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BRIEF COMMUNICATION

An electrophoretic analysis of the seed protein body proteins from *Pinus nigra*

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Abstract

Protein bodies (PBs) of European black pine (*Pinus nigra* Arn.) were isolated from mature seeds. Extracted soluble matrix proteins and crystalloid proteins PBs proteins were investigated by SDS-PAGE electrophoresis in presence and absence of 2-mercaptoethanol. The proteins of molecular masses 16, 17, 18, 61 and 65 kDa were presented only in crystalloid protein samples. Only 15 kDa protein was present in soluble matrix proteins and not in crystalloid proteins. Another protein bands were present in both soluble matrix and crystalloid proteins. 20, 37, 38, 39 and 48 kDa proteins were strongly visible among crystalloid proteins. Bands of 23 and 32 kDa were more visible in soluble matrix protein samples. Different composition in crystalloid proteins was found in absence of 2-mercaptoethanol: no proteins with molecular mass 71 kDa and more proteins in soluble matrix. In case of crystalloid proteins we detected 7 protein bands in interval from 71 to 212 kDa.

Additional key words: Pinus nigra, protein bodies, matrix and crystalloid proteins, SDS-PAGE.

Storage proteins are characteristically accumulated during mid- to late-maturation of seeds (Gatehouse and Shirsat 1993). The synthesis and sequestration of storage proteins is a highly regulated and multifaceted tissue specific process. Specific storage protein mRNAs accumulate according to a defined chronology during seed development (Okamura and Goldberg 1989, Gatehouse and Shirsat 1993). The proteins are either deposited in the cytoplasm or are transported via the Golgi apparatus to membrane-bound organelles called protein bodies. Misra (1994) demonstrated that major seed storage proteins of conifers have structural homologies with angiosperm seeds. In the conifers, the protein bodies consist of globoid and crystalloid inclusion embedded in a proteinaceous matrix (Misra and Green 1990, Hřib and Janisch 1995). The globoid region contains phytin and mineral deposit. The matrix and crystalloid proteins are often separated in protein extractions on the basis of differential solubilities (Misra and Green 1991, Green *et al.* 1991). Studies of seed proteins from a number of conifer species have shown that the crystalloid proteins are the major storage proteins, *e.g.* in *Pinus* species. In mature seeds of white spruce and Douglas fir these proteins accounted for 70 - 80 % of the total storage proteins and are localised in protein bodies (Gifford 1988, Jensen and Lixue 1991).

Protein bodies of European black pine isolated from mature seeds were investigated by scanning electron microscopy (SEM) (Hřib *et al.*, unpublished). Lectin-like activity was observed in these protein bodies (Nahálková *et al.* 1999). However, complete protein analysis has not been reported until now. In the present work, protein pattern of soluble matrix proteins and insoluble crystalloid proteins from protein bodies of European black pine (*Pinus nigra* Arn.) seeds were investigated.

Mature seeds of European black pine (*Pinus nigra* Arn.) were collected from selected trees in region Piešťany, Slovakia. The isolation of protein bodies was based on method of Yatsu and Jacks (1968). The seeds (30 g) were homogenised with 100 cm^3 of glycerol in a kitchen coffee mill, filtered through the gauze, centrifuged twice at 800 *g* for 5 min and then twice at

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1 500 *g* for 10 min to remove crude impurities (the segment contained shells and large seed fragments), then 4 times at 5 600 *g* for 10 min to remove the nuclear fraction (black sediment) and 3 times at 41 000 *g* for 15 min to remove other fine impurities, after which the white protein body (PB) sediment was obtained. The PB sediment was stored in glycerol at 4 °C for 2 - 3 months.

Extraction was performed according to Gifford *et al.* (1982). Isolated protein bodies were rinsed with 100 % ethanol to remove glycerol. Soluble matrix proteins were extracted 3 times with 10 mM phosphate-buffered saline (pH 7.2). After centrifugation at 40 000 *g* for 40 min crystalloid proteins were extracted using 65 mM Tris-HCl (pH 6.8) with 2 % (m/v) SDS and 10 % (m/v) glycerol. After centrifugation were soluble matrix and crystalloid proteins dialysed 48 against 50 volumes of distilled water and lyofilized.

Three concentrations of 10, 100 and 200 μg of soluble matrix and crystalloid proteins isolated from protein bodies were investigates in presence or absence of 2-mercaptoethanol. SDS-PAGE electrophoresis was performed on slab gels (160 × 140 × 1.5 mm) using 15 % separation and 4 % stacking gel respectively (Laemmli 1970). One volume of protein sample (10, 100 and 200 μg of proteins) was mixed with one volume of 2x SDS sample buffer prepared with and without of 2-mercaptoethanol according to Ausubel *et al.* (1989),

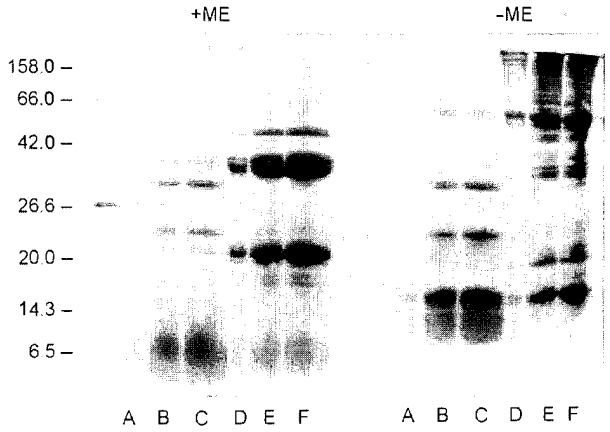

Fig. 1. Coomasie blue stained SDS-PAGE profiles of the soluble matrix proteins (*A*, *B*, *C*) and crystalloid proteins (*D*, *E*, *F*) from protein bodies of European black pine under reducing (+*ME*) and non-reducing (-*ME*) conditions. In each case 10 μg (*A*, *D*), 100 μg (*B*, *E*) and 200 μg (*C*, *F*) was loaded. Relative molecular masses [kDa] are shown adjacent to the gel profiles.

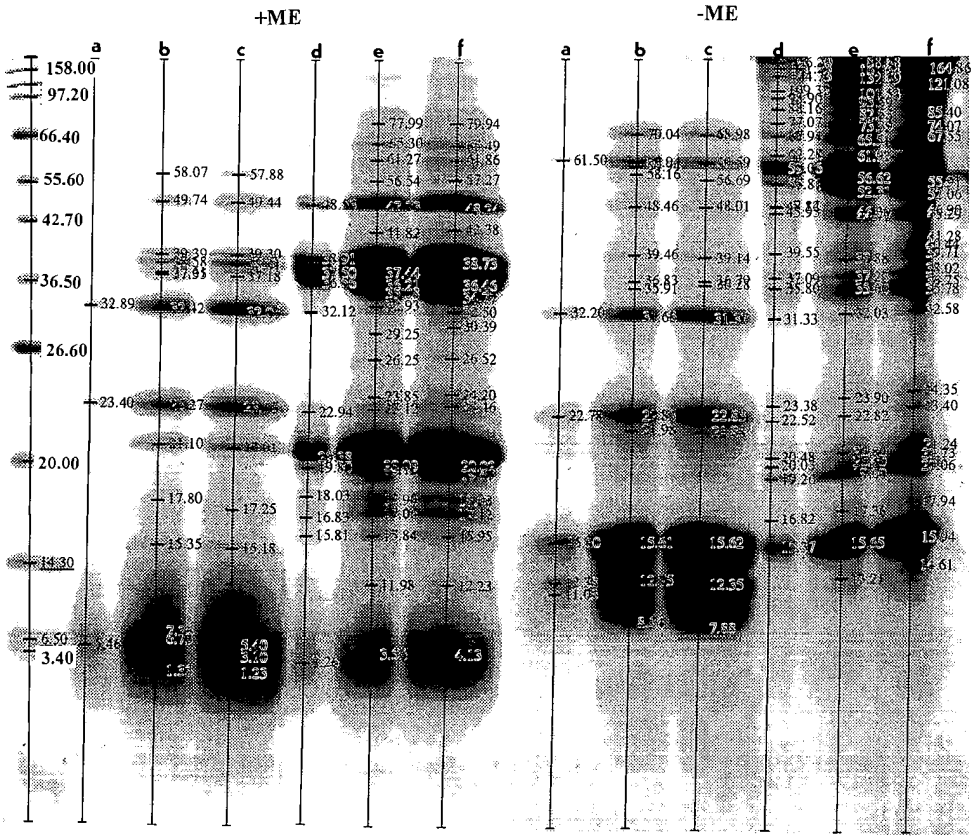

Fig. 2. Coomasie blue stained SDS-PAGE profiles of the soluble matrix proteins (*a*, *b*, *c*) and crystalloid proteins (*d*, *e*, *f*) from protein bodies of European black pine under reducing (+ME) and non-reducing (-ME) conditions evaluated by scanning densitometry. In each case 10 μg (*a*, *d*), 100 μg (*b*, *e*) and 200 μg (*c*, *f*) was loaded. Relative molecular masses [kDa] are shown adjacent to the gel profiles.

boiled for 10 min and placed on ice. After electrophoresis of the samples, the gels were stained with Coomassie Brilliant Blue R-250 (Ausubel *et al.* 1989).

We found different protein composition in matrix and crystalloid sample under both reducing and non-reducing conditions (Fig. 1). Coomasie blue stained SDS-PAGE profiles of proteins were evaluated by scanning densitometry (Fig. 2). In soluble matrix, 13 bands of proteins (from 8 kDa to 68 kDa) were identified under non-reducing conditions. Three major protein bands of 12.5, 23, and 32 kDa were detected. The proteins of molecular mass 60 and 68 kDa were presented only under non-reducing conditions. Some of the proteins were not affected by 2-mercaptoethanol treatment. Proteins of 23 and 32 kDa were presented under both non-reducing and reducing conditions. Bands 23 kDa and 32 kDa were more visible in soluble matrix protein samples. In crystalloid sample 24 protein bands (molecular masses 13 - 196 kDa) were found under non-reducing conditions. The bands of 16 and 20 kDa proteins were strongly visible. Seven proteins of molecular masses from 71 to 196 kDa visible on Fig. 2 were presented only under non-reducing conditions. In presence of 2-mercaptoethanol the main protein bands 20, 23, 32, 37, 38, 39, and 48 kDa were presented in crystalloid proteins as well as in soluble matrix under this conditions. The most abundant group in crystalloid proteins under reducing conditions were proteins of molecular masses 16, 17, 18, 61, and 65 kDa. They were presented only in crystalloid samples.

Two major protein bands of 17 and 16 kDa were found in *Picea glauca* embryo tissue but not in megagametophyte tissue (Flinn *et al.* 1991). The 61 kDa protein (37 and 24 kDa under reducing conditions) was also found in the insoluble fraction of embryos *Picea glauca* (Gifford and Tolley 1989). Crystalloid proteins were found in the megagametophyte as well as in embryonic axis of Douglas fir. These proteins in their non-reduced form have molecular mass 55 - 63 kDa and their reduced form migrate as two distinct groups of proteins in the molecular mass range of 32 to 35 and 20 to 23 kDa (Green *et al.* 1991).

Storage proteins 21 to 22.5, 29, 31 to 35, and 43 kDa were found in seeds of several *Pinus* species (Gifford 1988). The major proteins of *Picea abies*, *Picea glauca* and *Pseudotsuga menziesii* were also found to fall into these classes of proteins (Gifford and Tolley 1989, Roberts *et al.* 1989, Green *et al.* 1991). This suggests that the storage proteins may be conserved among the conifers, although the relative amount of the different proteins differ among the species (Gifford and Tolley 1989).

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