SHORT COMMUNICATION

INTRASPECIFIC VARIATION IN CHLOROPLAST DNA *psb*AI GENE REGION OF SILVER FIR (*ABIES ALBA* MILL.)

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ABSTRACT

Based on Southern-blot analysis the eight restriction enzyme specific variants were revealed in silver fir (*Abies alba* Mill.) confirming intraspecific variation in its chloroplast DNA *psb*AI gene region. The paternal inheritance of detected variants was illustrated in three interspecific hybrids of firs using *Bam* HI digestions. At the population level, a differential occurrence of individual variants was found in the two natural stands of silver fir in Slovakia. It is suggested that nature of the revealed cpDNA polymorphisms makes them useful for genetic and population studies of the species.

Key words: *Abies alba*, chloroplast DNA, *psb*AI gene region, intraspecific variation

INTRODUCTION

The molecular genetic markers derived from direct analysis of genetic polymorphism in DNA sequences represent a novel approach in evaluation of forest tree genetic variability. In comparison with isozyme markers they share several advantages among which the absence of tissue specifity and ontogenetic variability, stability towards environmental variation and potentially unlimited number are worth of mentioning (NEALE & SEDEROFF 1988). In case of forest trees it is the variation in their chloroplast DNA (cpDNA) which has been paid preferential attention, mainly due to its occurrence in multiple copies. This makes its analysis easier. Also, a cpDNA molecule is smaller and structurally simpler than nuclear DNA what allows straightforward molecular interpretation of its polymorphism (PALMER 1985). With special reference to the chloroplast genome of conifers, the cpDNA variants are sought to be distributed non-randomly, the vast majority of them being localized in one or a few "hot spots" only. These regions are likely to be composed of DNA that does not encode functional products owing to which they may mutate with little deleterious effects (STRAUSS *et al.* 1992). In silver fir such regions has been detected between the genes *trn*S and *psb*C as evidenced by the restriction site polymorphisms in the corresponding polymerase chain reaction (PCR) products revealed in 10 populations of the species by ZIEGENHAGEN *et al.* (1995).

As far as *Abies* species are concerned, it is only a third illustration of the intraspecific variation at this level. Using *atp*A probe (ATPase a-subunit) from cpDNA of *A. sachalinensis*, HAYASHI *et al.* (2000) reported of 2 *Eco* R I variants revealed among 19 populations of the species. The same was true of *A. mariesii* populations which were shown to be differentiated with respect to the enzyme/probe combinations *Hind* III/pCS7, *Hind* III/pCS10 and *Bgl* II/pCS7. The cpDNA probes were isolated from *Cryptomeria japonica* and from tobacco. No variation has on the other hand been found in three spacer regions between tRNA genes of cpDNA in *A. alba* and *A. nebrodensis* populations as reported by VICARIO *et al.* (1995). Our approach based on Southern blotting of single-digests of total DNA and subsequent hybridization with a heterologous probe of *Pinus contorta psb*AI gene has resulted in revealing some additional intraspecific polymorphisms in *A. alba* cpDNA.

MATERIALS AND METHODS

The two populations of silver fir (*Abies alba* Mill.) from the polluted area of the Upper Nitra region (Middle Slovakia) were involved into study. The

population Kamenec is located on the exposed northern side of the Vtá...nik Mountains, while the population in Jedµové Kostolany on the avert side of the mountain ridge. The number of tested individuals ranged between 20 mature trees from Kamenec and 21 trees from Jedµové Kostolany.

Along with mature silver fir trees, the seedlings of the interspecific crosses *A. alba* × *A. pinsapo*, *A. pinsapo* × *A. numidica* and *A. numidica* × *A. cephalonica* were used together with the corresponding parental trees. Five randomly chosen seedlings per each cross were analysed. The hybrids were obtained previously by the artificial hybridization of the species growing in arboretum MlyÁany (KORMUØÁK 1994).

Total DNA was extracted from young needles of firs using modified protocol by MURRAY & THOMPSON (1980). Only 3 g of fresh mass needle instead of 100 g material prescribed and 7 ml instead of 100 ml of „Ct extraction buffer" were used at the homogenization step.

10 µg of total DNA from the mature silver fir trees was digested with *Pst* I, *Rsa* I, *Dra* I, *Bam* H I, *Bcl* I, *Hind* III, *Eco* R I, *Xho* I, *Sac* I, and *Sal* I, respectively, (*Boehringer*, Mannheim), according to the producer`s instructions. The DNA samples of hybrid seedlings were digested using *Bam* H I only. Electrophoresis of restriction fragments was carried out in 0.8 % agarose gels for 10–12 h at 2.7 V cm^{-1} and 4 EC in 1 TBE. As a molecular mass standard, 1 kb DNA ladder (BRL) was used which comprises a ladder of 1018 bp increments ranging from 1018 to 12216 bp. The restriction fragments of cpDNA transferred from a gel to the *Hybond N* matrix by Southern blotting were hybridized with the nick-translated probe using 10 kb *Hind* III fragment of *Pinus contorta psb* AI gene region. The fragment was cloned into pUC19 vector as the pPCH273 clone (LIDHOLM & GUSTAFSSON 1991).

The differences at the frequencies of cpDNA variants in two *A. alba* populations were tested statistically by a binomial test.

RESULTS AND DISCUSSION

Screening of such a large segment of cpDNA resulted in revealing extensive polymorphism in silver fir individuals. Except for the *Pst* I and *Rsa* I generating identical restriction fragment patterns, the remaining digestions confirmed the presence of individual variation in the respective segment. The two cpDNA haplotypes (variants) were detected following *Hind* III, *Eco* R I, *Sac* I, *Dra* I, *Bcl* I and *Xho* I digestions, respectively, whereas the three variants in *Sal* I and *Bam* H I digestions (Fig. 1). The one and/or two fragment based haplotypes refer to the *Hind* III, *Dra* I and *Bam* H I digestions with one fragment differing in size, while those with a three fragment pattern to *Sac* I, *Sal* I and *Bcl* I. The *Xho* I variants consist of 6 and 7 restriction fragments, respectively.

Utility of these polymorphisms in population and genetic studies of firs has subsequently been tested using two *A. alba* populations and three interspecific crosses. All the above variants were found to occur in a varying proportion in the silver fir populations in Kamenec and in Jedµové Kostolany. It follows from Table 1 that except for the *Dra* I variants the population Kamenec consists of equal or nearly equal number of the haplotypes of a given restriction nuclease type. The population Jedµové Kostolany deviates in this respect having less balanced proportions of the variants. Of interest is in this connection a complete absence of the *Dra* I – 2 variant in the population Kamenec.

At the species level, the paternal inheritance of these variants was illustrated on the example of *Bam* H I digestions in hybrids. The *Bam* H I – 2 variant of the male tree *A. pinsapo* in *A. alba* × *A. pinsapo* crossing has also been exhibited by the hybrid seedlings as contrasted with the *Bam* H I – 3 variant of the female tree *A. alba* (Fig. 2, lanes 1–7). The reverse was true for the *A. numidica* × *A. cephalonica* combination with *Bam* H I – 1 variant shown by the male species *A. cephalonica* and the corresponding progeny (lanes

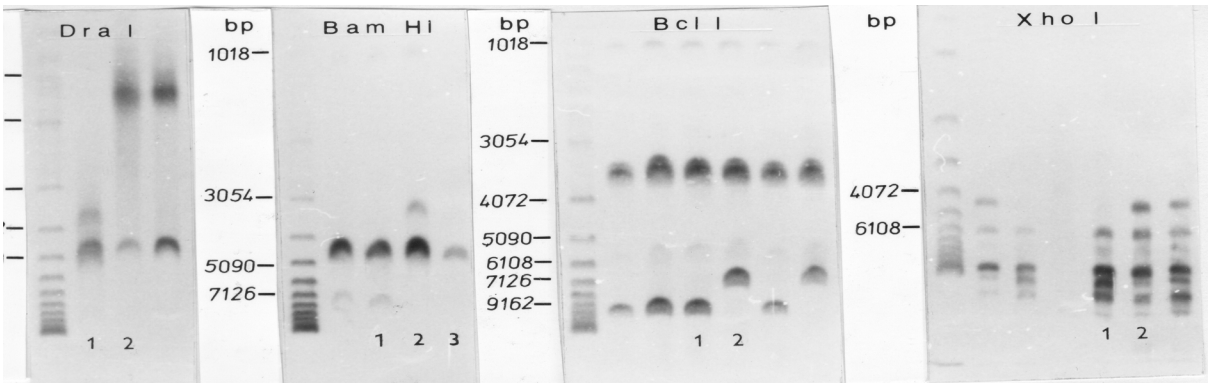

Figure 1 Restriction enzyme specific variants revealed in *psb*AI gene region of silver fir cpDNA

Table 1. The number and frequency of silver fir variants at the populations Kamenec and Jedµové Kostolany.

Enzyme	Haplotypes	Population		*P*
		Kamenec	Jedµové Kostolany	
Dra I	1	20	15	0.021*
	2	0	6	
Bam H I	1	10	11	1.000
	2	9	9	
	3	0	1	
Bcl I	1	10	7	0.345
	2	10	14	
Hind III	1	10	8	0.536
	2	10	13	
Eco R I	1	10	8	0.535
	2	10	13	
Xho I	1	8	12	1.000
	2	7	9	
Sac I	1	10	12	1.000
	2	8	9	
Sal I	1	5	11	0.314
	2	7	5	
	3	6	5	

13–19). Being of the same variant (*Bam* H I – 2) the parental trees *A. pinsapo* (lane 7) and *A. numidica* (lane 13) were indistinguishable from each other as well as from their progeny (lanes 9–12). The only exception was the hybrid seedling positioned under lane 8 (Fig. 2) possessing *Bam* H I – 1 variant which did not correspond to any of the parental variants. Most probably it represents a contamination.

In spite of generally postulated conservative nature of the chloroplast genome, there are frequent reports about cpDNA variations in forest trees. They refer preferentially to the inter-specific level but in *Pinus contorta*, *Pinus banksiana* (WAGNER *et al.* 1987), *Quercus robur* (DUMOLIN-LAPÈGUE *et al.* 1997), *Fagus sylvatica* (DEMESURE *et al.* 1996) and *Abies alba* (ZIEGENHAGEN *et al.* 1995) the intraspecific variation in PCR-RFLP profiles of their cpDNAs has also been reported. As far as *Abies* is concerned, the two variants were detected among *A. alba* individuals using the *trn*S-*psb*C/*Hae* III fragment/enzyme combination (ZIEGENHAGEN *et al.* 1995). The region includes coding and non-coding sequences between flanking regions of both the above genes with estimated size of 1500 bp. SZMIDT & PARDUCCI (1999) have extended their analysis of cpDNA of nine additional regions covering nearly 18 % of the molecule size. They have revealed numerous polymorphisms in *Abies* species. No intraspecific variability has however been detected so far, except for the *trn*S-*psb*C/*Hae* III. Referring to the interspecific variation, the conclusion has been drawn postulating the existence of multiple variable regions that appear to be distributed throughout the whole genome. The authors suggest that cpDNA in the genus *Abies* may be more polymorphic than in other conifers. The results presented in this study validate this idea at least partially. The size of a screened cpDNA fragment was incomparably larger than that of *trn*S-*psb*C and adequately increased was also the number of revealed

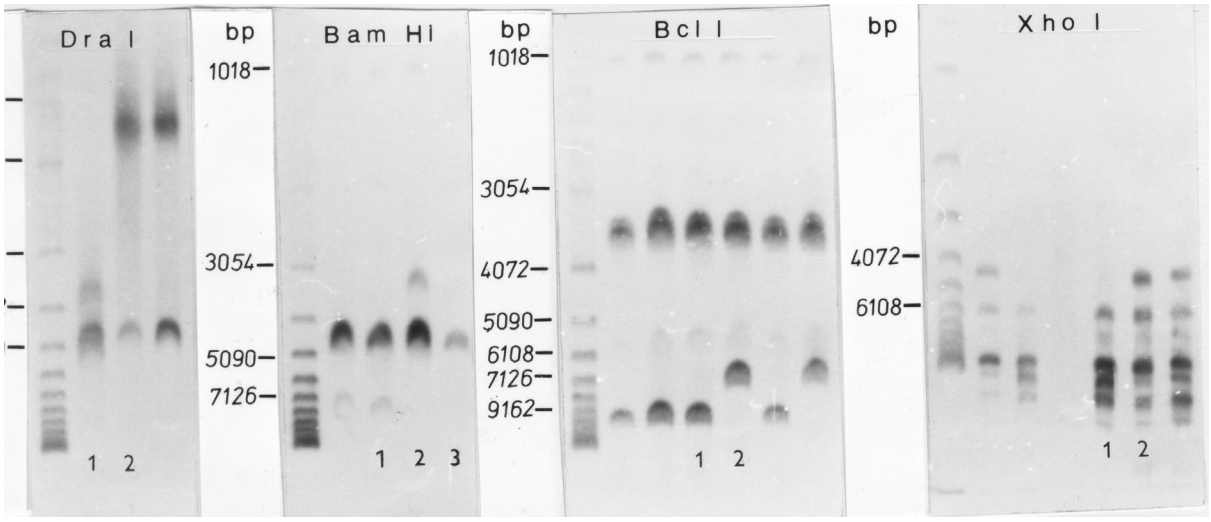

Figure 2 *Bam* HI restriction profiles of cpDNA in *A. alba* (1), *A. pinsapo* (7), *A. numidica* (13), *A. cephalonica* (19) and in their respective hybrids *A. alba* × *A. pinsapo* (2–6), *A. pinsapo* × *A. numidica* (8–12) and *A. numidica* × *A. cephalonica* (14–18). Arrows indicate less intensively stained fragments.

individual polymorphisms. Of the 10 endonucleases used, the 8 restriction enzymes yielded polymorphic restriction patterns which follow paternal inheritance. WAGNER *et al.* (1987) demonstrated that certain regions of conifer cpDNA appear as "hot spot" showing intraspecific variation which otherwise is rare due to the conserved nature of *cp* genome. SZMIDT (1991) considers it interesting that this variable region is located in a close proximity of the *psb*A genes.

The nature of revealed restriction patterns makes them useful for population studies of silver fir. Individual variants were found to occur differentially in the two populations studied. The most conspicuous was a complete absence of the *Dra* I – 2 variant in Kamenec. Binomial test confirmed statistical significance of this difference what may indicate the potential of this probe/enzyme combination for differentiation between silver fir populations.

Taking into account presented data we may conclude that intraspecific polymorphisms revealed within a *psb*A gene region of silver fir cpDNA proved their utility in genetic and population studies of the species. Along with the *Hae* III restriction patterns of the flanking region between the *trn*S and *psb*C genes they represent a new possibility for individual variation analysis in silver fir. This type of cpDNA variation has in general been considered to be underestimated what is particularly true for the conifers (SOLTIS *et al.* 1991). Therefore, additional analyses are necessary aiming at describing the genetic nature of revealed polymorphism in silver fir at the nucleotide sequence level and at subsequent confirming postulated variation of cpDNA on a broader scale.

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