THE INDOLEACETIC ACID CONTENT OF ROOT NODULES AND ROOTS OF CYCAS CIRCINALIS L. WITH REGARD TO OTHER ROOT-NODULE SYSTEMS

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SUMMARY

The indoleacetic acid contents of root and root-nodule tissue of *Cycas circinalis* L. were estimated quantitatively by spectrofluorimetry. The quantities were rather low and did not differ significantly. The results are discussed with reference to other root-nodule systems.

1. INTRODUCTION

From the start of research on growth substances, these compounds and especially auxins, have been thought to have something to do with the morphogenesis of the nitrogen-fixing root nodules of the leguminous as well as a number of non-leguminous species. The auxin with the widest distribution in the plant kingdom, indoleacetic acid, deserves special attention.

In connection with a comparative investigation on the IAA content of root nodules and roots of the legume *Lupinus luteus* L. (DULLAART 1967) and of the non-legume *Alnus glutinosa* (L.) Vill. (DULLAART, in preparation), it seemed interesting to estimate the amounts of IAA in nitrogen fixing root-nodules and roots of a third object.

2. MATERIAL

Cycas circinalis L. was chosen for this purpose. The nodules of this plant have blue-green algae as endophytes, although nodules without these symbionts also occur. According to WITTMANN *c.s.* (1965) and SCHAEDE (1944), it seems unlikely that the algal endophytes are responsible for the formation of the nodules; instead, during the normal development of the plant deformed, coralloid roots are formed, and later become infected with the blue-green algae. These root nodules – coralloid roots – show a negative geotropic growth pattern.

Since *Cycadaceae* are tropical or subtropical plants, material was obtained from the glass-house of the Leiden Botanic Garden, where the plant from which the nodule and root material was taken shows healthy growth.

3. METHODS, RESULTS AND DISCUSSION

A piece of a very compact nodule-cluster (weighing about 1 kg) was cut out, and a number of freshly formed, young roots were gathered. The material was

thoroughly washed with water and then lyophilized. After a standard cold methanol extraction procedure followed by the isolation of the acid ether-soluble fraction, a two dimensional thin-layer chromatography technique was used for identification. The resulting chromatograms of samples of the extracts showed no spot of IAA for either the root extract or the nodule extract. Only a weak spot of indolecarboxylic acid was detected in the chromatogram of the nodule extract.

Although this method is rather sensitive, a further attempt was made to detect IAA in these extracts by use of the very sensitive spectrofluorimetric method and to measure the IAA amounts quantitatively. For separation and purification, a standard double paper chromatography technique was used.

The eluates from the chromatogram regions corresponding to the IAA reference spot showed a weak but significant fluorescence with the same characteristics as those of pure IAA solutions: excitation maximum at 290 nm and fluorescence maximum at 348 nm. The fluorescence at these maxima was measured, and by interpolation in a calibration curve for IAA, the IAA content of the tissues was calculated with the measured correction factor for the loss of IAA during the total procedure, giving for the nodule tissue: 20–50 μg IAA/kg fresh weight, and for the root tissue: 20–40 μg IAA/kg fresh weight.

A detailed description of the methods used in this study has been given elsewhere (DULLAART 1967).

The IAA content of these tissues is very low, and it seemed rather surprising that no significant difference between the IAA content of roots and nodules was found. Tested in an *Avena* straight growth assay, the paper chromatograms of the acid fraction of extracts of portions of these tissues equivalent to 10 g fresh weight gave no significant growth-stimulating response ascribable to IAA; furthermore, no other growth-stimulating substances could be demonstrated. This is quite different from the situation in leguminous and *Alnus* species, where more IAA is found in the nodules than in the roots (PATE 1958; DULLAART 1967; DULLAART, in preparation). SILVER *c.s.* (1966) found no detectable auxin in nodule roots of *Myrica* and *Casuarina*, which show a negative geotropy, whereas non-nodulated roots of these species show a normal geotropy and have an auxin content within an anticipated range of 10 mg/kg fresh weight. They suggested a correlation between the geotropic behaviour and the IAA content of these tissues.

The lobes of the *Cycas* nodule cluster, part of which was extracted, grow upwards and thus show a negative geotropy, but there is no significant difference between the rather low IAA contents of the nodule and root tissues. Consequently, the auxin situation in the *Cycas* material differs fundamentally from that in the other cases. This difference can probably be explained by the fact that the initiation of the *Cycas* nodules occurs spontaneously, and the nodules are invaded by the blue-green algae later on. In leguminous plants and some non-leguminous plants such as *Alnus*, to the contrary, the infection by the symbiont is the primary cause of the development of the nodules. Therefore, it seems justifiable to conclude from the foregoing evidence that although the nodule

structure of *Cycas* superficially resembles that of, for example, *Alnus* nodule clusters, they are not identical. The difference in the auxin situation can be considered to provide support for this opinion.

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REFERENCES

DULLAART, J. (1967): Quantitative estimation of indoleacetic acid and indolecarboxylic acid in root nodules and roots of Lupinus luteus L. *Acta Bot. Neerl.* **16**: 222–230.

PATE, J. S. (1958): Studies on the growth substances of legume nodules using paper chromatography. *Austr. J. Biol. Sci.* **11**: 516–528.

SCHAEDE, R. (1944): Ueber die Korallenwurzeln der Cycadeen und ihre Symbiose. *Planta* (Berlin) **34**: 98–124.

SILVER, W. S., F. E. BENDANA & R. D. POWELL (1966): Root nodule symbiosis II. The relation of auxin to root geotropism in roots and root nodules of non-legumes. *Physiol. Plantarum* **19**: 207–218.

WITTMANN, W., F. J. BERGERSEN & G. S. KENNEDY (1965): The coralloid roots of Macrozamia communis L. Johnson. *Austr. J. Biol. Sci.* **18**: 1129–1134.