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Phenolic compounds in the Karelian birch (*Betula pendula* Roth. var. *carelica* (Merklin) Hejtmánek)

INTRODUCTION

The Karelian birch (*Betula pendula* Roth. var. *carelica* (Merklin) Hejtmánek) is believed to be a variety of silver birch (*Betula pendula* Roth.).

Komšilov and Selivanova 1962 have conducted basic studies on the chemical composition of the wood of Karelian birch and of silver birch, collected in Karelia. They have studied the content of lignin, celluloze and pentosans. On the basis of the results obtained they are inclined to claim that there are no differences between these two birches in the chemical composition of wood.

Surmiński 1964 has studied the chemical composition of the pathological wood tissues from cancerous burls growing on silver birch trees. The content of methoxylic groups in the wood samples correlates with the pigmentation of the pathological tissues. In darker wood the content of methoxylic groups was greater.

The wood of Karelian birch, is darker compared with the wood of silver birch, which is probably caused by a greater content of phenolic substances or the products of their oxidation.

The aim of the present study was to establish the reason for the darker colour of Karelian birch wood and to determine the biochemical mechanism responsible for it. The establishment of qualitative differences in the chemical composition could be useful in the early differentiation of the Karelian birch seedlings from the silver birch ones, which would be of considerable importance for the cultivation of this birch.

MATERIALS AND METHODS

For the study use was made of wood, leaves and cambium from Karelian birch (trees with the typical characteristics of Karelian birch), from silver birch of the Karelian birch population (trees obtained from seeds collected from Karelian birch trees growing in Gorce, but showing no signs of the characters typical for Karelian birch) and from silver birches of local stock.

The material for studies originated from trees growing in the natural stand of Karelian birch in Gorce or from seedlings raised from open pollinated seeds collected on the Karelian birches from that region. The seedlings were raised in Kórnik nr. Poznań (Zwierzyniec forest) by T. Jakuszewski. A detailed characteristic of the population of Karelian birch now growing in Kórnik has been presented by Jakuszewski (in press).

Also wood samples of Karelian birch from Finland have been investigated. These were obtained through the courtesy of dr. M. Hagman from the Forest Tree Breeding Station in Maisala.

The phenolic compounds have been extracted with acetone (wood and cambium) or with water (leaves). The water extracts after being acidified with 3% HCl have been extracted with diethyl ether. Also hydrolyzates of the tissues were studied after they have been heated for 20 minuts with 3% HCl on a boiling water bath and extracted with diethyl ether.

For the identification of phenolic compounds in the extracts use was made of ascending two dimensional paper chromatography and thin layer chromatography. The chromatograms were observed under UV light at $\lambda = 365$ and 252 nm in ammonium fumes, after being sprayed with 3% Na_2CO_3, 3% NaOH and 1% $AlCl_3$ in ethanol (reagent for flavonoids), and developed in diasotized sulfanilic acid.

For chromatography the following solvents were used:

Thin layer chromatography on silica gel FG_{254} – Merck

Chloroform – acetic acid (95 : 5),

Chloroform – ethyl acetate – formic acid (50 : 40 : 10).

Paper chromatography – Schleicher and Schuell 2043 b

1) Butanol – acetic acid – water (4 : 1 : 2), 2) 3% acetic acid.

1) Benzene – acetic acid – water (1 : 1 : 1), 2) 3% acetic acid.

1) Isopropanol – ammonia – water (10 : 1 : 1), 2) 3% acetic acid.

The content of chlorogenic acid in the leaves of birches has been determined by the Zucker and Ahrens method (1958). The results obtained have been expressed as the weight in mg of chlorogenic acid in 100 g of leaf dry weight.

The activity of phenolases has been determined by the manometric method as described by Tomaszewski (1961). It was expressed in µl of oxygen used per mg of protein by an acetone preparation and calculated from the determination of nitrogen in the sample by the Kieldahl method.

The activity of peroxidases has been determined colorimetrically by the method described by Safonov (1970). The activity of peroxidases oxidizing benzidine has been expressed in the units of the colorimetric scale (linear extrapolation of extinction) calculated for 1.0 mg of an acetone preparation of birch cambium.

Separation of the peroxidase isoenzymes from birch cambium has been performed by the method of disk electrophoresis on polyacrilamide gel as described by Davis (1964). The amounts of enzymatic preparations used for the studies have been equal in terms of activity. The isoenzymes have been developed by a benzidine solution according to Safonov (1970).

RESULTS AND DISCUSSION

Extensive studies on the biosynthesis of phenylopropane derivatives and on lignification have been conducted by Neish in 1960. He has established the main metabolic pathways leading to the most common phenylopropane derivatives and their participation in the synthesis of flavonoids. The synthesis takes place primarily in the leaves.

In the leaves of birches studied by us we have not been able to find any differences as regards the composition of phenolic compounds. The studies covered leaves of 11 trees of Karelian birch, 11 trees of silver birch from the progeny of Karelian birches and 3 trees of local silver birch. The leaves were collected in three vegetative periods (in June, July and September). Between the collection times no differences were observed in the qualitative composition of phenolic compounds.

In the leaves of the studied birches the following phenolic compounds have been identified: 1. chlorogenic acid, 2. *p*-coumarylquinic acid, 3. caffeic acid, 4. quercetin, glucosides of quercetins nos. 1, 2, 3 and 4 (glucoside no. 4 was not found in all leaves of the studied trees), 5. kaemferol, glucosides of kaemferol nos. 1 and 2, 6. elagic acid, 7. *d*-catechin, 8. *l*-epicatechin, 9. *p*-coumaric acid, 10. ferulic acid, 11. synapic acid, 12. gentisic acid, 13. vanilic acid, 14. syringic acid, 15. *p*-hydroxybenzoic acid.

The phenolic compounds, and in particular the phenylopropane derivatives participate in the biosynthesis of lignin, which is a product of the condensation of alcohols: *p*-coumaric, coniferylic and synapic (Freudenberg 1965). These processes take place in wood with the participation of oxidases.

Komšilov and Selivanova (1962) have found that there are no differences in the chemical composition of wood between silver birch and Karelian birch. Our studies have shown that the wood of Karelian birch does not contain *d*-catechin. The wood for our studies came from 12 year old trees of Karelian birch from the natural stand in Gorce, Poland, and from 4 and 8 year old trees raised in Kórnik from seed collected from Karelian birches in Gorce. In the studies were included wood samples from one year old branches on 8 year old trees, collected in the fall and winter. Also we have studied a wood sample of Karelian birch from Finland. In all these samples of Karelian birch we have not been able to establish the presence of *d*-catechin. It appears that *d*-catechin becomes oxidized in the wood.

In 1964 Surmiński has studied the cancerous growths on the stems. He reports results of analyses concerning the content of methoxyl groups in the wood. In the darker pathological wood tissue he has found a greater content of methoxyl groups, which determine the degree of lignification. The wood of Karelian birch compared with the wood of silver birch is darker. In order to clarify the cause for this difference, the cambial juice has been subjected to analysis.

The cambial juice has been collected in May 1971. For this purpose an 8 year old tree of Karelian birch has been cut down as well as an 8 year old silver birch tree from the progeny of Karelian birches and also a tree of local silver birch. As regards the content of phenolic compounds cambium of these birches does not appear

to differ much. In the cambium of Karelian birch we have found *d*-catechin, but also large quantities of dark substances which have lower R_F values in the studied solvent systems, give a dark pigmentation with NaOH and with diasotized sulfanilic acid. One has to conclude that these are products of condensation of phenolic compounds such as *d*-catechin. Presumably these substances cause the darker pigmentation of the wood of Karelian birch.

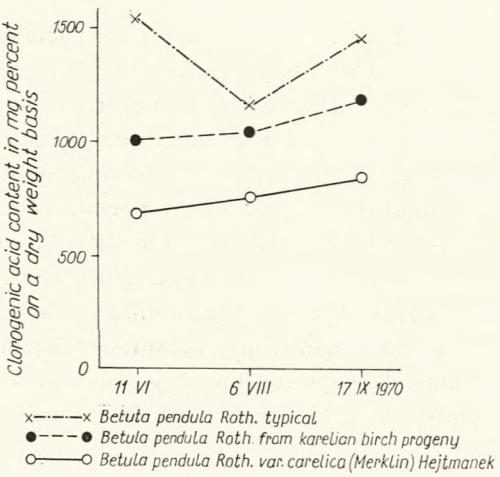

Fig. 1 Seasonal changes in chlorogenic acid content in leaves in mg per cent on a dry weight basis

Presumably differences in the activity of oxidizing enzymes determine the specificity of the chemical composition of the wood of Karelian birch. For this reason the study of enzyme activity was also undertaken — of polyphenoloxydases in the leaves and of peroxidases in the cambium. We have also identified the content of chlorogenic acid in the leaves of birches, which is related to the activity of the polyphenoloxydase.

Leaves for the study of chlorogenic acid content have been collected at three vegetation periods (in June, August and September). The content of chlorogenic acid in the leaves is presented in fig. 1. The leaves of birches differ in the content of chlorogenic acid, and these differences are maintained through-out the vegetation period. The results obtained have been subjected to a statistical analysis including the Duncan test, with the help of which it was possible to prove the significance of the difference between the studied populations of Karelian and silver birch from the same general progeny of Karelian birches in the content of chlorogenic acid. For the determinations leaves were collected from 11 silver birches from the Karelian birch progeny, 11 Karelian birches and 3 silver birches of local origin.

In August and September 1970 determinations of the activity of polyphenoloxydases isolated from the leaves of birches were made. In the studied samples it was found that there exist significant differences in the activity of polyphenoloxydases. Results of these studies are presented in fig. 2. The polyphenoloxydase from the leaves of

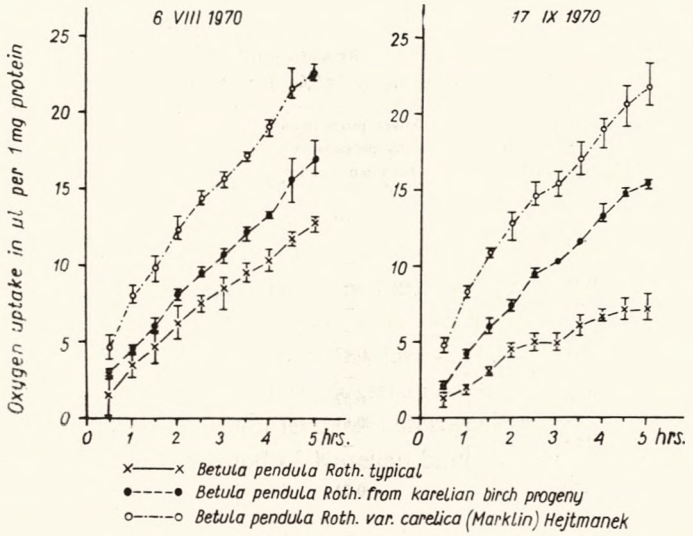

Fig. 2 Phenoloxydase activity in leaves

Reaction mixtures: enzyme in 5×10^{-3} M. phosphate, buffer pH 6.9, chlorogenic acid 5×10^{-3} M. in final conc. 10% KOH added in the central cup.

the Karelian birch is most active which is also indicated by the results of chlorogenic acid determinations in these leaves, where they are least concentrated.

In the cambium of the birches peroxidases were also studied. The activity was measured and the isoenzymes were separated. The results of activities of the peroxidases isolated from the cambium of birches are presented in table 1.

The activity of the peroxidase isolated from the cambium of Karelian birch is about 100 times greater than the activity of the peroxidase isolated from the cambium of silver birch from the Karelian birch progeny, and 40 times greater than the activity of the peroxidase from the cambium of local silver birch.

The very active oxidizing enzymes present in the Karelian birch oxidize *d*-catechin. The products of oxidations of the phenolic substances participate in the synthesis

Table 1

Activity of peroxidases oxidizing benzidine isolated from the cambium of birches expressed in the units of the colorimetric scale (linear extrapolation of extinction) calculated per 1.0 mg of acetone extract according to Safanov

	Time in sec.			
	30	60	90	120
Silver birch (*Betula pendula* Roth.)	0.174	0.257	0.294	0.331
Silver birch from the progeny of Karelian birches	0.084	0.105	0.119	0.133
Karelian birch (*Betula pendula* Roth. var. *carelica* (Merklin) Hejtmánek)	7.1	10.36	12.43	14.06

Table 2

Spectrum of isoenzymes isolated from the cambium of birches. Numerical values represent the electrophoretic velocity in R_F for the peroxidase isoenzymes

Karelian birch (*B. pendula* Roth. var. *carelica* (Merklin) Hejtmánek	Silver birch from the progeny of Karelian birches	Silver birch (*B. pendula* Roth.)
–	0.20	–
0.22	–	0.22
–	–	0.34
0.38	–	–
–	–	0.40
–	–	0.42
0.47	0.47	0.47
–	–	0.51
0.56	0.57	0.56
0.61	0.61	–
0.64	–	0.64
–	–	0.66
0.70	0.71	0.70
0.73	–	0.72
–	–	0.74
0.77	–	–
–	–	0.80
–	–	0.84

of lignin (Neish 1960) and they can also lead to a denaturation of proteins, particularily of enzymes, which can be the reason for the abnormal cambial activity.

After having used the electrophoresis it was possible to show that the peroxidases isolated from cambium of the studied birches differ in the composition of the isoenzymes. Results of the separation of the isoenzymes of peroxidases isolated from cambium of birches are presented in table 2. The numerical values represent the R_F of the individual isoenzyme bands separated on a polyacrilamide gel according to the method of Davis. The amounts of the separated samples where identical as regards their activity.

The oxidizing enzymes isolated from the Karelian birch, particularily from the cambium are more active, and this explains the darker colour of the wood of Karelian birch compared with the silver birch in which the non oxidized *d*-catechin is present.

SUMMARY

Comparative studies on the qualitative composition of phenolic compounds in the wood, cambium and leaves of Karelian birch, silver birch from the progeny of a Karelian birch and a typical silver birch, have led to the identification of qualitative differences in the chemical composition of wood.

The wood of Karelian birch does not contain *d*-catechin.

The leaves of the studied birches have similar composition of the phenolic compounds. In the leaves the presence of the following phenolic compounds was established: chlorogenic acid, *p*-coumarylquinic acid, elagic acid, *p*-coumaric acid, ferulic acid, caffeic acid, synapic acid, gentisic acid, vanilic acid, syringic acid, *p*-hydroxy-

benzoic acid, *d*-catechin, *l*-epicatechin, kaemferol, glucosides of kaemferol, quercetin and its glucosides.

The cambium of the Karelian birch contains *d*-catechin and dark products of phenol condensation.

Studies on the activities of enzymes in the cambium have shown that the peroxidase of Karelian birch is about 100 times more active than the peroxidase of cambium from silver birches from the progeny of Karelian birches and 40 times more active than the peroxidase from cambium of a local silver birch. After the use of electrophoresis on a polyacrilamide gel it was shown that the peroxidases from the cambium of the birches differ in the composition of the isoenzymes.

Studies on the activity of the phenoloxydase in the leaves have shown that the enzyme is most active in the leaves of the Karelian birch. On the other hand the leaves of Karelian birches have the lowest levels of chlorogenic acid.

The darker colour of the wood of Karelian birch is caused by the high activity of the oxidizing enzymes.

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Związki fenolowe w brzozie karelskiej (Betula pendula Roth. var. carelica (Merklin) Hejtmánek)

Streszczenie

Porównawcze badania składu jakościowego związków fenolowych w drewnie, kambium i liściach brzozy karelskiej oraz brzóz brodawkowatej z populacji karelskiej i brodawkowatej typowej pozwoliły na znalezienie jakościowych różnic w składzie chemicznym drewna.

Drewno brzozy karelskiej nie zawiera *d*-katechiny.

Liście badanych brzóz mają podobny skład związków fenolowych. W liściach stwierdzono obecność następujących związków fenolowych: kwasy: chlorogenowy, *p*-kumarylochinowy elagowy, *p*-kumarowy, ferulowy, kawowy, synapinowy, gentyzynowy, waniliowy, syryngowy *p*-hydroksybenzoesowy; *d*-katechina, *l*-epikatechina, kaemferol i glukozydy kaemferolu, kwercetyna i jej glukozydy.

Kambium brzozy karelskiej zawiera *d*-katechinę, prócz tego ciemne produkty skondensowanych fenoli.

Badania aktywności enzymów w kambium wykazały, że peroksydaza z kambium brzozy karelskiej jest około 100 razy aktywniejsza niż peroksydaza z kambium brzozy brodawkowatej z populacji karelskiej oraz 40 razy aktywniejsza niż peroksydaza z kambium brzozy brodawkowatej. Przy zastosowaniu elektroforezy na żelu poliakrylamidowym wykazano, że peroksydazy z kambium brzóz różnią się składem izoenzymów.

Badania aktywności fenoloksydazy w liściach brzóz pokazały, że fenoloksydaza z liści brzozy karelskiej jest najaktywniejsza. Liście brzozy karelskiej natomiast zawierają najmniej kwasu chlorogenowego.

Ciemniejsze zabarwienie drewna brzozy karelskiej spowodowane jest wysoką aktywnością enzymów utleniających.

КАЗИМЕЖ КРАВЯЖ

Фенольные соединения карельской берёзы (Betula pendula Roth. var. carelica (Merklin) Hejtmánek)

Резюме

Проведено сравнительное исследование состава фенольных веществ в древесине, камбии, листьях берёз карельской и бородавчатой обычной, а также из популяции карельской. Обнаружено, что, в отличие от берёзы бородавчатой, древесина карельской берёзы не содержит *d* — катехина.

Состав фенолов в листьях был идентичен, а именно: хлорогеновая, *p*-кумарилхинная, эллаговая, ванилиновая, сиреневая, кофейная, синаповая, гентизиновая, *p*-оксибензойная кислоты; *d*-катехин, *l*-эпикатехин, кемпферол, кверцитин и их глюкозиды.

В камбии карельской берёзы найден *d*-катехин, а также тёмноокрашенные продукты конденсации.

Активность пероксидазы у камбия была в 100 раз выше у карельской берёзы, чем у бородавчатой из карельской популяции и в 40 раз выше, чем у обычной. Электрофоретическое разделение на полиакриламидном геле показало разницу в изоэнзимах пероксидазы.

Листья карельской берёзы имели наивысшую активность полифенолоксидазы и самый низкий уровень хлорогеновой кислоты.

Более тёмная окраска древесины карельской берёзы, повидимому, вызвана высокой активностью окислительных ферментов.