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Seasonal Alteration of Plant Peroxidase Isoenzyme Pattern in *Larix decidua*

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With 6 figures

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Summary

KORORI S. A. A, HINTERSTOISSER B., LANG H. P. & EBERMANN R. 1992. Seasonal alteration of plant peroxidase isoenzyme pattern in *Larix decidua.* – Phyton (Horn, Austria) 32 (2): 307–313, 6 figures. – English with German summary.

Twigs and seeds of three 50 years old *Larix decidua* MILL. from low, middle and high location, which had been grown under natural conditions, were collected in June, July, September, October, November and January in the year 1989. The investigation has been repeated in the years 1990 and 1991 and yielded the same results. The enzyme peroxidase was extracted according to a procedure described by EBERMANN & STICH 1982. The alterations of peroxidase isoenzymes were investigated by polyacrylamide – gel electrophoresis (PAGE) (EBERMANN & STICH 1982, KORORI 1989).

The results point out that the isoenzyme pattern of peroxidase changes dramatically during the different seasons. The change of isoenzyme patterns in twigs manifests itself mainly by the appearance of new isoenzymes with lower as well as higher mobility at the beginning of fall (October). The isoenzymes with extremely higher mobility mostly disappear again until January whereas the bands with low mobility increase in intensity. The striking fact is, that only two of the zones with

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enzymatic activity remain unchanged in the course of the year. The alterations of peroxidase isoenzymes in the seeds due to the season show a different course. During the early development (June, July) of the seeds isoenzymes with high as well as low electrophoretical mobility are present. At the beginning of ripening these zones with high mobility vanish. In the course of seed development only a single peroxidase isoenzyme stays invariant.

Zusammenfassung

KORORI S. A. A, HINTERSTOISSER B., LANG H. P. & EBERMANN R. 1992. Jahreszeitliche Änderung des Peroxidaseisoenzymmusters in *Larix decidua* . – Phyton (Horn, Austria) 32 (2): 307–313, 6 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Von drei fünfzigjährigen Lärchen (*Larix decidua* MILL.), die unter natürlichen Bedingungen gewachsen waren, wurden in den Jahren 1989 bis 1991 im Juni, Juli, September, Oktober, November und Jänner Ast- und Samenproben genommen. Die Äste hatten einen Durchmesser von ca. 1 cm. Die Lärchen stammten aus Tief-, Mittel- und Hochlage.

Die Peroxidase-Enzyme wurden aus den Proben nach der von EBERMANN & STICH 1982 beschriebenen Methode extrahiert. Mittels PAGE (Polyacrylamidgelelektrophorese) wurden die wechselnden Isoenzymmuster verglichen (EBERMANN & STICH 1982, KORORI 1989). Das Ergebnis zeigt, daß sich das Peroxidaseisoenzymmuster im Verlauf eines Jahres drastisch verändert. Der Wechsel im Isoenzymmuster in den Zweigen zeigt sich in erster Linie in dem Vorhandensein von Isoenzymen sowohl mit geringer als auch mit hoher Mobilität zu Beginn des Herbstes (Oktober). Die Isoenzyme mit hoher elektrophoretischer Mobilität bilden sich im weiteren Verlauf des Jahres zurück. Im Jänner sind sie nur noch in Spuren nachzuweisen. Nur zwei Zonen mit enzymatischer Aktivität bleiben im Verlauf des Jahres konstant.

Während der frühen Entwicklung der Samen (Juni, Juli) besitzen die Samen-Isoenzyme eine hohe und niedrige elektrophoretische Mobilität. Mit Beginn der Reifung der Samen verschwinden die Isoenzymbanden mit hoher Mobilität. Im Fall der Samenentwicklung bleibt nur ein Peroxidase-Isoenzym invariabel.

Introduction

The British biochemist DIXON described living matter as a system of unstable catalysts being kept in existence by the occurence of the reaction which they catalyse. Keeping the instability of these catalysts (enzymes) in mind, growing old has been referred to as an unfavourable change in the ability of protoplasma to maintain itself. This implies that the ratio of catabolic to anabolic enzymes increases because of aging (SALISBURY & ROSS 1969).

Environmental conditions have a great influence on living organisms. Temperature, e.g., as one of the stress releasing-factors is involved in the creation of an adapted flora in temperate zones. Several metabolic changes happen during the seasonal variation, controlled by the course of the temperature (LEVITT 1980).

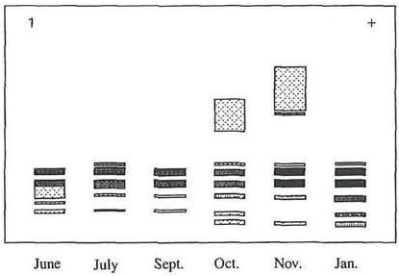
An example is the hardening process of trees, which depends on the adaptation of the development of plants to the climate in its seasonal variation (KORORI 1989). It is widely assumed that the conversion of coniferyl alcohol and other p-hydroxy-cinnamyl alcohols to lignin and formation of H_2O_2 is catalysed by peroxidase (MÄDER & FÜSSL 1982, HARKIN & OBST 1973). Lignin is an important component of xylem cells, and as these cells begin to develop there is a noticeable build – up of peroxidase enzymes (indicated by a histochemical colour reaction). In the temperate zones heartwood formation takes place mainly in the transient season. Therefore an adaption of the metabolism of the trees is necessary in the transition period between summer and winter (SHAIN & MACKAY 1973, NELSON 1978). The annual change in freezing tolerance depends on three factors: temperature, internal rhythm of the plant and photoperiod (SCHWARZ 1968).

Peroxidase as well as amylase are enzymes, which are involved in metabolic changes. Both occur in the heartwood and sapwood of trees (EBERMANN & STICH 1982). Peroxidase is also discussed as a marker of stress (CASTILLO 1986, SIEGEL & SIEGEL 1970, 1986). Because of the great variability of the isoenzymes due to environment the peroxidase hardly seems to be useful for genetical investigation on conifers (GRILL & al. 1982). A maximum of activity of peroxidase could be observed during the dormant season. EBERMANN & STICH (1984) found that heartwood formation in *Quercus robur* mainly occurs during winter. The peroxidase isoenzymes isolated from wood are stable over several months after cutting (LICKL & EBERMANN 1987). The peroxidase isoenzymes have slightly different substrate specifities (SIEGEL & SIEGEL 1970). The isoenzymes of peroxidase isolated from sick plants are often different from those of healthy ones (PRILLINGER 1973). The peroxidase activity and the isoenzyme pattern is altered according to temperature treatments (EBERMANN & al. 1991).

This paper presents evidence that there is a seasonal variation in the isoenzyme pattern of peroxidase which takes a different course in twigs and seeds of the investigated larch (*Larix decidua* MILL.).

Material and Methods

Twigs (diameter about 1 cm) and cones (30 seeds of the any cone) of three 50 years old *Larix decidua* MILL., from low (700 meter above sealevel) – middle (1000 meter above sealevel) – and high (1350 meter above sealevel) locations were collected. The trees had grown under natural conditions. The samples were taken on June 17th, July 17th, September 18th, October 31th, November 27th and January 18th. The twigs and cones were collected from the same main branches at the same date. After harvesting the twigs were cut into splinters and the seeds (30 seeds in each case) were homogenized. 1 g of these splinters and each sample of the seeds were extracted over night at +4° C (1.0 g twigs in 3 ml and 30 seeds in 1 ml buffer) according to EBERMANN & STICH 1982. Polyacrylamide – gel electrophoresis (PAGE) and the isoenzyme detection procedures were performed as described by EBERMANN & KORORI 1990. All of the



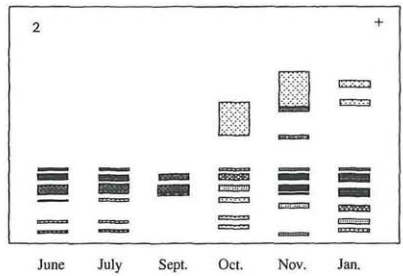
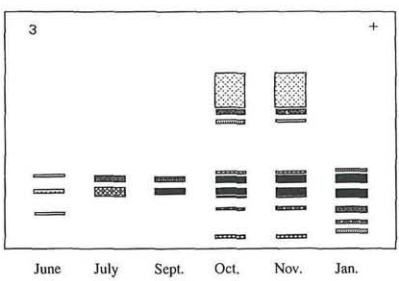


Fig. 1. Peroxidase isoenzyme pattern of twigs from larch, from low location.
Fig. 2. Peroxidase isoenzyme pattern of twigs from larch, from middle location.



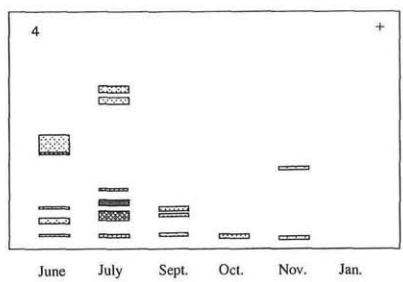
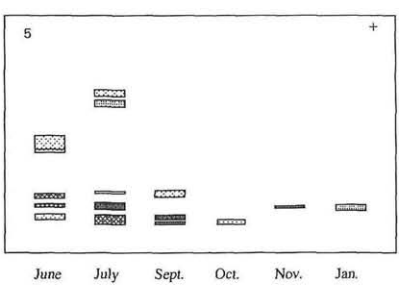


Fig. 3. Peroxidase isoenzyme pattern of twigs from larch, from high location.
Fig. 4. Peroxidase isoenzyme pattern of seeds from larch, from low location.



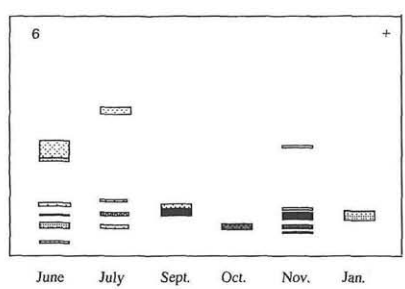


Fig. 5. Peroxidase isoenzyme pattern of seeds from larch, from middle location.
Fig. 6. Peroxidase isoenzyme pattern of seeds from larch, from high location.

samples were separated on the same gel. The detection of peroxidase isoenzyme was performed using o – Dianisidine and H_2O_2 (GABRIEL 1971). Stained patterns were compared by visual inspection.

Results

The comparison of the peroxidase isoenzyme pattern of twigs as well as of seeds from larch from different seasons clearly shows that the pattern changes dramatically during the course of the year. Trees of three different locations had been examined and the results point out that the changes in the isoenzyme pattern depend on the location as well as on the time of sampling. This underlines again the assumption that the period of vegetation time is a very important regulator for the peroxidase isoenzyme pattern.

Fig. 1-6 show the peroxidase activity staining with o – Dianisidine and H_2O_2 of the samples (twigs and seeds) from June to January.

In Fig. 1–3 the isoenzyme pattern of the samples of twigs from low, middle and high location are illustrated. The visual comparison of the staining intensities of the bands shows no major alteration in peroxidase patterns from June to July except the pattern of the peroxidase isoenzymes of the branches of high location. The lowest intensity was found in the sample of branches from high location (Fig. 3) and the highest intensity in middle location (Fig. 2). In the samples which were taken at the end of October, new zones with low and high mobility can be found. This fact signifies that the enzyme systems of the investigated trees start to react to the cold season. At the end of November there have appeared several new isoenzymes with high and low electrophoretical mobility in all of the branches. In the middle of January most of the isoenzymes with high mobility have disappeared whereas the zones in low mobility region increase until January. Only two of the peroxidase isoenzyme bands remain constant.

In Fig. 4–6 the isoenzyme pattern of the samples of seeds from low location, middle location and high location are illustrated. In June the peroxidase activity in the seeds is in a period of decrease. The patterns of peroxidase isoenzyme in the samples of the three different locations have nearly equal intensity. There are isoenzymes with low as well as with high mobility. In July the activity staining of the peroxidase of the investigated seeds differs from that of June, especially in intensity and in the high mobility region. In September – after two months of development in nature – the isoenzyme systems of the seeds have changed drastically in comparison to June and July. The isoenzyme-zones with high electrophoretical mobility have disappeared completely. There can be seen a drastic reduction of enzyme activity in the low location. In fall the peroxidase activity is diminished. This fact is shown by the isoenzyme pattern of the samples

taken at the end of October. There exist only zones with low mobility and low intensity. At the end of November the peroxidase enzyme activity in the analysed seeds has disappeared nearly completely. Only in the seeds of the high location some activity remains. The peroxidase activity of seeds taken in the middle of January has decreased to a very low level in the middle and high location and has disappeared completely in the low location.

Discussion

We have investigated the qualitative and semiquantitative changes of peroxidase enzymes isolated from branches and seeds of larch. The samples were taken at different times of the season of trees from three different locations. The obtained results show that the enzymic system of the trees reacts to the changes of the season by a change in the isoenzyme pattern of peroxidase. The changes of the isoenzyme spectrum is thought to be caused by the change of the lenght of the day, the phytohormones, and the change of temperature. Some of the isoenzymes are more sensitive to low temperatures than others. There are only two main bands which remain uneffected in their electrophoretic mobility in the branches of larch. Due to the seasonal changes – beginning in September – peroxidase isoenzyme alteration is observed in the 1000 meter above sealevel region primarily caused by temperature decline. In October changes appear in all investigated trees.

An opposite course takes the peroxidase development in seeds of *L. decidua*. At the beginning of formation of the seeds at the end of June until November – the time of seed ripening – the peroxidase isoenzyme pattern changes. First there are both, low and high mobility zones present in the peroxidase isoenzyme pattern from the three locations (June and July). In September the pattern has changed in all locations. There are only zones with low electrophoretical mobility remaining. Two main bands have been constant in electrophoretical mobility, but even the activities of these bands vary in their intensities. In November, the pattern of the peroxidase isoenzyme in seeds starts to disappear. At least – in January – the activities are greatly reduced.

The results – obtained in a three year investigation – point out clearly that peroxidase is greatly influenced in its molecular composition by environmental parameters, like temperature. The variation of peroxidase isoenzymes is strongly related to the change of the seasons and it is different in branches and seeds. The characteristic alteration of peroxidase isoenzyme in the course of the season reflects the continous adaption of the tree to its environment and its behaviour parallels the schedule of the year.

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