A taxonomic analysis of seed proteins in *Pinus* spp. (*Pinaceae*)

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**Abstract:** Seed storage proteins have proved to be a powerful biochemical marker for taxonomic research, but they have not been extensively employed in forest tree studies. In order to improve the understanding of the taxonomy of the genus *Pinus*, total seed proteins of 12 pine species have been analyzed by means of SDS-PAGE (Sodium dodecylsulphate-polyacrylamide gel electrophoresis). The results showed the presence, in the genus *Pinus*, of two main sub-taxa, corresponding to the subgenera *Haploxylon* and *Diploxylon*. Differences and affinities between Mediterranean pine species were found in agreement with classification of Klaus (1989).

Seed storage proteins are an important semantide for taxonomical studies (Ladizinsky & Hymowitz 1979, Vaughan 1983). They can be used for the identification of the geographical origin of different taxa, the determination of parents of hybrid species, the characterization of polyploids and amphiploids, the establishment of similarities between species and the formulation of hypotheses on their phylogeny (Miege 1982). Therefore, Konarev & al. (1987) suggested that the analysis of the seed total storage proteins should be used as a routine test in the identification of plant taxa.

As reviewed by Miege (1982), protein analysis can be carried out by means of aminoacidic sequences, serological properties, and electrophoresis patterns. There are numerous examples of SDS-PAGE carried out on seed proteins from species or sub-specific taxa of angiosperms (Stegemann 1983, Lalonde & al. 1984, Burges & Shewry 1986, Collada & al. 1988, King 1986, Aliaga-Morell & al. 1987, Lafiandra & al. 1990). Nevertheless, references for the gymnosperms and particularly for the genus *Pinus* are very few and seemingly without taxonomic implications (Gifford 1988, Gifford & al. 1989, Jensen & Berthold 1989).

The taxonomic models proposed for *Pinus* are numerous and very detailed (Shaw 1914, Pilger 1926, Duffield 1953, Gaussen 1960, Critchfield & Little 1966, Farjon 1984, Price 1989), but there are still several unsolved problems, especially regarding interspecific phylogenetic relationships. This is believed to be due, in part, to interspecific hybridization. Indeed, many pine species, often belonging to distant taxonomic groups, can be intercrossed so as to produce fertile
hybrids (e.g., *Pinus halepensis*, *P. sylvestris*, *P. monticola* Doug., *P. ponderosa* Laws), while other species have strong barriers that prevent hybridization (e.g., *P. pinea*, *P. bungeana*, *P. sabiniana* Doug.). Furthermore, several wide-ranging species show considerable intraspecific variability, especially in morphological characters (*P. sylvestris*, *P. nigra*, *P. mugo*) and, in several regions, such as Mexico or SE. Asia, the genus appears to be undergoing active speciation (Mirov 1967).


In the present paper we have used monodimensional electrophoretic analysis of total seed proteins to search for taxonomically interesting differences within the genus *Pinus*.

**Material and methods**

**Plant material.** Examined species and seed provenances are listed in Table 1. Seeds were stored in the dark at 4 °C. No less than 100 mature and viable seeds from each taxon were used. Moreover, a screening among 150 specimens for each of four *P. pinaster* provenances (Tuscany, Pantelleria, Corsica and Sardinia) was carried out in order to characterize the intraspecific variability.

**Protein extraction.** Dried seeds, without coats, were ground to flour. Then the proteins were extracted following Payne & al. (1981) method (modified). 12 h later, the extracts were centrifuged at 13,000 rpm for 10 min and the supernatant containing the total proteins was collected.

**Electrophoresis.** The proteins were electrophoresed following Laemmli (1970) method (modified) and stained with Coomassie Brilliant blue R-250 (0.05% w/v) in trichloroacetic acid (12% w/v). Proteins molecular weights were estimated through the SDS-PAGE Molecular Weight Low Range System (BIORAD). Once destained, the gels were photographed with Agfaortho 25 film and the electrophoresis profiles were examined with an Ultroscan LKB 2202 Laser Densitometer.

**Data analysis.** Characteristic protein patterns for each species were constructed averaging Rf values calculated for different specimens. Banding data were treated as a series of a binary characters recording the presence or absence of bands. The obtained data served for the calculation of a normalized euclidean distance matrix between the examined species. A cluster analysis was performed using the single linkage method (nearest neighbour) and a dendogram was constructed. Computation was carried out using the SYSTAT 2 package designed by Wilkinson (1985).

**Results**

A great variability was found in the total protein electrophoretic patterns among species belonging to different families and orders. Figure 1 shows the main differ-