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Relationships between Nodulation and Auxin Level in Pea Roots

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Abstract. In the roots of 4-day-old pea plants germinated in unsterile soil from *Rhizobium*-inoculated seeds, a higher level of native IAA was determined than in roots of pea plants germinated in sterile soil from superficially sterilized seeds. The IAA level in plants grown from inoculated seeds increased markedly up to the age of 6 days of the plant, while in plants growing under sterile conditions it did not significantly change during the same period. Between the 6th and 10th days of the age of the plant, a decline in the IAA level was observed in roots of plants growing from inoculated seeds. It was not until after 10 days of age of the plant that the level of IAA in nodulated roots again increased.

THIMANN (1936) was the first author to draw attention to the fact that root nodules in legumes contain more auxin than the normal root tissue. The theory that the nodule arises as a direct result of the production of auxin by *Rhizobium* within the infected root was supported by the fact that nodules of a variety of plants (including the non-legumes) contain relatively large amounts of auxin (THIMANN 1939). Later, there was much speculation that IAA might play a role at various stages in the symbiotic relationship between *Rhizobium* bacteria and leguminous plants (DART 1977, NEWCOMB 1980). In particular, it has been postulated that IAA is the causal agent of root hair curling (FÅHRAEUS and LIUNGGREN 1968).

IAA has been tentatively identified in culture supernatants of *Rhizobium* strains grown in the presence of tryptophan (HARTMANN and GLOMBITZA 1967, RIGAUD 1970a,b, BADENOCH-JONES *et al.* 1982) and in root growth medium of plants inoculated with *Rhizobium* (KEFFORD *et al.* 1960). DANGAR and BASU (1984a,b, 1985, 1987) studied the level of IAA in nodules in relation to the seasonal variation and the nodule age.

However, there are no studies dealing with the level of native IAA in roots at the time preceding nodule formation. Therefore, the aim of the present study was to gather some new information about the endogenous level of auxin (IAA) in pea roots before and after nodules were visible.

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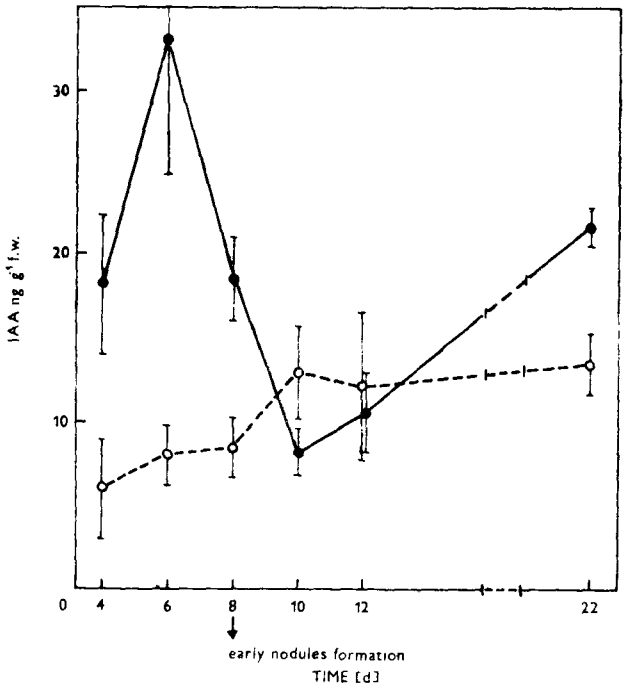
MATERIAL AND METHODS

Pea (*Pisum sativum* L. cv. Smaragd) seeds were planted into sterilized soil (Var. 1) and unsterilized soil (Var. 2). Experiments were carried out in a greenhouse in pots (30 cm in diameter). Fertile garden soil which was used in these experiments was divided into two groups.

The first group was autoclaved twice for 3 h under two atmospheric pressures in bags and the seeds of this part were superficially sterilized in 70 % ethanol for 5 min and then washed three times with sterilized water; they were then soaked in sterilized water before cultivation. The pots of this part of sterilized soil were also sterilized with 1 % formaldehyde solution and then were left for 15 days before cultivation.

The second group of soil was not sterilized (and neither were the pots and seeds). After soaking in water for 24 h, the seeds were inoculated with a liquid medium of *Rhizobium leguminosarum* (5 ml/20 seeds) immediately before cultivation. The level of endogenous IAA and/or natural auxin-like substances was determined fluorimetrically (MOUSDALE *et al.* 1978) or by bioassay (SIROIS 1966). Root samples were taken at plant age of 4, 6, 8, 10, 12 and 22 days for both fluorimetry and bioassay determinations.

The experiment was repeated three times and the fluorimetrical results were evaluated statistically. In the case of the bioassay the results shown here represent the first replication, the other two being analogous.



. ı ree AA measured in pea roots by the pyrone fluorometric assay. Plants grown under sterilized condition s (non-nodulated, ○), and under unsterilized conditions (nodulated, ●).

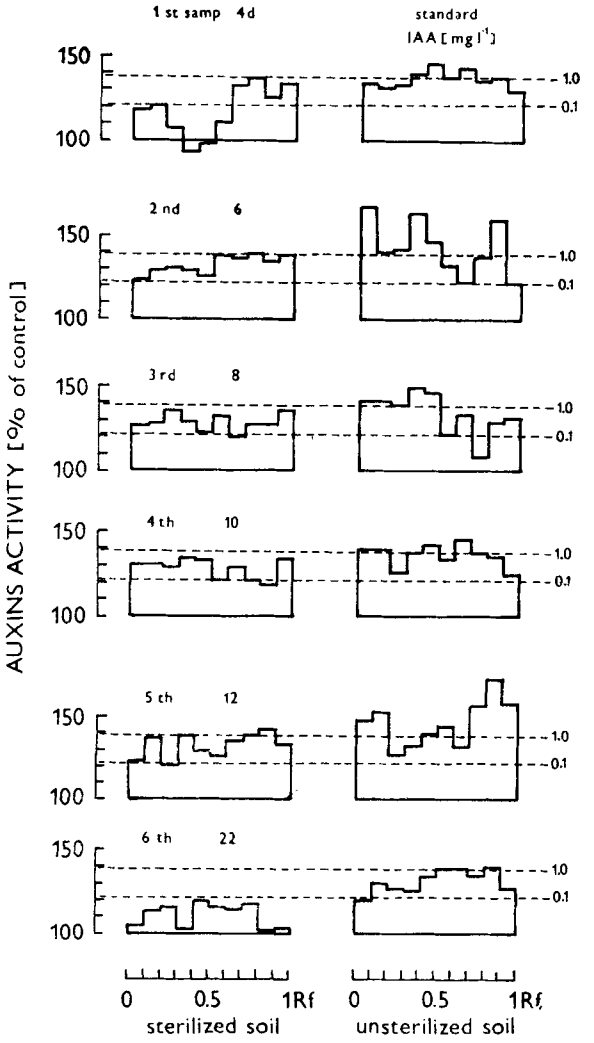


Fig. 2. The estimation of endogenous auxins in pea roots cultivated in sterilized and unsterilized soil. — Ordinate: coleoptile segments [%] in comparison with the control in the wheat (*Triticum*) coleoptile segment straight growth bioassay. — Abscissa: Rf values.

RESULTS AND DISCUSSION

In the roots of 4-day-old pea plants germinated from *Rhizobium*—inoculated seeds in unsterile soil a higher level of native IAA was determined than in roots of pea plants germinated in sterile soil from superficially sterilized seeds (Fig. 1). The IAA level in plants grown from inoculated seeds was found to increase markedly up to the age of 6 days of the plant, while in plants growing under sterile conditions it did not significantly change during the same period. Hence, the preparation of nodule initiation on pea roots is associated with a marked increase in the level of native IAA in the roots. Since the first nodules on roots of plants grown from the inoculated seeds were not visible until the 8th day of age of the plants, it is apparent that from the age of 4—6 days the preparation of nodule initiation is associated with a marked increase in the level of native IAA. After the 6th day of age of plants grown from the inoculated seeds under unsterile conditions,

the level of IAA in the roots of these plants declined. Since the level of auxin in an organ reflects the balance between the rates of its synthesis, degradation, conversion to conjugates and transport into and out of the organ, the decline in the IAA level between the 6th and 10th days of the plant age is probably associated with a consumption or conjugation of IAA necessary for nodule formation.

All the previous studies resulted in a conclusive identification of IAA in culture supernatants of several *Rhizobium* strains (*e.g.* BADENOCH-JONES *et al.* 1982) or confirmed the fact that nodules of a variety of plants (including the non-legumes) contain higher levels of auxin than the normal tissue of the root (*e.g.* THIMANN 1939). Only KEFFORD *et al.* (1960) reported that when clover (*Trifolium subterraneum* L.) was grown in distilled water for 3 weeks, tryptophan was detected in the root medium of both sterile cultures and in those inoculated with *Rhizobium trifolii* three days earlier. However, auxin with chromatographic properties of IAA was detected only in the inoculated medium suggesting that tryptophan exuded by clover roots was converted to IAA by nodule bacteria. It was not until after 10 days of the age of the plant than an increase in the level of IAA in the roots could be observed. This increase in the level of IAA between the 10th and 22nd day of age of the plants is probably associated with a further wave of nodulation which is typical of most leguminous plants.

The presence of IAA in pea root nodules was also unequivocally established by Gc-Ms (BADENOCH-JONES *et al.* 1984) either largely on chromatographic properties, bioassay and chromogenic reaction (THIMANN 1936, DANGAR and BASU 1984a,b, 1985) or on spectrofluorimetry (DULLAART 1967, 1968, 1970a,b, DANGAR and BASU 1984a, 1987). The results obtained by bioassay were analogous with results obtained fluorimetrically. The only difference was that in roots of 10-day-old plants grown from *Rhizobium*-inoculated seeds a decrease in the level of native IAA was observed when using the fluorimetrical method, while no evident decrease was found when using the bioassay.

This difference is apparently associated with the fact that the bioassay responds to the whole auxin activity, while fluorimetry responds to free IAA only. Therefore, in bioassays in all the time determinations a higher level of auxin was recorded in roots of plants grown from inoculated seeds (Fig. 2).

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