AMAZONIANA	X	2	173 – 180	Kiel, Oktober 1987

From cooperation between Max-Planck-Institute for Limnology, Working group "Tropical Ecology", Plön, West Germany, and Instituto Nacional de Pesquisas da Amazônia, Manaus – Amazonas, Brazil

Da cooperação entre Max-Planck-Institut für Limnologie, Arbeitsgruppe Tropenökologie, Plön, Alemanha Oc., e Instituto Nacional de Pesquisas da Amazônia, Manaus – Amazonas, Brasil

Ecological and physiological investigations on *Eichhornia crassipes* (MART.) SOLMS. II. Studies of root structure and the dependance of pigment deposition on the age and physiological state of the roots

by

Thaila Mattern & Bodo Furch

Dipl.-Biol. Thaila Mattern, Priv. Doz. Dr. Bodo Furch, Botanisches Institut der Universität Kiel, Olshausenstraße 40, 2300 Kiel (FRG).
(accepted for publication: January 1987).

Abstract

The Amazonian water hyacinth, *E. crassipes*, a Pontederiaceae, is a rarity among higher plants due to the production of blue colour in its roots in response to nitrogen deficiency in the medium. Qualitative and quantitative aspects of pigment deposition in the various root regions, from epidermis to hypodermis, throughout the entire primary cortex, is described. By determining the activity of three enzymes of the pentosephosphate cycle and the shikimate pathway, in combination with the successive pigment deposition in the cell walls, it has been shown that de novo synthesis must occur and that the pigments are probably anthocyanin-like compounds.

A possible ecological function of the deposition of secondary products in the roots is discussed.

Keywords: *Eichhornia crassipes*, anatomy and morphology of roots, biosynthesis of root pigments.

Introduction

As shown by SCHLÜTER & FURCH (1987), roots of *Eichhornia crassipes* (Pontederiaceae, material from central Amazonia) produce a blue to blue-black colouration in nitrogen-poor or nitrogen-free water culture medium under controlled culture room conditions. This pigmentation is a result of the deposition of a presumably anthocyanin-like pigment in the root cell apoplasts (FURCH & ZIMMERMANN 1983). It only develops reliably and particularly intensely if the plants are deprived of nitrate or ammonium nitrogen. A low quantum flux of more than 0,5 μmol photons x m^{-2} x sec^{-1} (PAR, scalar) in the root region is necessary for the expression of this colouration.

This work will report on

1. studies on the anatomy and morphology of fully grown roots of *E. crassipes.*
2. qualitative and quantitative deposition of the pigment in root cell apoplasts during root development.
3. several aspects of the biosynthesis of pigment precursors and pigments.

Material and methods

Fig. 1 shows a 5 week old daughter plant. The development of long anchor roots with which the completely free-floating plant attempts to obtain contact with the bottom sediment is typical. The roots produce numerous lateral roots and remains of the stolons die off later. Both from the compact growth form and development of the bulbous petioles it can be seen that this is a floating individual grown under adequate illumination, without lateral pressure from other plants. A good description of this phenotype can be found in MITCHELL & THOMAS (1972).

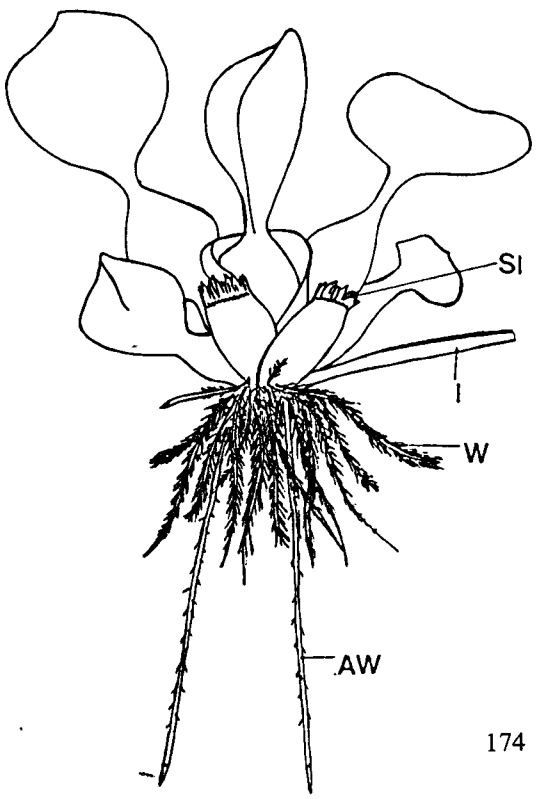

Fig. 1:
Developmental stage of a 5 week old daughter plant.
AW = anchor root; I = remains of the stolon; SI = stipular lobes; W = main root with numerous lateral roots.

Under extreme conditions, with respect to nutrients and site, the phenotype of this species can deviate considerably from that described (GOPAL pers. comm., own obs). After preculture in greenhouses, the plants were grown on water culture in growth chambers as described by SCHLÜTER & FURCH (1987). Daughter plants were used for the biochemical experiments in which root pigmentation was induced by transfer from full nutrient solution (20 % KNOP) to nitrogen-free medium (20 % KNOP without nitrogen compounds) for 14 days. From the start of nitrogen starvation, root samples were harvested daily from each plant and treated for pigment and enzyme activity determination as follows: The activity of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and shikimate dehydrogenase were tested by the usual methods and related to the protein content of the roots; this was established by the Bio-Rad protein identification method as described by BRADFORD (1976). In some cases the shikimate pathway was selectively blocked by the addition of glyphosate which is known to inhibit the synthesis of chorismate from shikimate and phosphoenolpyruvate.

Material was broken up in ice cold buffer (3 g root material as dry powder with 0,5 g polyvinylpyrrolidon, as protection against phenolic substances, in 10 ml triethanolaminohydrochloride/EDTA buffer, pH 8,50).

As a comparative measure of dissolved pigment concentration in hydrochlorid methanol, extinction at the absorption maximum of 558 nm was chosen.

Anatomical and morphological studies were conducted on hand sections using light and transmission electron microscopy. The determination of the contents of the newly discovered secretory passages in the central pigh were achieved using the HOEPFNER-VORSATZ test for phenols, modified after REEVE (1950).

Results and Discussion

Morphology of the roots

Numerous first order lateral roots effectively take over the role of root hairs which are lacking in *E. crassipes*. Only in exceptional cases are second order root branches developed. This is clearly proved by the calyptra ontogeny (MATTERN 1985). After about 3 or 4 weeks a second root type is found among the otherwise identically produced roots. This anchor root is noticeable due to its extended longitudinal growth, increased diameter and reduced number of side roots.

The root is differentiated (Fig. 2). A single layered, thin-walled epidermis and a 1 - 2 layered hypodermis with relatively thick-walled cells can be recongnised. The adjacent outer layer of the primary cortex is composed of large lumened thin-walled cells without intercellular spaces. There is then a massive aerenchyma of sponge-like elongate cells. Isolated idioblasts with calcium oxalate raphides occur. The innermost layer of the boidal intercellular spaces. This is bordered by a primary endodermis.

The central pith is composed of polyarch conducting bundles typical of monocotyledons in which, in addition to annular and spirally thickened tracheids, genuine vessels occur. Within the phloem, comprised of sieve tubes companion cells and parenchyma, schizogenously produced secretory passages are found. Their contents could be identified as phenol derivatives by the use of specific histochemical steins. Up to 15 of these lateral axially orientated, presumably unbranched, secretory passages could be found per central cylinder. Precise identification of the secretory products has not yet been possible, but since phenol storing cells have been demonstrated in the leaves (MARTYN et al. 1983) it is presumed that these phenols are also involved here.

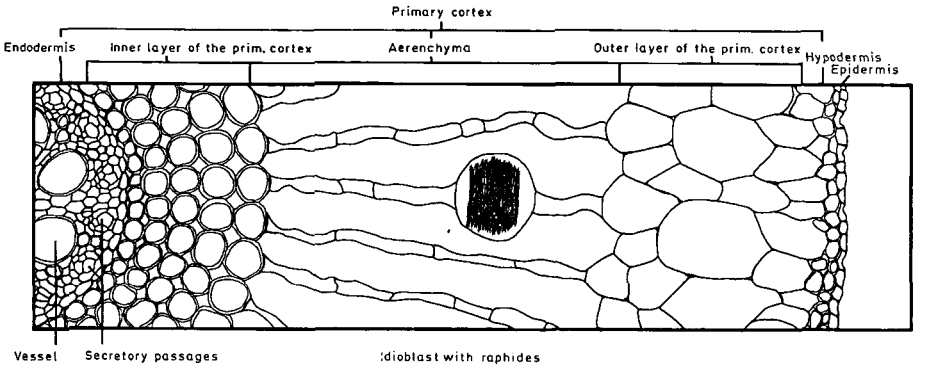

Fig. 2:
Representation of the cross-section of an older primary root; for further explanation see the text.

Tracheids occur in the central cylinder; not only schizogenously produced protoxylem lacunae as cited by HASMAN & INANC (1957). Sclerified elements and a pericambium are totally absent. Side roots (not illustrated) are much more simply constructed showing no lacunar intercellular spaces, idioblasts or secretory passages (MATTERN 1985).

Pigment deposition during the development of a mein root

Pigment deposition in the root cell walls can basically be resolved in two ways:

1. Young rootless daughter plants are grown on complete nutrient medium without any nitrogen.

During development of the main root, which is complete after about 80 - 100 days, pigment deposition can be traced histologically (using sections) or photometrically (by isolating the pigment and determining its relative concentration).

2. Young rootless daughter plants are grown on complete medium with a nitrogen source for 14 days and the plants, with their newly developed colourless roots, are then planced on complete medium without nitrogen. Pigment deposition is more rapid than in the first case. After only 5 - 7 days after transfer to nitrogen-free, but otherwise complete medium the previously colourless roots have acquired a blue colour.

Fig. 3 (Plants grown according to 1.) shows that pigment deposition in the cell walls follows a defined pattern and is not diffuse. During root development accumulation of the nitrogen deficiency induced pigment progresses from the outside towards the centre; after only 3 days the epidermis and hypodermis are coloured, after 3 more days part of the outermost cortex, after 20 - 40 days the entire cortical layer and after 40 - 90 days the entire cortex. The central cylinder remains largely unpigmented. As shown by the photometric analyses by the end of the study the relative concentration of extractable pigment has risen from an initial 2 % to 100 %. Roots of this age are usually pigmented throughout and appear blue-black.

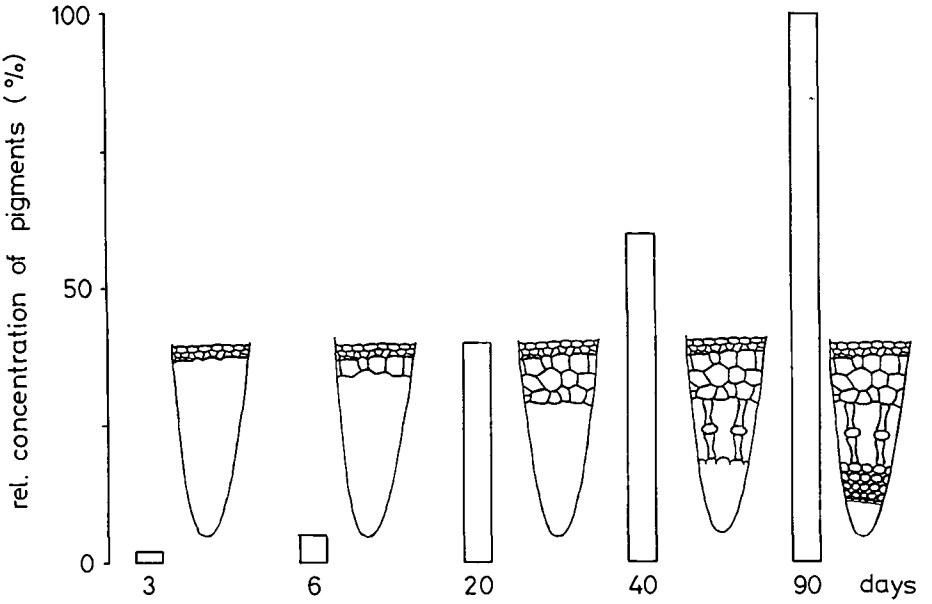

Fig. 3:
Progress of nitrogen-deficiency induced pigment deposition during the ontogeny of a main root from 3 to 90 days. Ordinate – relative concentration of the pigment in %.

Studies indicating the metabolic origin of the pigments

Even with modern methods such as HPLC it has not yet been possible to unequivocally determine the chemical structure of the pigments. However the compounds show several typically anthocyanin features in their solubility behaviour, ability to form complexes, bathochromic effect and the initiation of pigment synthesis only in the light (FURCH & ZIMMERMANN 1983; SCHLÜTER 1985; MATTERN 1985). Assuming at least that anthocyanin-like compounds are involved, they must be synthesized via the shikimate pathway. For this reason the activity of some important key enzymes in the primary and secondary anthocyanin-synthesis metabolism was tested in relation to nitrogen-deficiency induced pigment deposition. Simultaneous marked increase in the activity of glucose-6-phosphate-dehydrogenase, 6-phospho-gluconate-dehydrogenase, the initial enzyme of the pentose-phosphate shunt and of shikimate dehydrogenase, with delayed appearance of root colouration, allow us to conclude that fresh pigment synthesis via their respective reaction pathways has occurred (Fig. 4). At the end of the synthetic phase enzyme activity levels characteristically drop back to the control level (0 days after removal of nitrogen). The discovery that supplying 0.75 nmol/l glyphosat to the nutrient medium almost completely inhibits pigment synthesis (up to 90 %) is further evidence for de novo synthesis under nitrogen deficiency. Because this inhibitor prevents chorismate production rather than affecting an immediate pigment precursor, undisturbed progression

of the entire pentose-phosphate cycle and the shikimate pathway is necessary to obtain a distict pigment production reaction. The pigments presumably originate from phenylpropane metabolism. Although they do not provide unequivocal evidence of anthocyanins (results not given here), the pigments are at least anthocyanin-like. They are however not deposited in vacuoles, but are tightly bound into the cell wall matrix, as the numerous extraction attempts have demonstrated (MATTERN 1985). This may also explain why pigment, once deposited, is not re-metabolized. It has not been possible to observe any loss of colour from pigmented roots during culture experiments.

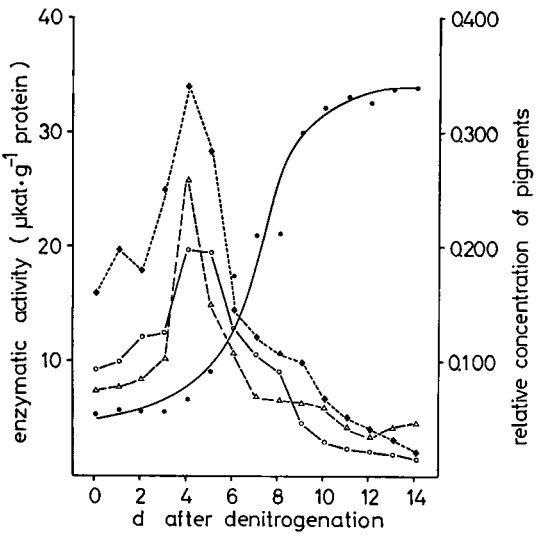

Fig. 4:
Activity of several important key enzymes after the induction of pigment deposition in mature roots by nitrogen starvation. Increase of the extractable pigments during the experiment. Sigmoidal curve = relative pigment concentration (right-hand ordinate); dotted line = 6-phosphogluconate-dehydrogenase; solid line= glucose-6-phosphate-dehydrogenase; broken line = shikimate-dehydrogenase. Abscissa = days after denitrification of the medium (20 % KNOP).

The possible ecological significance of secondary products in *E. crassipes* roots

Given that roots of *E. crassipes*

1. contain many large idioblasts with raphides,

2. the central cylinder of the main root possesses secretory passages which contain phenolic substances and

3. the roots are frequently strongly coloured with anthocyanin-like compounds, the question arises whether these products, which are all produced via extremely energy-costly metabolic sequences, have an ecological function. There is evidence in support of this contention. It is known that *E. crassipes* is largely unsuitable as cattle feed, and produces diarrhoea in grazing animals. When given as the only fodder to black bengal goats death, due to liver and heart-muscle lesions (DUTTA et al. 1984), occurs within a month. Organisms which partially consume or destroy *Eichhornia* are known (e. g. FORNO & WRIGHT (1981) on Australian Eichhornias), but all parasitic beetles, moths and fungi fail to attack the *Eichhornia* roots, further evidence of defence against graizing via phenylpropane metabolism products. Other possible functions of pigment deposition in *E. crassipes* roots have also been discussed (SCHLÜTER 1985).

Zusammenfassung

Wie bei SCHLÜTER & FURCH (1987); (s. vorstehender Bericht) beschrieben, ist *Eichhornia crassipes* eine Zeigerpflanze für NO_3-N- und/oder NH_4-N-Mangel: Durch diese Bedingungen im Substrat wird eine Blaufärbung der vorher fast farblosen Wurzeln ausgelöst, die Pigmente werden dabei im Apoplasten der Wurzelzellen gelagert und dort sehr fest an die Matrix gebunden.

In dieser Arbeit wird berichtet

1. Über die Anatomie und Morphologie der ausgewachsenen *Eichhornia*-Wurzeln. Dabei werden erstmals schizogen entstandene, im Zentralzylinder lokalisierte Sekretgänge beschrieben, die phenolische Substanzen beinhalten.
2. Über die Progression der Einlagerung der Pigmente, beginnend bei der Epidermis über Hypodermis und primäre Rinde. Die Inkorporation stoppt beim CASPARY'schen Streifen der primären Endodermis.
3. Über den wahrscheinlichen Syntheseweg dieser Pigmente. Durch Bestimmung von Enzymaktivitäten und Hemmversuche kann die bisherige Annahme gestützt werden, daß es sich bei den Farbstoffen, die de novo synthetisiert werden, zumindest um anthocyanähnliche Verbindungen handeln muß.

Die ökophysiologische Bedeutung einer für den Stoffwechsel der Pflanze aufwendigen Farbstoffeinlagerung in die Zellwände und die Ablagerung von phenolischen Substanzen in Sekretgängen wird diskutiert.

Summary

By virtue of increasing blue colouration of its roots *E. crassipes* can be an indicator of nitrogen deficiency under certain conditions (SCHLÜTER & FURCH 1987). The pigments are deposited in the root cell apoplasts and then bound to the wall matrix.

An account is given of

1. Studies on the antomy and morphology of vegetative roots and the histological localization of the phenol-like compounds.
2. The development of pigment deposition during the main root ontogeny, and
3. various aspects of the biosynthesis of pigment precursors and compounds after pigment development has been induced (nitrogen starvation).

The possible eco-physiological significance of the energetically costly pigment metabolism and deposition, and the storage of phenolic substances in secretory passages is discussed.

Resumo

Como descrito por SCHLÜTER & FURCH (1987; veja o relato anterior), *Eichhornia crassipes* é uma planta indicadora de carência de NO_3-N e/ou NH_4-N: tais condições no substrato provocam uma coloração azul das raízes antes quase incolores. Neste processo, os pigmentos são depositados no epoplasto das células radiculares e ali fixados mui firmemente na matriz.

Neste trabalho relata-se

1. sobre a anatomia e a morfologia das raízes crescidas de *Eichhornia*. Descrevem-se, pela primeira vez, vasos de secreção formados esquizogenicamente e localizados no cilindro central, os quais contêm substâncias fenólicas.
2. sobre o desenvolvimento da deposição dos pigmentos, começando na epiderme via hipoderme e casca primária. A incorporação cessa na faixa de CASPARI da endoderme primária.
3. sobre a via provável da síntese destes pigmentos. Pela determinação de atividades de enzimas e por ensaios de inibição pode-se reforçar a atual suposição de que, nos pigmentos sintetizados de novo, deve tratar-se pelo menos de compostos semelhantes a antocianina.

O significado eco-fisiológico de uma incorporação de pigmento nas paredes celulares, dispendiosa para o metabolismo da planta, e da deposição de substâncias fenólicas nos vasos de secretores é discutido.

Acknowledgments

Supported by the Brazilian Research Council (CNPq), the Max-Planck-Gesellschaft (MPG) and by a grant SCHIMPER-Stipendium der H. und E. WALTER-Stiftung to one of us (B. F.). Thanks to H. SIOLI who translated the Summary into Portuguese.

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