of elongation growth in the internodes. Further investigations are required before these findings can satisfactorily be interpreted.

1. Ingensiep, H. W. PNL 13:21-23.
2. Ingensiep, H. W. PNL 14:19-20.

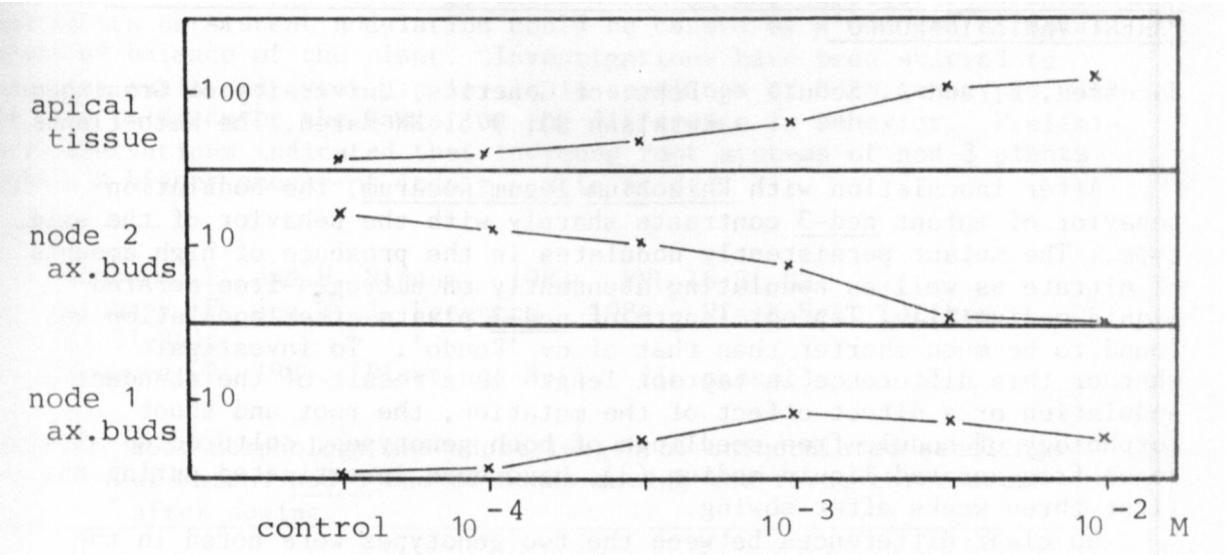

Fig. 1. Growth of apical tissue and axillary buds at the first and second node of decapitated pea seedlings (IL) two weeks after apical application of 2,4-D 10^{-4} - 10^{-2} M. Apical tissue in mg weight of the tissue above the second node. Elongated shoot buds at node 1 and 2 in cm length.

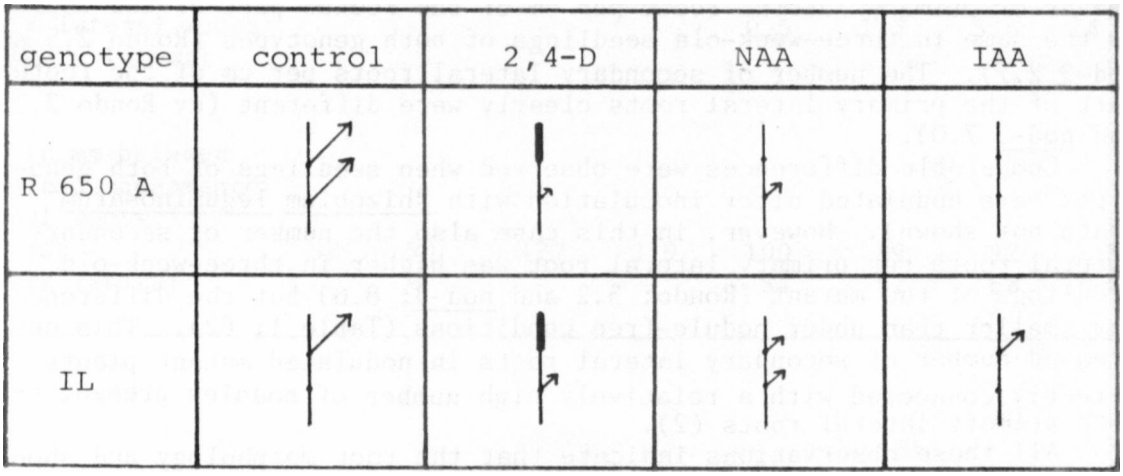

Fig. 2. Morphogenetic reaction pattern of pea seedlings (IL and R 650 A) one week after decapitation and application of the auxins 2,4-D, NAA, and IAA (10^{-2}M) to the apex. The arrows indicate the degree of bud elongation at the first and second node. The thick lines indicate strong tissue swelling and callus structures.