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Restriction fragment length polymorphism of chloroplast DNAs in some species of fir (*Abies* sp.)

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Abstract

Restriction fragment analysis of the chloroplast gene psbA was carried out in twelve *Abies* species native to Asia, Europe and North America using the four restriction enzymes. The pair of Asiatic representatives of *A. koreana* and *A. veitchii* differed profoundly not only from each other but also with respect to the European and Northamerican species. The variation observed within the last mentioned groups of firs was mainly due to the diferent restriction profiles of the gene in *A. alba* and *A. nordmanniana* species of the former region as well as owing to its heterogeneous nature in all the four species from North America. The RFLP data presented to far only partially correlate with the taxonomic status and hybridological relationships of the species concerned.

Introduction

Within context of the plant molecular techniques the restriction fragment length polymorphism of DNA (RFLP) represents a novel approach which has enabled to reveal the type of variation hardly detectable by either morphological or karyological criterions (Rivin *et al.* 1986). Also, as an electrophoretic separation technique, it is believed to have a higher diagnostic potential than the isoenzyme data (Helentjaris *et al.* 1986). At the species level is prefered the chloroplast DNA (cpDNA) which owing to its small size and simple structure became a target of intensive molecular studies in various plant taxa (Kataoka *et al.* 1987). A conservative nature of cpDNA together with the size differences of its restriction fragments in related species have on the other hand contributed to the utilization of restriction analysis as an evolutionary tool in the study of phylogenetic relationships among plant species (Palmer 1985).

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According to Kaneko *et al.* (1986) it may be extremely useful for studies on woody plants, because other genetic means are usually difficult to apply to such large plants whose life-span is so large.

Being smaller, the conifer cpDNA occupies a unique position in plant kingdom, with the size amounting to only about 120 kbp (Kondo *et al.* 1986, Howe *et al.* 1988). This shortening is partially due to the absence of two inversely oriented repeated segments that are commonly present in cpDNA of most plants but which have supposedly been lost quite early in the phylogeny of 200 million year old group of conifers (Szmidt 1988). In order to verify the utility of chloroplast genome in discrimination between conifers, the attempt has been made to compare the restriction fragment profiles of its psb A gene in twelve species of the genus *Abies*.

Material and methods

In addition to the domestic species *Abies alba* Mill., the foreign representatives of firs introduced to our climatic conditions from Asia, Europe and North America have also been included into study. Their list consists of *A koreana* Wils., *A. veitchii* Lindl., *A. nordmanniana* (Stev.) Spach, *A. cephalonica* Loud., *A. cilicica* (Ant. *et* Kotschy) Carr., *A. pinsapo* Boiss., *A. numidica* De Lann., *A. concolor* (Gord. et Glend.) Lindl., *A. grandis* (Dougl.) Lindl., *A. balsamea* (Linn.) Mill., and *A. procera* Rehd., respectively.

Total cellular DNA was extracted from the young needles of fir trees by a modification of the method of Murray and Thompson (1980). Needle tissue (10 g, fresh mass) was homogenized with polytron (8 s) in 25 ml of extraction buffer consisting of 50 mM TRIS-HCl, pH 8.0, 2 % hexadecyltrimethylammonium bromide (CTAB), 1.25 M NaCl, 10 mM 2-mercapto-ethanol, 7 mM EDTA, 1 % bovine serum albumin, and 5 % polyvinylpyrrollidone, respectively. The homogenate was filtered through several layers of cheesecloth and one layer of Miracloth. After one chloroform/octanol (24:1) extraction, the mixture was centrifuged at 10 000 *g* and 4 °C for 10 min., DNA containing fraction was repeatedly extracted with chloroform/octanol and centrifuged. The fraction was brought to a final concentration of 1 % CTAB/0.7 M NaCl and incubated at 60 °C for 10 min. After one additioned extraction with chloroform/octanol and centrifugation the DNA was precipitated with two volumes of ethanol and collected by centrifugation at 5 000 rpm for 10 min (4 °C).

10 μg of total cellular DNA from each sample was digested with Taq I, Bgl II, Hind III and Alu II (*Boehringer*, Mannheim), according to the producer's instructions. Electrophoresis of restriction fragments was carried out in 0.8 % agarose gels for 10 - 12 h at 2.7 V cm^{-1} and 4 °C in TRIS-borate-EDTA buffer, pH 8.0 (Maniatis *et al.* 1982). 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 cpDNAs transferred from a gel to the *Hybond N* matrix by Southern blotting were hybridized with the nick-translated probe of psbA gene isolated from *Spinacea oleracea* L. and cloned into pUC 18 vector. The gene itself is localized in the chloroplast genome where it codes for the D1 protein

which is an integral part of the photosystem II reaction center. It appears to be duplicated in all higher plants, including the pines (Lidholm *et al.* 1988).

The genetic similarity between chloroplast genomes of individual species was calculated according to the formulae Kaneko *et al.* (1986) using the percentage of differential restriction fragments.

$$P_{ij} = \frac{d_{ij}}{n_i + n_j} \times 100$$

where n_i and n_j are the total fragment numbers observed in four restriction patterns of the ith and jth species, and d_{ij} is the number of differential fragments found between the two species.

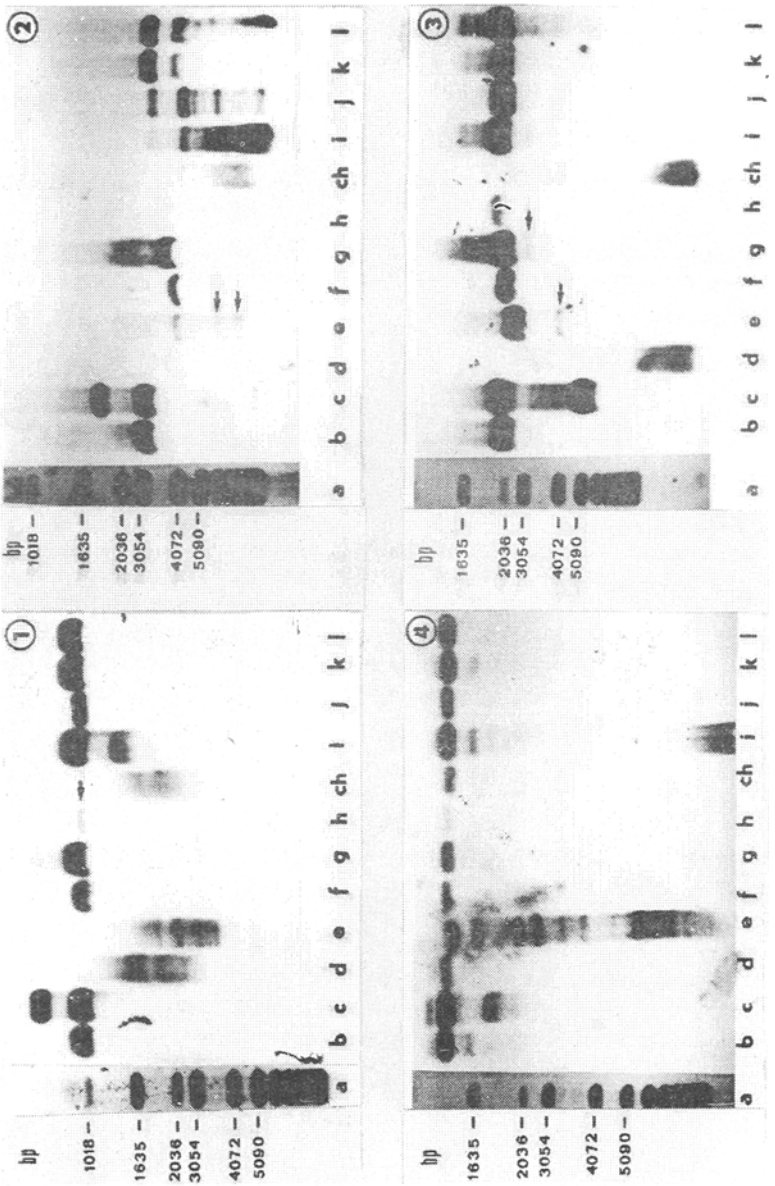
Results and discussion

The restriction profiles of psbA gene obtained by the four endonucleases have in a varying degree reflected the uniqueness of the species investigated but only partially correlated with their taxonomic positions.

The asystematic nature of the results is apparent in all the three groups of firs sampled so far but the most distinct was in this respect the pair of the Asiatic representatives of *A. koreana* and *A. veitchii* which in spite of a common occurrence within the section Elate (Tang 1971) differed strikingly in the number of their restriction fragments. As shown in Fig. 1 - 3, only one fragment of *A. koreana* (*b*) generated by Taq I, Bgl II and Hind III had as a rule contrasted with the two-fragment restriction patterns obtained correspondingly in *A. veitchii* (*c*). The digest of Alu I was the only exception producing one additional fragment in the former (Fig. 4b) and two supernumerary fragments in the latter (Fig. 4c) deepening thus a differentiation of these species. We can only speculate to what extent the observed diversity of psbA gene in *A. koreana* and *A. veitchii* affects their crossability relationship which according to Klaehn and Winieski (1962) is characterized by a striking inkompatibility.

Among the European species the highest variability was observed within the section *Abies* with *A. alba*, *A. nordmanniana* and *A. cephalonica* involved. Particularly conspicuous was the difference in restriction fragment profiles of psbA gene in *A. alba* and *A. nordmanniana*. As evidenced by their Alu I digests, one fragment of the former contrasted with as many as eleven fragments of the latter (Fig. 4d,e). It is the only difference between these species which has not however been possible to confirm by the Bgl II and Hind III nucleases, mainly due to the failure with digestion of *A. alba* cpDNA (Fig. 2d, 3d). Also, the variation between both the above species (Fig. 1d,e) is probably due to the problems with digestion of their cpDNAs by Taq I.

The species *A nordmanniana* seems to deviate by its psbA gene not only from *A. alba* but to some degree also from *A. cephalonica*. Figs. 2 and 3 illustrate the presence of some minor components in Bgl II and Hind III digests of *A. nordmanniana* (*e, arrows*) which have not been detected in *A. cephalonica*. The



Figs. 1 - 4.Restriction profiles of cpDNA obtained after digestions with Taq I (*1*), Bgl II (2), Hind III (*3*) and Alu I (*4*) in *A. koreana* (*b*), *A. veitchii* (*c*), *A. alba* (*d*), *A. nordmanniana* (*e*), *A. cephalonica* (*f*), *A. cilicica* (*g*), *A. pinsapo* (*h*), *A. numidica* (*ch*), *A. concolor* (*i*), *A. grandis* (*j*), *A. balsamea* (k), and *A. procera* (l), respectively.*a* - 1 kb DNA ladder

restriction pattern of the latter is characterized by only one fragment in all the four phenotypes obtaned (Fig. 1 - 4f).

Because of unsatisfactory cleavage of cpDNAs in *A. pinsapo* and *A. numidica*, the chloroplast genomes of these representatives of the section Piceaster as well as that of *A. cillicica* belonging to the same section are comparable only partially. Alu I phenotypes of all the three species are identical, consisting of only one fragment of psbA gene (Fig. 4). The same holds for the Taq I profiles of *A. pinsapo* and *A. numidica* (Fig.1g,h) and Bgl II phenotype of *A. pinsapo* (Fig. 2g). A small difference seems to exist only between restriction fragments of Alu I in *A. pinsapo* and *A. numidica* (Fig. 3g,h) as evidenced by the minor fragments in the first of the mentioned species (*arrow*).

It follows from the described restriction patterns of psbA gene that variation observed among the European species does not correlate perfectly with their taxonomic positions. Having in mind the fact that species as a regional group are characterized by the mutually compatible hybridological relationships which are especially close between the intrasectional combinations *A. alba* × *A. nordmanniana* and *A. cephalonica* × *A. nordmanniana* (Kantor and Chira 1972, Greguss 1984, Tang Shui-Lui 1971), we must admit the lack of homology between the RFLP data of the species and their genetic afinity. Surprisingly enough, but just these pairs of species were found to differ profoundly in their psbA genes. This conclusion can partially be applied also to the North American species of firs among which both extremities may be observed including a complete identity as well as a considerable heterogeneity of their cpDNAs. The species of *A. concolor* and *A. grandis* belong systematically into the section Grandes and hybridize spontaneously or artificialy very easily (Gathy 1957, Hawley and De Hayes 1985). Nevertheless, their Taq I (Fig. 1i,j) and Alu I (Fig. 4i,j) profiles differ considerably. A high genetic affinity between them may be inferred only on the basis of their Bgl II and Hind III phenotypes (Fig. 2i,j and 3i,j). The same holds for the species *A. balsamea* and that of *A. procera* which appear to be differentiated with respect to the Bgl II (Fig. 2k,l), Hind III (Fig. 3k,l) and Alu I (Fig. 4k,l) restriction fragments what correlates with their taxonomic positions within the sections Balsameae and Nobiles, respectively.

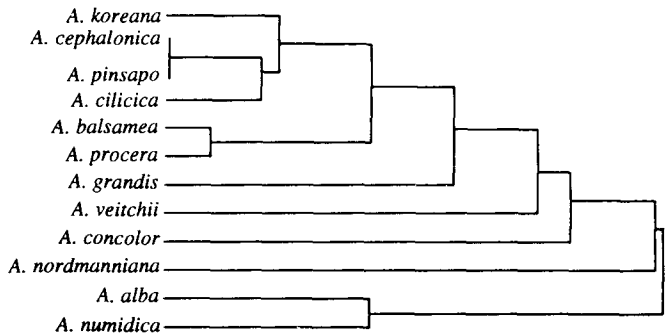


Fig. 5. Dendrogram showing the genetic distances between individual species of firs in terms of dissimilarity coefficients.

All the above described diferences are schematically expressed in a dendrogram which illustrates the relative distances between individual species (Fig. 5). It is evident that their positons reflect only partially the classification scheme based on morphological character differences. Arbitrary is in this respect a separation of European species *A. cephalonica*, *A. pinsapo* and *A. cilicica* from those of *A. nordmanniana*, *A. alba* and *A. numidica* by the intruded group of Northamerican species *A. balsamea*, *A. grandis* and *A. concolor*, respectively. Widely separated positions of Asiatic species *A. koreana* and *A. veitchii*, indicating a considerable divergency, coincide with their RFLP data but it is highly desirable to confirm their status by hybridization experiments.

In conclusion we can state that the Asiatic, European and Northamerican firs investigated so far are differentiated with respect to their psbA genes, the last mentioned group being more heterogeneous, than the European species. It is of interest to mention in this connection that both the European and Northamerican firs were shown to be reproductively isolated (Kormuťák 1985). A strong tendency towards divergence from the European and Northamerican firs have also displayed the pair of the Asiatic representatives under study. However, in order to obtain a more conclusive evidence related to the role of the chloroplast DNA in the process of genetic differentiation of firs it is necessary to verify the diagnostic value of additional genes of the chloroplast genomes with a special emphasis on those which share the properties of genetical markers.

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