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Temperature Dependent Alterations of Peroxidase and Amylase Isoenzymes in *Quercus robur*

By

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With 8 Figures

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Summary

EBERMANN R., KORORI S. A. A. & LICKL E. 1991. Temperature dependent alterations of peroxidase and amylase isoenzymes in *Quercus robur.* – Phyton (Horn, Austria) 31 (1): 121–128, 8 figures. – English with German summary.

Branches of four year old trees of English oak – descendants from one mother – had been cut after the entire individuals had been exposed to different temperatures in climatized chambers (temperature range from −10°C to +20°C, various times and series). Peroxidase and amylase enzymes isolated from branches were compared by PAGE and activity staining. The peroxidase activity and the isoenzyme pattern is altered according to temperature treatments. This effects are also depending on the harvest season of the branches (summer or winter). The amylase enzyme activity is altered appreciably due to temperature treatment in climatized chambers too.

Zusammenfassung

EBERMANN R., KORORI S. A. A. & LICKL E. 1991. Veränderungen von Peroxidase- und Amylase-Isoenzymen bei *Quercus robur* in Abhängigkeit von der Temperatur. – Phyton (Horn, Austria) 31 (1): 121–128, 8 Abbildungen. – Englisch mit deutscher Zusammenfassung.

In klimatisierten Kammern wurden vierjährige Topfpflanzen von *Quercus robur,* die von einer gemeinsamen Mutter abstammen, verschiedenen Temperaturen ausgesetzt (Temperaturbereich zwischen −10° C und +20° C, verschiedene

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Standzeiten und Temperaturfolgen). Die Peroxidase- und Amylaseenzyme aus Zweigen wurden nach polyacrylamid-gel-elektrophoretischer Trennung in bezug auf Aktivität und Isoenzymmuster verglichen. Die Aktivität und Muster der Isoenzyme der Peroxidase verändern sich bei Temperaturbehandlung in Abhängigkeit von der Erntezeit (Sommer oder Winter), die Amylasen verändern sich auch.

Introduction

Life cycle and growth of plants takes place within certain limits of temperature, extreme situations had been investigated on their influence on plants (LEVITT 1956, 1958, 1980, BIEBL 1962). In the temperate zones plants are stressed periodically by the coldness of the winter and have acquired frost resistance. Hardening depends on the adaptation of the development of plants to the climate in its seasonal variation, especially at the start of the winter rest. The seasonal variation of peroxidase activity in English oak shows an increased activity during the winter (EBERMANN & STICH 1985). In different species an inverse relationship between temperature and peroxidase activity has been reported (LEVITT 1980) with an adaptation to chilling rather than freezing.

Amylase activity is one indicator of the carbohydrate metabolism in plants, any influence of chilling and freezing on this metabolism should be shown by activity variation of amylase.

In this paper we investigate the peroxidase (E.C.1.11.1.7.) and amylase (E.C.3.2.1) activity in English oak after temperature treatment.

Material and Methods

Entire individuals, descendants from a single mother were placed in the cold (−10° C), (0° C) and moderate (+20° C) chambers, branches cut, protein extracted and the enzyme activity measured qualitatively by PAGE and activity staining. Branches (diameter about 1.0 cm) of six individuals (A, B, C, D, E, and F) of *Quercus robur* (4 years old, pot plants) were collected on September 17, at +23° C, the trees had been growing under natural conditions outdoor. Then the branches were collected and samples prepared for PAGE. Subsequently the six individuals of English oak were treated in a more complex temperature regimen in climatized chambers as follows: the first (A) 2 weeks at 20° C, the second (B) 1 week at 0° C and another week at +20° C, the third (C) 1 week at −10° C and 1 week at +20° C, the fourth (D) 2 weeks at 0° C, the fifth (E) 1 week at −10° C and another week at 0° C. The last individual (F) was stored for 2 weeks at −10° C. Finally the last samples were taken after one month of rest (growth under natural conditions outdoor) on 2nd of November at +4° C. Splinters were prepared by using a pencil-sharpener.

1.0 g of fresh shavings of branches were extracted overnight at +4° C (1.0 g in 3.5 ml buffer) according to EBERMANN & STICH 1982. Gel electrophoresis in polyacrylamide, detection of peroxidase with benzidine and H_2O_2 (ORNSTEIN 1968) and amylase isoenzymes (EBERMANN & STICH 1982) were performed as described. Polyacrylamide gels containing 4.0% of soluble starch (electrophoresis grade) were incubated at 37° C in 0.5 M acetate buffer, pH 5.0, 1.47 g $CaCl_2$ per liter added, after

precipitation of the starch transparent zones localize the regions with amylase activity. Stained patterns and transparent zones, respectively, were compared by visual inspection.

Results

Fig. 1 shows the peroxidase activity staining of six individuals of English oak after PAGE and staining with benzidine and H_2O_2. The samples had been taken in September. Clearly can be seen, that the genetic variation is expressed in the isoenzyme pattern. Conspicuous is the appearance of green bands in the enzyme pattern of all individuals, which indicates newly synthesized isoenzymes.

The visual comparison of the staining intensity of the bands shows the lowest intensity in Fig. 2. The storage of the trees for four days at different temperatures preserves the green bands, only their intensity varies. Lower temperatures enhance the intensity of the stain (Fig. 2, lanes 3, 5, and 6, samples taken after storage for four days at −10° C, lane 2 and 4 at 0° C).

After seven days of storage the colour of the green band has altered (Fig. 3). Besides the appearance of the new isoenzyme band no major alterations can be seen in the peroxidase pattern of *Quercus robur* after temperature treatment.

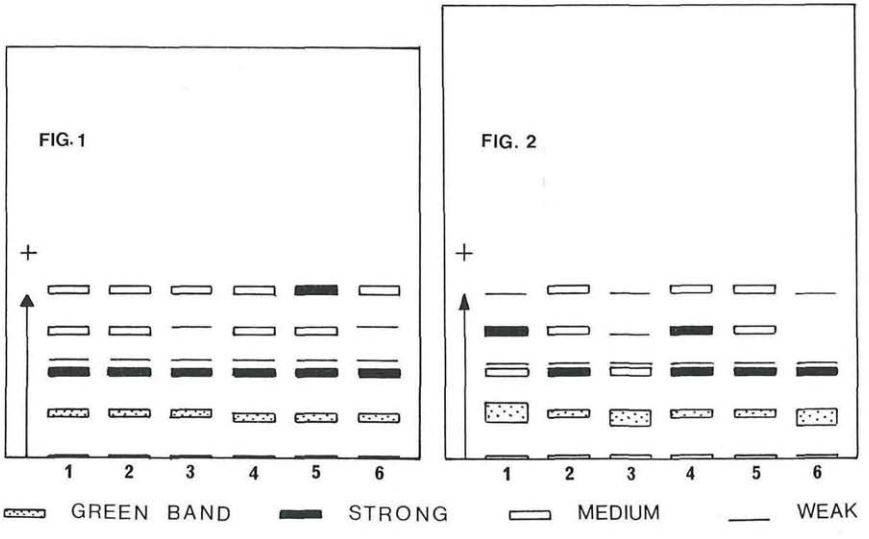
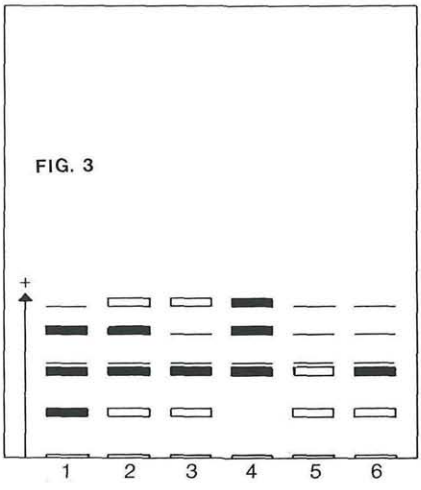


Fig. 1. Peroxidase isoenzymes of *Quercus robur* extracted from branches of six individuals after outdoor cultivation on September 17 at +23° C.

Fig. 2. Peroxidase isoenzymes of *Quercus robur* after 4 days of exposure to temperatures in climatized chambers. Lane 1 . . . individual A, +20° C, lane 2 . . . B, 0° C, lane 3 . . . C, – 10° C, lane 4 . . . D, 0° C, lane 5 . . . E, −10° C, lane 6 . . . F, – 10° C.



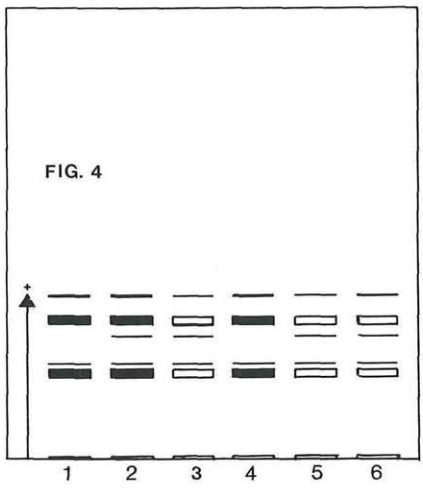


Fig. 3. Peroxidase isoenzymes of *Quercus robur* after 7 days of exposure to temperatures in climatized chambers. Conditions see Fig. 2.

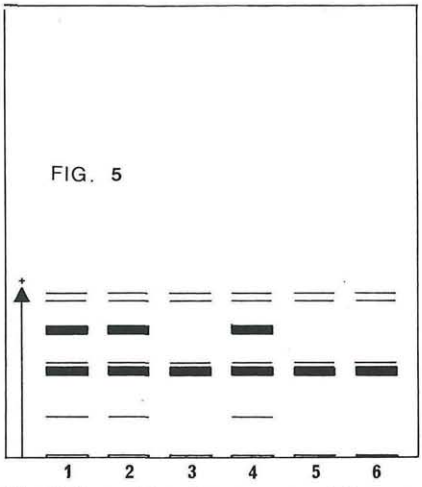
Fig. 4. Peroxidase isoenzymes of *Quercus robur* after 2 weeks of exposure to temperatures in climatized chambers. Lane 1 . . . individual A, 2 weeks at +20° C, lane 2 . . . B, 1 week 0° C, 1 week + 20° C, lane 3 . . . C, 1 week −10° C, 1 week at +20° C, lane 4 . . . D, 2 weeks at 0° C, lane 5 . . . E, 1 week at −10° C, 1 week at 0° C, lane 6 . . . F, 2 weeks at –10° C. – Signatures see Fig. 1 and 2.

After fifteen days of storage (Fig. 4) lane 1 at +20° C lane 4 at 0° C and lane 6 at −10° C, the slowest migrating isoenzyme bands have disappeared. There are increased activities at 20° C and 0° C and low activity at −10° C. It seems that any treatment of −10° C reduces the enzyme activity. One month of growth under natural conditions after the end of the temperature treatment stabilized the peroxidase isoenzyme pattern.

The six individuals from Fig. 1 are shown in their isoenzyme spectrum. In lane 1, 2 and 4 the peroxidases having disappeared (Fig. 4) are synthesized again. After the month without treatment these results are unequivocally, Fig. 5 shows definite differences in the staining intensities. The activity of peroxidase is enhanced in all individuals due to the reason – the samples had been taken on 2nd of November), but the staining intensity of bands with higher mobility is weak in lane 3, 5 and 6, or the individuals show a simplified pattern of 3 or 4 bands.

The individual variation of the amylase enzymes is shown in Fig. 6. The pattern is characterized by three main bands of different R_f-values. Any kind of temperature treatment does alter the pattern (Fig. 6 and 7), a change in the isoenzyme spectrum also in the total activity (compared visually) can be observed.

After the month of rest the pattern has diversified (Fig. 8). The activity of these bands has enhanced. Additionally in all samples several new isoenzyme bands of various R_f-values have appeared.



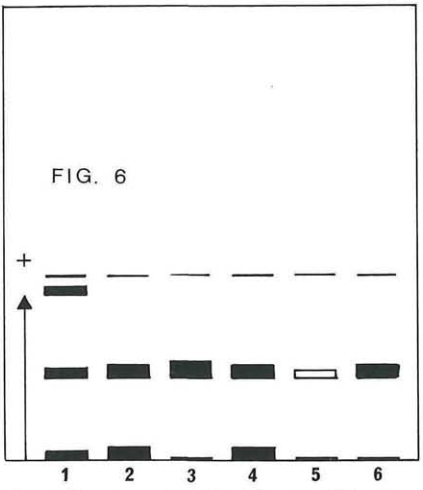
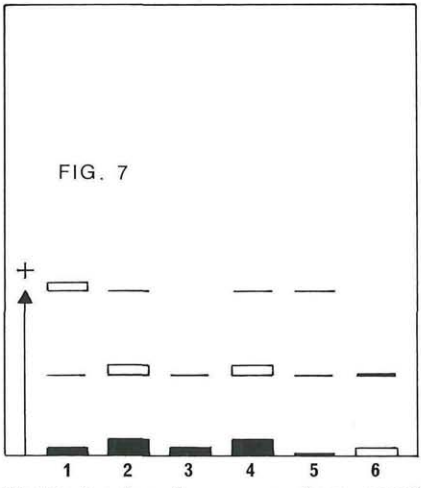


Fig. 5. Peroxidase isoenzymes of *Quercus robur* after 4 weeks of outdoor cultivation, samples taken on November 2 at +4° C. Lane 1 . . . A, lane 2 . . . B, lane 3 . . . C, lane 4 . . . D, lane 5 . . . E, lane 6 . . . F.

Fig. 6. Amylase isoenzymes of *Quercus robur* extracted from branches of six individuals after outdoor cultivation on September 17. – Signatures see Fig. 1 and 2.



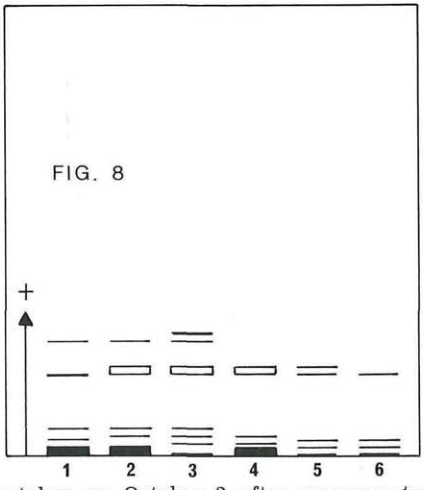


Fig. 7: Amylase isoenzymes from samples taken on October 2 after exposure to temperatures in climatized chambers since September 17. Conditions see Fig. 2, 3 and 4.

Fig. 8: Amylase isoenzymes after 4 weeks of outdoor cultivation taken on November 2. Conditions see Fig. 5. – Signatures see Fig. 1 and 2.

Diskussion

Experiments about frost resistance had been done mainly on test samples like branches which had been put in climatized chambers for standardized times and definite temperatures. Entire trees as objects for lab experiments dealing with frost resistance have the advantage, that the reaction of an individual in all its physical and biochemical behaviour can be watched comprehensively. We have investigated the qualitative and quantitative changes of peroxidase and amylase enzymes isolated from branches which had been cut after the end of the temperature treatment done on four year old trees of English oak in climatized chambers and in field experiments.

Six individuals of trees had been used having one mother in common, this guarantees a broad spectrum of isoenzymes, differing in their mobility in electrophoretic separations in polyacrylamide. The samples isolated from the six trees – the enzymes had been extracted from the wood of the branches – before any treatment had started show in all lanes the same peroxidase isoenzyme pattern though differences in enzyme activities exist. In amylase patterns (Fig. 6) differences occur in the course of the experiments, the individuals show varied reactions. The total activities of peroxidases are diminished unter cold stress (−10° C, Fig. 2 and 3, lanes 3, 5, and 6). The enzyme system of the trees reacts fast and, after four days significant differences in the activities can be observed. The activation is thought to be started by the change of the length of the day, the duration of sunshine, phytohormones, and the change of temperature verse cold (LARCHER 1981, BIEBL 1967, SCHWARZ 1968). Detailed experiments with several species confirm these results (KORORI 1989). the peroxidase of trees of which the enzyme had been isolated in summer reacts on any cool or cold temperature treatment with a reduction of the activity. If the same experiment is done in winter the peroxidase activity will be found to be enhanced. Peroxidases play a significant role in polymerization processes, in trees i. e. the lignification. Heartwood formation takes place during in the winter rest (NELSON 1978, SHAIN & MACKAY 1973). Seasonal attributes influence the reactions of peroxidase, p. e. temperature.

Longer exposure of the trees to moderate temperature (+20° C) results in a high activity (Fig. 4, lane 1); when the moderate temperature treatment is preceded by a treatment for one week at 0° C the enhancement is the same. But if in the first week −10° C had been applied, the reduction of the peroxidase activity is drastically (Fig. 4, lane 3). It seems that any treatment of −10° C (four days as well as two weeks) drastically reduces the enzyme activity. After a period of rest in which the trees had been held outdoor the pattern of the isoenzymes in the six individuals shows considerable variety. The individuals treated moderately show strong bands in the electropherogram, those treated cool have a weakly stained isoenzyme pattern. It seems

that some of the isoenzymes are more sensitive to low temperatures than others (compare Fig. 3). Two main bands are stable in their locations, but even their activities vary from tree to tree. Individuals E and F are treated in the same manner, but the intensities of the isoenzymes differ from the start of the experiment.

In Fig. 1 and 2 green and brown oxidation products of benzidine appear, the green colour disappears in the course of time, in samples taken after seven days of temperature treatment only brown stain remains. Apparently this phenomenon is a matter of newly synthesized isoenzymes. The brown and the green staining originates from different oxidation products of the substrate on the gel. It is known that depending on the concentration of the enzyme and of the peroxide a stable green colour can be produced, peroxidases of different origin, human and of plants, show this phenomenon in reactions on polyacrylamide gel or in solution (LICKL, unpublished data). A newly synthesized isoenzyme is possibly present in the location of these green zones. They appear only in samples which had been investigated in their peroxidatic activity in early autumn, at a time when the total activity of peroxidase in plants is rather low, and the electropherogram had not shown any band at these distinct R_f-values earlier.

The carbohydrate metabolism as indicated by the amylase activity is also affected by the temperature treatment. The pattern and the intensity of amylase isoenzymes do alter also, when the trees are placed in climatized chambers as described. After the month in the open fields subdued to the climate a marked change in the pattern is shown (Fig. 8). The characteristics of the main bands of activity remain the same, but additionally every sample shows seveal sharp bands of isoenzymes.

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