Journal of the Minnesota Academy of Science

Volume 33 | Number 1 Article 5

1965

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ABSTRACT: The root nodules of a series of legumes were investigated with and without inoculum in sterilized growing material as well as in open soil to find plants that synthesize extractable amounts of porphyrins. These experiments were a preliminary study toward the investigation of the influence of heavy metal salts on the increased production of porphyrins.

The occurrence of hemoglobin and porphyrins in the root nodules of leguminous plants has been of interest to many investigators of the problems of nitrogen fixation (Falk, Appleby, and Porra, 1959; Kluver, 1949; Little, 1948; Neumann, 1952; Virtanen, Jorma, Linkola, and Linnesalmi, 1947).

Stimulated by the campaign of eradicating insects with large amounts of lead salts and aware of the effect of lead on human and animal porphyrin metabolism (Granick, 1962; Rubino, 1962), the author added lead acetate to a nutrient solution[2] for beans. The leaves turned white within 24 hours and much larger accumulation of porphyrins was found in the treated root nodules than in those simultaneously grown without the addition of lead.

Before this study could be pursued, a general investigation of porphyrine production in root nodules was done to find varieties best suited for later intensive research.

Falk et al. (1959) have shown that in the symbiosis of roots with bacteroids, as well as in artificial cultures and breis, a great increase in cytochrome *C* occurred alongside the production of porphyrins other than proto-copro- and uro. Sironval (1958), in light exposure studies, found a correlation between chlorophyll formation in the tops, and accumulation of porphyrins in the nodules.

The investigation reported here dealt with the production of porphyrins in live plants in controlled and in open soil as well as in liquid cultures.

This work was supported by the Department of Medicine, University of Minnesota Hospitals and was, in part, carried out at Hamline University. Thanks are given to Dr. C. J. Watson, Department of Medicine, University of Minnesota, and to Patricia J. Rand, now at the University of Arkansas, Fayetteville.

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[2] Nutrient solution for plant growth (University of Minnesota Department of Botany, 1958): .0136M $Ca(NO_3)2$; .0136M KH_2PO_4 1Ml $FeCl_3$ 2ppm/1; 2.8ppm H_3; 1.8ppm $MuSO_4$; .1ppm $ZnSO_4$ .05ppm $CuSO_4$.

[3] The Nitrogen Company, Milwaukee, Wisc.

Methods

All seeds were nicked, mixed with several varieties of rhizobium in the form of commercial inoculum[3] and brought to germination in petri dishes. Parallel germinations were started without inoculum. The germinated seeds were then transferred either to flower pots with mixed soil, sterilized sand, artificial medium in pint bottles, or into open soil. Plants were removed at regular time intervals, quickly rinsed free of soil and the nodules transferred immediately into a minimal amount of 25 per cent hydrochloric acid and crushed. In the case of clover, in which the nodules were too small, the whole roots were ground into the *HC*1.

For paperchromatography, some of the *HC*1 extract was concentrated in vacuum over *KOH*, applied to Whatman #1 filter paper and ascending chromatography was done in lutidine-water mixtures (Nicholas and Rimington, 1949). Where larger amounts of porphyrin could be obtained, these were extracted (Schwartz, Berg, Bossenmaier, and Dinsmore, 1960) and converted to methylesters (e.g., 200 mg. from nine plants of Kentucky wonder pole beans); subsequent partition chromatography was carried out on calcium carbonate (Schwartz et al., 19).

Results

While uro and coproporphyrin were the more abundant components, the chromatographic evidence showed the presence of porphyrins with 7,6,5,3, and 2 carboxyl groups also. These resembled those obtained artificially by decarboxylation (Schwartz et al., 1960; Chu and Chu, 1959). Already three weeks after germination fluorescent nodules could be found on the root, but in most cases, the majority of the plants were harvested after six weeks. If no nodules could be found the plants were observed for 12 weeks. The summary below shows four categories:

A. Extractable amounts of porphyrins are produced from single plants.

B. Single nodules yield enough porphyrins for chromatography.

C. Chromatography could only be performed if many plants were extracted and the porphyrins conentrated.

D. Plants tested which did not yield nodules with any of the nitrogen inoculum.

A	B
Phaseolus: Kentucky wonder pole, Kidney wax, Lima Scarlet runner, Tender green. Peanuts.	Phaseolus: All in A, broad, Mung, Stringless green. Galiga bicolor. Glycine Soya. Kudzu Vine. Lathyrus Latifolis. Lupinus: Annal, Hartwig, Russel, Texas blue bonnet. Pisum, Sweet. Vetch: Common, Hairy, Purple Woolly pod. Vigna sinensus.

C	D
Trifolium: Alsike, Berseen, Black medic, Bur, Crimson, Ladino, Red, Sour, Strawberry, Suckling, Sweet White, White Dutch. Baptisia Vigna. Pisum: Canadian Field, Progress. Sebania. Trefoil.	Dolichos: Hyacinth Guar.

Since Kentucky wonder pole beans — Danish, 1954 — gave the best results, time studies were repeated in sterilized sand watered with artificial medium. There was an increase in nodule formation and porphyrin production to the fifth week, after which the pink fluorescent color of the nodules changed to brown and leghemoglobin could be found. The porphyrins were often absent in nodules older than 10 weeks. In open soil, it was difficult to obtain the nodules at the right time since they tended to become soft and to break. The best porphyrin yield was obtained before the flowering time. The Kentucky wonder pole bean was cultivated through seven generations. The porphyrin production, however, decreased and after the fifth generation nodules could not be elicited, neither by a variety of inoculum nor in open soil. Since the beans had been grown in a yard sprayed with DDT this could be a reason (Tompovshchikov, 1956) and should be further investigated.

In open soil, Mung beans, Soya beans, Lathyrus, Lupines, Peanuts, and Canadian field peas still showed the presence of porphyrins after 12 to 20 weeks and peanuts especially gave large fluorescent nodules. Alder nodules were also investigated and showed traces of porphyrin but mainly a blue-red indicator like color compound.

Conclusions

Although many years of work went into this investigation there is no clear-cut answer to which type of phaseolus is most consistent in yielding porphyrins. Investigations of growing rhizobium on blood-carrot agar (Neumann, 1952) should also be considered for a study of the influence of heavy metal salts on the porphyrin "leghemoglobin" and nodule producing bacteria.

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