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Characterization of Oak (*Quercus* L.) Seed Proteins by Electrophoresis

By

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Summary

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Total seed proteins of four *Quercus* species extracted from bulked seed samples were analyzed by modified SDS-PAGE in order to obtain additional taxonomically useful descriptors. A total of 7 alternative protein bands with different mobility rates were identified within a molecular weight range of 24 kDa to 36 kDa. *Quercus robur* L. and *Q. petraea*/Matt./Leibl. showed equal electrophoregrams. *Q. pubescens* Willd. can be discriminated from *Q. robur* L. and Q. petraea/Matt./ Leibl. by its two additional bands. *Q. rubra* L. showed a significantly different electrophoregram with completely novel protein bands.

Introduction

Taxonomic problems of oak are related to its high morphological variability which is probably related to the outbreeding nature of *Quercus* species. It is known that cross-pollination promotes the maintenance of high levels of genetic variability in certain species or populations which can make their accurate identification difficult. In addition, the appearance of natural hybrids among different species in the genus *Quercus* hampers successful resolution of taxonomic nomenclature. In Europe several oak species have been reported as potentially interbreeding, especially *Q. robur* and *Q. petraea*. Variability of the genus *Quercus* has been studied by many authors with morphological descriptors, as well as by isoenzyme and some novel

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DNA markers, but differentiation within the genus *Quercus*, especially with *Quercus robur* and *Q. petraea*, has not yet been fully solved.

The seed protein profile, obtained by electrophoresis, was frequently used in taxonomic and evolutionary studies on account of several advantages: (i) the seed protein profile is highly stable and species specific (LADIZINSKY & HYMOWITZ 1979), because (ii) proteins are relatively direct gene products and can be considered as markers of these genes, and differences in the electrophoretic profiles are presumably proportional to the genetic divergence among the plants being compared (LADIZINSKY & HYMOWITZ 1979, CRAWFORD 1990); (iii) the electrophoretic protein phenotype is not affected by growing conditions; (iv) seeds represent a defined physiological state so different samples are fully comparable; (v) due to the general heterogeneity of seed proteins, this technique has given good results with many plant species; (vi) protein electrophoresis is rapid and inexpensive in comparison with new DNA molecular methods, although the latter are more informative.

Although the structure of seed storage proteins from economically important crops has been intensively studied, little is known about the seed proteins of forest trees. However, COLLADA & al. 1988 electrophoretically analyzed seed storage proteins in *Fagaceae* species. The results showed that species of the genus *Quercus* L. predominantly store glutelins, while the major protein fraction of *Fagus* and *Castanea* was found to be globulins. Therefore, the present study was undertaken first to optimize the SDS-PAGE technique for the separation of oak seed proteins and second to investigate the possibility of using the seed protein electrophoretic profiles for the identification of *Quercus* species.

Materials and Methods

Acorns were collected from different trees in five locations of pedunculate oak (*Quercus robur*) and in one location of sessile oak (*Q. petraea*), pubescent oak (*Q. pubescens*) and red oak (*Q. rubra*). Proteins were extracted from single seeds and bulked seed samples of a particular tree and location.

Total seed proteins were extracted from the dry crashed acorns for two hours at room temperature in the ratio 1:5 w/v, using a 0.07 M Tris-HCl electrophoresis sample buffer (pH 6.8), 4% SDS, 3% 2-mercaptoethanol, 10% glycerol, 0.02% Bromphenol Blue. In the SDS-PAGE discontinuous system the separating gel of GIULIAN & GRAHAM 1992-1993 was applied using pH 8.8 gel buffer instead of pH 9.3. The final concentration of the separating gel was 20% acrylamide (T%) with 0.5% of crosslinker (C%), 0.75 M Tris-HCl (pH 8.8), 0.1% SDS, 10% glycerol. The stacking gel contained 4% acrylamide (T%) with 2.7% of crosslinker (C%), 0.125 M Tris-HCl (pH 6.8), 0.1% SDS, 10% glycerol. The electrode buffer contained 0.025 M Tris, 0.192 M glycine, 0.1% SDS. Electrophoresis was carried out for about 5 hours at a constant current of 10 mA per gel. A vertical slab electrophoresis system was used. The 1.0 mm thick gels were stained twice with a solution of 0.05% Coomassie Brilliant Blue R, 5% ethanol, 12% trichloroacetic acid, for 24 hours.

Results and Discussion

At first, the most commonly used SDS-PAGE system of LAEMMLI 1970 with the acrylamide concentration of 10%T, 12.5%T and 15%T was examined but the separation of protein bands was not satisfactory (results not shown). Therefore the modified, low molecular weight SDS system described by GIULIAN & GRAHAM 1992-1993 was tested. With this rather unusual gel system a notably better separation of protein bands was achieved, so this system was applied for all further analyses.

Electrophoretic identification of cross-pollinated species is rather difficult because of the genotypic variation of an individual within a certain species or population. A similar situation can be observed in *Quercus* species where single seed analysis of four species showed certain variation of the electrophoretic protein banding pattern among different trees, as well as within a particular tree (results not shown). Electrophoresis of seed proteins of a bulked seed sample has proved to be a more effective method for distinguishing cultivars of cross-pollinating crops (GARDINER & FORDE 1992, as an example). A bulk sample is a composite seed sample that represents the mixture of electrophoretic phenotypes present in a certain cultivar, population or species (ROGL & JAVORNIK 1996).

The oak protein banding patterns of the bulk samples were divided into three distinct regions A, B, C, equivalent to decreasing molecular weights (Fig. 1). Regions A and C contained very thin, weakly stained and rather unreproducible bands. Region B consisted of 7 major, dark protein bands within a molecular weight range of 29 kDa to 45 kDa (Fig.1) which were used for oak species discrimination. Evaluation of protein profiles was done visually on gels, scoring only qualitative band differences, i.e. the presence/absence of a protein band. The numerical formulas of the four species (Q. robur, Q. petraea - [2b, 6b], Q. pubescens, [1b,2b,4b,6b], Q. rubra - [3b,5b,7b]) were constructed. With Q. robur and Q. petraea no major differences were found among the protein bulk electrophoregrams consisting of two bands 2b and 6b (Fig. 1). There is also an additional band on Q. robur and Q. petraea electrophoregrams (marked with an asterix on Fig. 1). This thick and smeared protein band consists of a bulk of several co-migrating bands and consequently was not included into band classification. The electrophoregram of Q. pubescens can be discriminated from Q. robur and Q. petraea by the two addittional bands 1b and 4b (Fig.1) while Q. rubra showed a significantly different electrophoregram with the completely novel bands 3b, 5b and 7b (Fig.1).

From our study we can conclude that electrophoretic seed protein profiles of four *Quercus* species are in agreement to a certain degree with their general systematic order. According to KRÜSSMANN 1978 *Q. robur*, *Q. petraea* and *Q. pubescens* are closely related species belonging the European group, while American *Q. rubra* is more distantly related. The use of seed protein profiles in systematic studies is based on the presumption that closely related species show more similar electrophoretic protein patterns than those that are phylogenetically less related (CRAWFORD 1990). Due to the fact that the seed protein profile is species-specific (LADIZINSKY & HYMOWITZ 1979), the question arises whether

pedunculate and sessile oak, showing identical protein electrophoregrams, are from the biological species concept indeed separate species. According to the protein profile the *Q. pubescens, Q. robur* and *Q. petraea* are more closely related , while American *Q. rubra* showed the expected significant distance from the other three species. The results of our study have confirmed that SDS-PAGE method presented could be an important additional tool for differentiation of *Quercus* species.

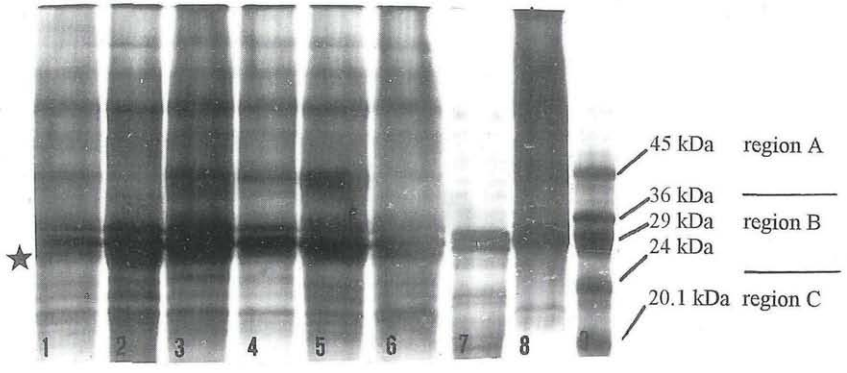

Fig. 1. SDS-PAGE bulk electrophoregrams of seed proteins of Quercus species: *Quercus robur* L. (lanes 1 - 5), *Q. petraea* /Matt./ Leibl. (lane 6) *Q rubra.* L. (lane 7), Q. pubescens Willd. (lane 8) and marker proteins (lane 9).

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