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Phyton (Austria)	**Vol. 28**	Fasc. 1	109–114	20. 7. 1988

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Localization on Peroxidase Isoenzymes in Different Parts of Some Trees

By

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With 2 Figures

Received May 15, 1987

Key words: Angiosperms, Sapwood, heartwood, peroxidase, isoenzymes, lignification, *Quercus robur, Aesculus hippocastanum.*

Summary

STICH K. & EBERMANN R. 1988. Localization of peroxidase isoenzymes in different parts of some trees. – Phyton (Austria) 28 (1): 109–114, 2 figures. – English with German abstract.

Peroxidase isoenzymes from different parts of trees *(Quercus robur, Aesculus hippocastanum)* were investigated electrophoretically. Isoenzymes with identical electrophoretical mobilities could be detected in bark, branch, sapwood and heartwood, although the intensity of a specific peroxidase isoenzyme differs between organs. Essentially, no major differences in isoenzymatic pattern can be found at peroxidases from lignifying and heartwood forming tissues. Therefore, no special isoenzymes which are solely responsible for lignification or heartwood formation, seem to exist. On the contrary, the results support the conclusion that identical wood peroxidases participate in lignification as well as in heartwood formation.

Zusammenfassung

STICH K. & EBERMANN R. 1988. Lokalisation von Peroxidase – Isoenzymen in verschiedenen Teilen einiger Bäume. – Phyton (Austria) 28 (1): 109–114, 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Peroxidaseisoenzyme aus verschiedenen Teilen des Baumes *(Quercus robur, Aesculus hippocastanum)* wurden elektrophoretisch untersucht. Isoenzyme mit identischer elektrophoretischer Mobilität konnten in der Rinde, im Zweig, dem Splint- bzw. Kernholz nachgewiesen werden, obwohl die Enzymaktivität der Isoenzyme je nach Art des untersuchten Gewebes deutliche Unterschiede aufwies. Jedoch bestehen

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keine wesentlichen Unterschiede im Peroxidaseisoenzymmuster von lignifizierendem bzw. kernholzbildendem Gewebe. Es scheint daher keine spezifischen Isoenzyme zu geben, die ausschließlich der Lignifizierung bzw. der Kernholzbildung zugeordnet werden können. Diese Befunde unterstützen im Gegenteil die Annahme, daß die in Hölzern vorkommenden Peroxidaseisoenzyme sowohl an der Lignifizierung als auch an der Kernholzbildung beteiligt sind.

Introduction

Since countless ages wood is one of the most important raw materials for human beings. The wood of many species occurring in our hemisphere has during its development differentiated into sapwood and heartwood. Sapwood is responsible for transportation of water and has also storage functions for reserve materials. Besides distinct differences in the chemical composition, the main physiological difference between sapwood and heartwood is, that a major amount of living cells still exist in sapwood.

Further evidence for this fact gives the occurrence of considerable enzymic activities in sapwood (HILLS 1977). During the transformation from sapwood to heartwood an increase of metabolic activity is observed (HÖLL & LENDZIAN 1973). This increase is localized in the transition zone joining the heartwood boundary. Especially enzymes showing peroxidase and polyphenol oxidase activity play an important role in the final conversion of monomeric phenols into polymers deposited in the heartwood (WARDROP & CRONSHAW 1962).

Peroxidases also play an essential role in lignification (NAKAMURA 1967, NAKAMURA & NOZU 1967, NOZU 1967, HARKIN & OBST 1973). Many of the peroxidase isoenzymes possess IAA oxidase activity (GASPAR & al. 1982) as well as polyphenol oxidase (STICH & EBERMANN 1984, 1987) acitivity. Therefore, peroxidases can be considered as multifunctional enzymes having at least three enzymic functions (peroxidase, polyphenol oxidase, IAA oxidase) which are able to exert catalytical influence on metabolism in different ways.

Subject of this report is the question, whether the same peroxidase isoenzymes are responsible for lignification and heartwood formation or whether on the other hand special isoenzymes do exist, which belong to one of those metabolisms.

Material and Methods

a) Plant material

Two oak trees and their branches *(Quercus robur L.)* were used. They had grown in a forest owned by the City of Vienna. The trees were cut down at the beginning of July.

Besides that, branches and leaves of a horse-chestnut *(Aesculus hippocastanum L.)* were used. These branches were cut off in a public park.

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b) Preparation of wood extracts and determination of peroxidase activity

The preparation of wood extracts was done according to EBERMANN & STICH (1982).

Previous experiments have shown that EDTA-Na_2, contained in the extraction medium, did not have any inhibiting influence on the peroxidase activity (HASCHKE & FRIEDHOFF 1978). Furthermore, it was tested if the applied extraction method was also suitable to extract the cell wall bound peroxidase isoenzymes. Extraction results were comparable with those obtained by MÄDER & al. (1975).

The determination of peroxidase activity was made according to „THE WORTHINGTON MANUAL" (1972). The change of absorption was measured by a Perkin Elmer Spectralphotometer 550 and reported by a Perkin Recorder 56.

c) Electrophoresis of peroxidase isoenzymes

Gelelectrophoresis in polyacrylamide gel was performed as described earlier (EBERMANN & BODENSEHER 1968) with the pH of the separation gel changed to 6,0. From each extract 50 μl were used for PAGE.

d) Staining of peroxidase isoenzymes on polyacrylamide gel

Two different methods were used. The first one was done with benzidine (Merck) and hydrogen peroxide (Merck) according to ORNSTEIN (1971). In the second method peroxidase isoenzymes were visualized with coniferyl alcohol and hydrogen peroxide (EBERMANN & STICH 1984). The gels were incubated in 200 ml 0,1 M acetate buffer pH = 6,0, containing 0,5 mM coniferyl alcohol. To this solution 2 ml 3% hydrogen peroxide solution was added.

Results

Fig. 1 shows peroxidase isoenzymes occurring in different parts of an oak *(Quercus robur)*. By comparing the isoenzyme patterns from the different plant organs obtained, it can be easily seen that the most intense zone of enzymic activity is always present. Changes in the relative and absolute intensities of single peroxidase enzymes lead to differences in enzymic patterns shown in Fig. 1. This can be demonstrated by comparing total peroxidase activities in sapwood and heartwood. The peroxidase activity of the tested heartwood sample (0,03 A/min per 50 μl wood extract) is much lower than the activity measured in the sapwood sample (0,21 A/min per 50 μl wood extract). So only the isoenzymes with the highest activity can be detected electrophoretically and the isoenzymes having lower enzymic activites remain below the detection limit.

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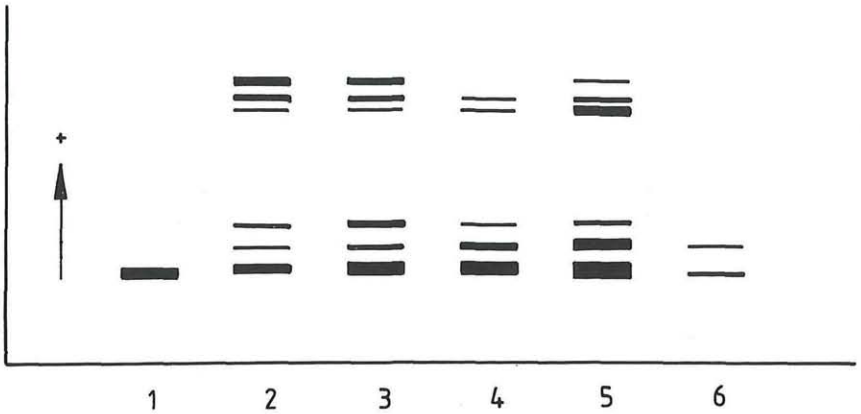

Fig. 1: Peroxidase isoenzymes in different parts of an oak *(Quercus robur)* stained with benzidine and hydrogen peroxide.

1 Leaf, 2 Branch with Bark, 3 Branch without Bark, 4 Bark, 5 Sapwood, 6 Heartwood.

Fig. 2 shows the peroxidase isoenzymes occurring in different parts of horse chestnut *(Aesculus hippocastanum)*. The enzymic activities are visualized with coniferyl alcohol instead of benzidine. At the location of peroxidase activity, white dim zones of a lignin like polymer are formed. The isoenzymic pattern obtained by staining with coniferyl alcohol completely corresponds to the pattern obtained by staining with benzidine (data not shown). From this result we can draw the conclusion that all isoenzymes are capable of synthesizing lignin.

As can be seen from Fig. 2 the electrophoretic patterns of peroxidase isoenzymes reveal differences according to the part of the plant tissue. It is remarkable that in the parts showing the highest enzymatic activities an additional isoenzyme with low activity can be detected. This isoenzyme is even slightly visible in the sample branch without bark.

Discussion

From Fig. 1 and 2 can be seen that, with a few exceptions, the same peroxidase isoenzymes are occurring in different parts of the trees investigated. It could also be found that the absolute and relative activities of single isoenzymes are not equal in each part of the trees investigated, but predominant isoenzymes can be detected in each part of the tree. No specific isoenzyme could be detected which is solely responsible for lignification or heartwood formation. Probably both of the processes are catalysed by the same isoenzymes.

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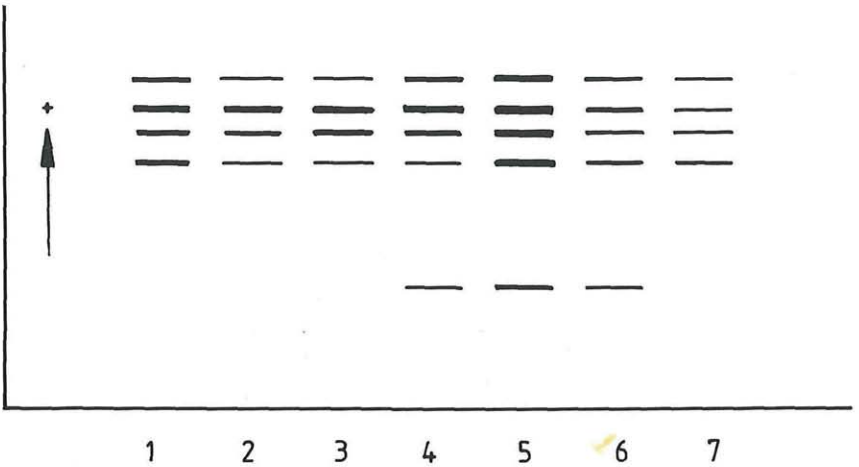

Fig. 2: Peroxidase isoenzymes in different parts of a hourse-chestnut *(Aesculus hippocastanum)* stained with coniferyl alcohol and hydrogenperoxide.

1 Sapwood, 2 Sapwood with bark, 3 Branch with bark, 4 Branch without bark, 5 Cambial layer, 6 Bark, 7 Leaf.

In Fig. 2 the additional slow moving peroxidase isoenzyme is found in tissues with presumably the highest metabolic activity (HILLIS 1977) and has a rather low activity compared to the other isoenzymes. Therefore, it seems that the enzymatic activity of this isoenzyme in other samples is below the detection limit. On the other hand, it seems unlikely that this isoenzyme would have an important function during lignification.

It is interesting that a peroxidase isoenzyme with weak activity can be detected in heartwood. In the opposite to sapwood no living cells should exist in heartwood (HILLIS 1977). The detectable peroxidase activity seems to survive in dead cells.

This experimental result gives an explanation for the findings of ROUX & EVELYN (1958). Monomeric phenolic compounds like leuco-fisetinidin and low molecular weight tannins are subjected to oxidative polymerisation reactions in heartwood. The average molecular weight of this condensed tannins (MW = 650) increases from the sapwood–heartwood junction to the central heartwood (MW = 1050). Responsible for these reactions are without any doubt the peroxidase isoenzymes detectable in heartwood.

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Zeitschrift/Journal: Phyton, Annales Rei Botanicae, Horn

Jahr/Year: 1988

Band/Volume: 28_1

Autor(en)/Author(s): Stich Karl, Ebermann Robert

Artikel/Article: Localization on Peroxidase Isoenzymes in Different Parts of Some Trees. 109-114