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Nuclear microsatellites as a tool in the genetic certification of forest reproductive material. A case study in sessile oak (*Quercus petraea*, Matt., Lieb.)

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Introduction

Oaks, as long-living forest tree species, need to maintain their amount of genetic variation in order to adapt themselves and their progenies to complex heterogeneous environments. For companies trading with seeds and plants, it is very important to know whether their products reveal high quality of reproductive material with respect to adaptive potential. Genetic certification can essentially contribute to the verification of such quality. This category of certification mainly addresses the following topics:

1) quantification of genetic variation;
2) monitoring of inbreeding;
3) detection of the geographic origin.

The aim of a commercial seed and plant trading company is to offer to forestry genetically characterized reproductive material. For this reason we mainly focused on the validation of a quick and efficient method, based on molecular tools, for investigating genetic variation in nursery material. We have chosen *Quercus petraea* because it is an important deciduous tree species for plant production in nurseries.

Earlier inventories on genetic variation made in *Quercus* species mainly used various isoenzyme markers (Müller-Starck *et al*. 1991, 1993; Bacilieri *et al*. 1994; Zanetto *et al*. 1994).

Objective

Our objective was to perform a genetic inventory employing highly polymorphic DNA markers, called microsatellites or SSR (simple sequence repeats consisting of a set of tandemly repeated nucleotide motifs), because they have been shown to be easily applicable for the following reasons:

- they are amplified by standard PCR - amplification protocols;

- the PCR products can be pooled, allowing multiple loading (an advantage for rapid genotyping of large sample sizes);
- genotyping is a relatively simple process, when the fluorescently labelled fragments are, for example, scored on an automated laser sequencer.

Material and methods

We evaluated genetic diversity for 8 oak populations that originated from different areas of Germany. After acorns were collected, the material was sown in the nursery and then 100 seedlings per population were randomly chosen for our investigation. At the time of the collection of the material for the molecular analysis, four of the populations were part of a commercial livestock of three-year-old plants, and the other four populations were included in an international provenance trial.

DNA was isolated from buds. PCR amplifications were performed with five variable nuclear microsatellites, developed by Steinkellner *et al.* (1997). The PCR products were separated in an acrylamide/urea gel and detected by an automated laser sequencer ALFexpress (Pharmacia).

Results

1) Quantification of genetic variation.

Polymorphism in a population is due to the existence of different genetic variants. The basis of variation is thus the number of polymorphic loci together with the number of alleles and their frequency distributions in a population. Based on this well known concept, we checked for each locus the number of different alleles and the genetic diversity. A large number of alleles was identified in the populations. All five microsatellite loci were highly polymorphic with an average of 15.6 alleles per locus. The level of variation is much higher than at isoenzyme loci. As an example, in Müller-Starck *et al.* (1991) the mean average number of alleles in *Quercus petraea* was 3.1.

The diversity *v*, calculated following Gregorius (1978), indicated small deviations among the populations surveyed (range 6.33 - 8.40), although no statistical test was applied to test the significance of these deviations.

2) Monitoring of inbreeding.

Theoretically, if one allele is present at high frequency and all others show low frequencies, then the population will be characterized by a low degree of heterozygosity because, by necessity, most individuals will be homozygous for the common allele. For our populations, the allelic frequency profiles were characterized by few frequent alleles and several rare alleles. Both expected (He) and observed heterozygosity (Ho) do not deviate substantially among the populations surveyed; however, the He values exceeded the observed ones (mean value 0.86 vs. 0.60). Further statistical analyses will be applied to these data to assess the significance of the departures.

The observed heterozygosity deficiency was also clearly indicated when we computed Weir's fixation index (F=1-Ho/He). This index measures the deviation of the proportion of the observed heterozygotes from Hardy-Weinberg expectation. Negative values indicate that the population is characterized by an excess of heterozygotes. On the other hand, values above zero indicate an excess of homozygotes. The overall mean F-value in our populations was 0.29 and revealed a significant deviation (at the 1% significance level) from H-W expectation.

The present results may be interpreted as indication of inbreeding or, in a few cases, of genetic selection following the given environmental conditions. This assumption is supported by the fact that the excess of homozygote genotypes and the corresponding deficiency of heterozygote genotypes hold for all frequent genotypes at four nuclear SSR loci of the five investigated. Isoenzyme investigations, performed in our lab, on two of these populations showed similar trends with low levels of heterozygosity (data not shown). An exception from these results was found, however, only in one seedling population in the case of one primer pair, where an excess of heterozygous genotypes was observed (data not shown, La Scala *et al*. in preparation). It has however to be noted that for microsatellites heterozygosity may be overestimated and misinterpretation can arise, because of the slippage effects during the amplification process.

3) Analysis of geographic origin.

One region-specific allele was found in the population Müllrose and the population Eberswalde at one of the five microsatellite loci. The presence of this allele could explain why the two populations, both of which are located in northeastern Germany, reveal greater similarities to each other than to the other populations analysed (La Scala *et al*. in preparation). Even though the nuclear microsatellites tested turned out to be hypervariable with nearly 30 alleles detected in the populations surveyed, differentiation among populations was very low.

For a better understanding of possible geographic variation of the eight sessile oak populations, further investigations are going on using chloroplast microsatellites (cpSSRs). The use of variants present in cytoplasmic organelles (such as mitochondria or chloroplasts) seems to be more appropriate to study geographic variation. This is due to the lack of recombination because of uniparental mode of inheritance (in oaks chloroplasts are transmitted only by seeds).

Conclusions

The present study on genetic inventories by means of molecular tools in sessile oak populations suggests the following conclusions:

- application of the nuclear microsatellite techniques in oak trees has successfully indicated a higher degree of genetic variation than was detected by previous isoenzyme analyses;
- the high level of homozygosity allows the quantification of inbreeding, which is an important parameter to verify genetic quality of reproductive material;
- when the purpose of the study is to investigate geographic variation, cytoplasmic markers, such as chloroplast microsatellites or other uniparentally inherited markers, should be applied because they can be expected to be much more indicative.

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