Short Note: Electrophoretic Characterization of the Euramerican Poplar Clones 'I-214' and 'Campeador'

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Summary

Biochemical phenotypes of two Euramerican poplar clones, *Populus* × *euramericana* 'I-214' and 'Campeador' were determined by starch gel electrophoresis. As it is not possible to distinguish both clones apart when utilizing morphological characters as criteria, some have thought in the last few years that they were identical. This hypothesis can be discarded as both clones show clearly different banding patterns with two isoenzyme profiles: Phosphoglutamase (PGM) and peroxydase (PX).

Key words: *Populus* × *euramericana* clones, starch gel electrophoretic characterization, peroxydase, phosphoglucomutase.

Resumen

Mediante técnicas electroforéticas en gel de almidón se ha conseguido determinar en hojas los fenotipos bioquímicos de dos clones euramericanos de chopo: *Populus* × *euramericana* 'I-214' y 'Campeador'. Al no ser posible la distinción de ambos clones mediante caracteres morfológicos, en los últimos años se venía planteando la posibilidad de suidentidad. Con el trabajo que se presenta, queda anulada esta hipótesis, al presentar ambos clones, patrones de bandas claramente diferentes para dos sistemas enzimáticos: Fosfoglucomutasa (PGM) y Peroxidasa (PX).

Zusammenfassung

Bei zwei *Populus* × *euramericana*-Klonen (I-214 und Campeador) wurden die biochemischen Phänotypen mittels Stärkegel-Elektrophorese bestimmt. Da es nicht möglich ist, die beiden Klone anhand morphologischer Merkmale zu unterscheiden, wurden sie in den letzten Jahren für identisch gehalten. Diese Hypothese kann widerlegt werden, da beide Klone deutlich unterschiedliche Isoenzym-Muster aufweisen und zwar PGM (Phosphoglucomutase) und PX (Peroxidase).

Introduction

Phenotypical resemblance among commonly used clones is frequent. The morphology, alsometry and phenology of many clones are often found to be nearly the same. This is the case of the two major clones of *Populus* of the Spanish poplar culture, namely: *Populus* × *euramericana* (Dode) Guinier 'I-214' and 'Campeador'.

Because of this resemblance, in the last few years a strong controversy has raised at the international level regarding the assumed identity of both clones (Chardenon, 1981).

The resemblance between both clones is evident from a phenotypic viewpoint. Actually their behaviour in terms of vegetative propagation, growth and resistance to pathogens as well as their general phenology is similar. They are even similar from the alsometric point of view, as shown exemplarily by Padro (1984) for 'I-214' and by Antonanzas (1978) for 'Campeador'.

Electrophoretic identification of isoenzymes has proved to be a highly efficient method to differentiate genotypes. This technique has also been found to be applicable to different poplar species, such as *P. tremula* (Guzina, 1978), *P. trichocarpa* (Weber and Stettler, 1981) and *P. tremuloides* (Cheliak and Pitel, 1984).

The object of this study was, therefore, to discriminate the two clones 'I-214' and 'Campeador' by means of electrophoretically detectable isozyme patterns.

Material and Methods

Dormant buds, bark of shoots and leaves were tested. Part of this material was freeze-dried and stored under dry conditions and at room temperature for further use. The rest of the material was used two hours after collection.

As for leaves, the first fully-grown one from the top on each shoot was chosen from stool beds growing in nursery. These leaves had to be diseasefree, undammaged and without symptoms of aging. In order to avoid seasonal variations in zymogram patterns, samples were collected in spring, summer and autumn from annual shoots of several evenaged stumps subject to uniform cultural practices.

The samples consisted of one bud or 1 cm^2 of bark, fresh leaf or freezedried leaf, ground in a mortar where we had previously poured two drops of the extraction buffer.

For leucine amino peptidase (LAP) and peroxidases (PX), the extraction buffer was Glutation (0.1 M Tris at pH = 8.5 and 1% reduced Glutation, adjusting the whole to pH = 7.5—8.0), and for phosphoglucomutase (PGM) it was Polyvinyl-Pyrrolidone (PVP) (0.5% ascorbic acid; 1% Glutation; 1 mM EDTA; 0.1 M Tris; 0.25% Triton X 100 and 10 mM $MgCl_2$), adjusting the whole to pH = 7.5—8.0 and supplemented with 15% Polyvinyl-Pyrrolidone (PVP).

Once the sample was crushed, the crude juice was absorbed in 5 mm × 2 mm filter paper wicks (Whatman No. 3), trying to avoid the absortion of impurities.

The samples were put into slots cut in 1 mm thick starch gel (12%) prepared the day before, these slots were placed at a distance from the cathode equal to one third of the total length of the gel.

Electrophoresis was run at a temperature of 0—4° C for 10 minutes at 100 V, and then the wicks were removed. Electrophoresis was continued at 250 V for two more hours. The intensity was kept below 40 mA during the whole process.

The gel and tray buffer as well as the extraction buffer were chosen according to the recommendations for the different isoenzymes under study. (Scandalios, 1969; Tanksley, 1979).

We used the following staining methods: for PX the staining method of Graham *et al.* (1964), for PGM, Tanksley's (1979) method and for LAP two systems were applied, i.e., Scandalios (1969) method with and without Tanksley's (1979) modifications.

Results and Conclusions

The bark showed very low activity. The buds proved to be somewhat more active but not enough to make clear banding patterns.

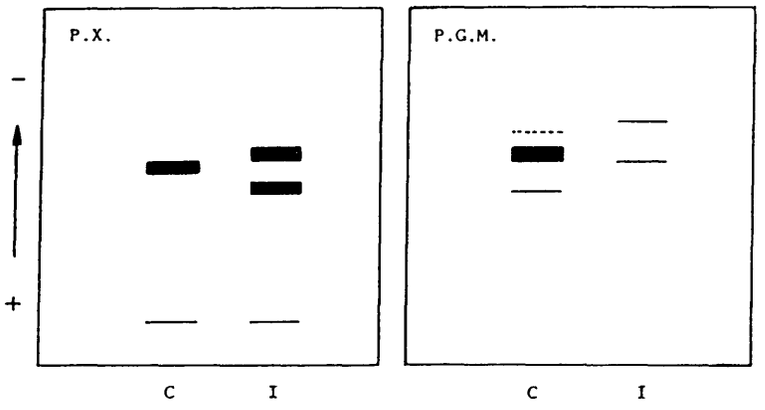

Figure 1. — Electrophoretical patterns of peroxidase (PX) and phosphoglucomutase (PGM) in two *P.* × *euramericana* clones 'I-214' (I) and 'Campeador' (C).

The leaves, both fresh and freeze-dried, showed a similar behaviour in terms of number of bands, intensity and position for each clone studied, regardless of collection time and stump. The freeze-dried material remained active for seven months.

LAP showed activity with SCANDALIOS' (1969) method, which revealed six clear bands with no perceptible differences between the two clones but did not show any activity with SCANDALIOS' method modified by TANKSLEY (1979).

PGM and PX also showed clear banding patterns, this time clearly differentiated for 'I-214' and 'Campeador' as shown in *Figure 1.*

No cathodic activity was found in any instance.

As the differences found in the banding patterns of both clones cannot easily be attributed either to electrophoresis identification or to the sampling procedure, these differences are bound to be the visible sign of different genotypes. This substantiates the hypothesis that Euramerican clones 'I-214' and 'Campeador' are different.

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Buchbesprechungen

Provenances and Forest Tree Breeding for High Latitudes. By D. LINDGREN (ed.). Proceedings of the Frans Kempe symposium in Umeå June 10—11, 1986. Report No. 6, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Umeå, 1986.

This proceedings is available free of charge from: Department of Forest Genetics and Plant Physiology, SLV, S-901 Umeå, Sweden.

Biochemical Genetics and Legislation of Forest Reproductive Material. By H.-J. MUHS (edited). Proceedings of the IUFRO Joint Meeting held at Grosshansdorf, FR of Germany, from June 25 to 28, 1985, Institute of Forest Genetics and Forest Tree Breeding, Sieker Landstr. 2, D-2070 Grosshansdorf 2. Mitteilungen der Bundesforschungsanstalt für Forst- und Holzwirtschaft, 154. Hamburg, 1986. 211 pp. DM 23,—.

The proceedings contain papers of a joint meeting of IUFRO Working Parties S2.04-05 Biochemical Genetics and S2 03-14 Legislation of Forest Reproductive Material held at the Federal Research Centre of Forestry and Forest Products, Hamburg, at the Institute of Forest Genetics and Forest Tree Breeding at Grosshansdorf (near Hamburg), FR of Germany, from June 25 to 28, 1985. Of the 25 papers included in the proceedings the much larger portion of 21 papers deal with legislation of forest reproductive material and only the remaining four about biochemical genetics. These four papers all have the use of the isoenzyme technique in common. Two are concerned with the proof of changes in the genetic structure due to air pollution, while one has the genetic structure of *Castanea sativa* provenances and the other mass pollination in *Pinus sylvestris* seed orchards as subject.

Concerning the second topic, legislation of forest reproductive material, first a synopsis is given about some national and international rules. Then two papers follow, one by an East and one by a West European member about certification problems. A block of 5 papers are concerned about the problem of finding a consensus between the laws of nature and man-made rules and regulations in order that evolutionary forces can still be effective in "wild" tree populations which most today and future forest stands are. Also ways are shown to characterize and identify forest reproductive material (mainly by isozymes) to have means of verifying observance of the different rules and regulations. Following this, some special problems are dealt with like problems with the category "tested reproductive material", optimal number of standards in tests, priority for resistance testing in the regulations, legal aspects of early testing, and transfer of newly bred varieties of public research institutions into practical use. At last, a group of 7 papers is concerned with the legal aspects of clones in forestry: production of clones by cuttings or in-vitro culture, necessary number of clones in clonal mixtures, influences affecting optimal clone numbers, protection of forest plant varieties, rules for certification of multiclonal varieties and a synopsis of some national regulations for multiclonal mixtures.

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