

THE LIBERATION OF INHIBITORS OF INDOLEACETIC ACID OXIDASE ACTIVITY OUT OF COLEUS INTERNODES TREATED WITH POTASSIUM HYDROXIDE OR SULPHURIC ACID

R. SOEKARJO and M. G. H. JANSSEN

Botanisch Laboratorium, Utrecht

INTRODUCTION

The presence of indoleacetic acid as the natural growth substance in *Coleus* has been demonstrated by SCOTT & JACOBS (1964), whereas indoleacetic acid oxidase activity of preparations of *Coleus* has been reported by KAMINSKI (1966).

In earlier experiments (SOEKARJO 1965), it was shown that treatment of cuttings of *Coleus* with potassium hydroxide or sulphuric acid results in a stimulation of the formation of adventitious roots. It seemed possible that the mechanism by which these treatments caused the enhanced formation of adventitious roots depended upon interference with the concentration of endogenous auxin. Therefore, we investigated the effect of the diffusate of treated internodes on the activity of indoleacetic acid oxidase.

MATERIAL AND METHODS

Internodes number 4 and 5 (counted from the apex downward) of *Coleus scutellarioides* Benth. plants were cut off 5 mm under and above the nodes. In our first experiments, one internode each time was ground in a chilled mortar with sand and 20 ml of a refrigerated phosphate-citrate buffer solution of pH 5. The resulting brei was centrifuged for 10 minutes at $27000 \times g$. The supernatant, the *Coleus* extract, was tested for the presence of inhibitors of indoleacetic acid oxidase.

The indoleacetic acid oxidase preparation was obtained from roots of *Pisum sativum* cv. "Vlijmsche Gele Krombek" and partially purified by acetone precipitation as described by JANSSEN (1969).

The reaction mixture in these experiments contained: 1.0 ml of pea enzyme solution (10 mg/ml distilled water), 0.5 ml p-coumaric acid 10^{-5} , 0.2 ml indoleacetic acid 10^{-3} and a varying amount of the *Coleus* extract. The reaction mixture was completed to a volume of 5.0 ml with phosphate-citrate buffer solution of pH 5.

Treatment of the internodes with potassium hydroxide or sulphuric acid was done by bringing the lower 1 cm of the internodes in contact with a 0.5% solution of either substance for 5 minutes. The internodes were then thoroughly rinsed.

The diffusate was obtained by putting two internodes in the refrigerator at 4°C for 24 hours, in darkness, with their basal parts in 2 ml of water.

The reaction mixture in these experiments was: 1.0 ml diffusate, 0.5 ml p-coumaric acid 10^{-5} , 0.2 ml indoleacetic acid 10^{-3} , 0.5 ml pea enzyme solution and 2.8 ml buffer solution pH 5.

The residual amount of indoleacetic acid was measured colorimetrically with Salkowski reagent (TANG & BONNER 1947).

RESULTS AND DISCUSSION

In our first experiments we tried to ascertain the presence of substances inhibiting the activity of indoleacetic acid oxidase in internodal tissue of *Coleus*. The results of an experiment of this type are given in fig. 1. It is clearly shown that an increasing amount of *Coleus* extract results in a stronger inhibition of the indoleacetic acid oxidase activity.

In later experiments we determined the effect of the diffusate of untreated and treated internodes of *Coleus*. In fig. 2 are given the results of an experiment of this kind for treatment with potassium hydroxide and fig. 3 gives the results for sulphuric acid.

In both cases it can be seen that the diffusate of untreated internodes only slightly inhibits the indoleacetic acid oxidase activity, if at all. The diffusate of treated internodes, however, strongly inhibits the activity of the enzyme.

Therefore, it seems possible that the effect on root formation of these injurious treatments may be caused by the presence of the diffusate from the damaged cells in the intact cells near by. The result would then be a higher concentration of endogenous auxin in those intact cells, eventually giving rise to the formation of adventitious roots, if kept under appropriate conditions.

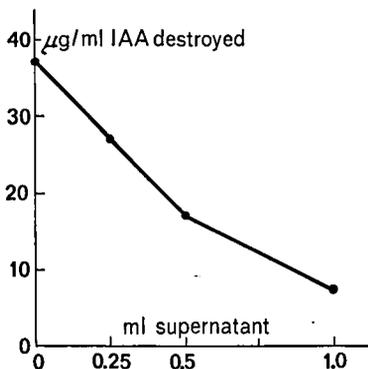


Fig. 1. The relationship between the amount of *Coleus* extract supernatant and the indoleacetic acid destroying activity of the pea enzyme. Incubation time: 30 minutes.

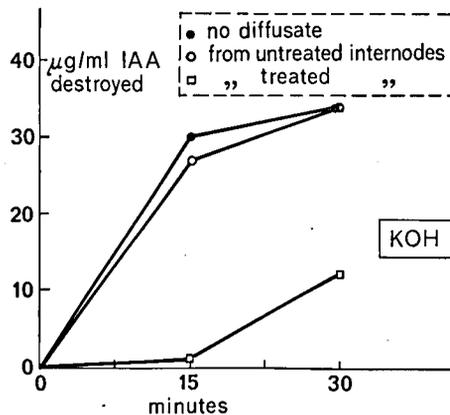
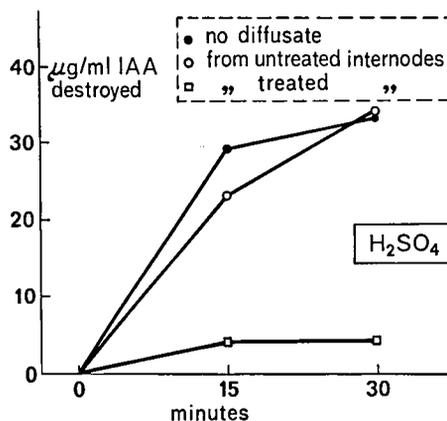


Fig. 2. A comparison between the activities of the pea enzyme under influence of added diffusate from internodes of *Coleus*, untreated or pre-treated with potassium hydroxide, after different times of incubation.

IAA OXIDASE INHIBITORS FROM COLEUS INTERNODES

Fig. 3. A comparison between the activities of the pea enzyme under influence of added diffusate from internodes of *Coleus*, untreated or pre-treated with sulphuric acid, after different times of incubation.



This possibility would suggest that wound hormone activity as first described by Haberlandt might at least partly be an inhibition of indoleacetic acid oxidase.

The results reported here, however, do not exclude a direct effect of the injurious treatment on the cells that are not completely damaged: an alteration of membrane conditions or the disruption of some membranes may have an effect on the stability of the differentiation of a particular cell.

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