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SDS-PAGE of *Ophioglossum vulgatum* Proteins

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Authors' contributions

All authors contributed this work equally. Author MSC collected the samples and extracted proteins. Author VOC performed SDS-PAGE and densitometry. Author LIM contributed to the work on all stages and also developed an ideology of experiments and interpreted the data obtained. All authors read and approved the final manuscript.

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ABSTRACT

Background: Despite that the protein composition is one of the main characteristics of plant organism, there are not many studies dedicated to the fern proteins characterisation. Protein composition as a part of biochemical characterisation of *Ophioglossum vulgatum* (an evolutionary distinct fern species related to *Psilotaceae*) is of interest from the point of view of ecology, taxonomy and plant biochemistry.
Aims: Study of the protein composition of roots and leaves of *O. vulgatum* during seasonal ontogenesis of its sporophytes.
Methodology: SDS-PAGE with following densitometry was used for the estimation of protein composition of roots and leaves of *O. vulgatum* collected from young plants, mature plants, spore-bearing and not spore-bearing plants at the peak of their seasonal development. To determine the phosphorylation of studied proteins the electrophoresis using Phos-tag™ Acrylamide Gels were applied.
Results: The simple and effective method of protein extraction from the leaves and roots of *O. vulgatum* was developed and applied. Protein fractions with the molecular weights of 112±1.3, 57±1.0, 45±1.2, 35.5±0.6, 25.8±0.5, 18±1.5 kDa were shown as the major for both leaves and

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roots. Huge RuBisCO accumulation during the stages of plant initial growth and spore-bearing was shown (up to 55±10% in leaves and 89±5% in roots). All large chains of detected RuBisCO were phosphorylated which suggests that the active form of *O. vulgatum* RuBisCO is phosphoprotein.
Conclusion: The dynamic change of protein content was shown for different ontogenesis stages of *O. vulgatum*. The expression of phosphorylated RuBisCO was distinctly increased during the beginning of leaf development and spore-bearing.

Keywords: SDS-PAGE; Ophioglossum vulgatum; Phos-tag; protein composition; extraction of plant proteins.

1. INTRODUCTION

Ferns are a large and diverse group of pteridophytes, commonly referred to as the lower vascular plants. More than a hundred of them are edible [1].

Despite that the protein composition is one of the main characteristics of a plant organism; there are not many studies of fern protein composition spanning evolutionary diverse branches. Evidence of the correlation between the amino acid and protein compositions and plant taxonomy was shown [2,3]. Also, some studies showed the potential nutrition value of fern-derived proteins [4-6]. It is known, for angiosperms, that growth and bloom of plants leads to distinct proteomic changes [7,8].

Ophioglossaceae are a putatively ancient lineage of ferns in which the aerial portion of the plant is composed of a single leaf [9]. *O. vulgatum* is fern species which in Europe has a wide scattered distribution [10]. Initially heterotrophic gametophytes of *O. vulgatum* form mutualistic associations with fungi of the *Glomeromycota* but it is unknown whether mature *O. vulgatum* sporophytes have such mutualism [11]. Evolutionally *Ophioglossaceae* is an early diverging fern family with reduced features that is thought to be sister group to *Psilotaceae* [12].

All mentioned above makes the biochemistry of *O. vulgatum* of interest from the points of view of evolution studies, taxonomy, phytochemistry and proteomics. However there were no studies on the change of protein composition of this remarkable plant species. That is why the aim of present work was to study the protein composition of roots and leaves of *O. vulgatum* during ontogenesis of its sporophytes.

2. MATERIALS AND METHODS

2.1 Materials

Tris (161-0716, Sigma); Glycine (161-0718, Sigma); PBS tablets (2810305, MP Biomedicals); Disposable PD 10 Desalting Columns (17-0851-01, GE Healthcare); Ready-to-Use Phos-tag™ Acrylamide Gel (195-16381, Wako); Ethylenediaminetetraacetic acid (EDTA) (03620, Sigma); Phenylmethylsulfonyl fluoride (PMSF) (329-98-6, Sigma); Amicon Ultra-0.5 mL Centrifugal Filters (UFC500324, Merck Millipore); molecular weight markers (0671, Fermentas).

2.2 Methods

2.2.1 Protein extraction

For the extraction of total protein from *Ophioglossum vulgatum* leaves and roots the methods described in [13-15] were modified as follows. Plant tissue was collected in the field during different stages of plant ontogenesis: 1 – young vegetative plant (May 7th); 2 – developing vegetative plant (June 10th); 3 – mature spore-bearing plant (June 27th); 4 – mature vegetative (not spore-bearing) plant (June 27th). Collected samples were moved on dry ice to the laboratory where they were washed, dried with paper towels, divided into leaves and roots, and then 50 mg of each was weighed and then stored in the freezer at -20℃. Samples were placed in a mortar, over them was poured liquid nitrogen and pestled until no chunks were visible. 1 ml of extraction buffer (0.05 M Tris-HCl buffer pH 7.4 with 0.3 M NaCl, 1mM PMSF and 1mM EDTA) was added to the homogenised biomass. Samples were suspended in the extraction buffer and transferred to the sample tube. Mortar and pestle were rinsed with 0.5 ml of extraction buffer to remove all traces of sample. Obtained lysates of tissues were collected to 2 ml sample tubes and spinned-down at 4 000 rpm, + 4℃ for 30 minutes. Supernatants were collected and then gel-filtered on PD-10 column to exclude non-protein components from the samples. Eluted fractions were concentrated using Amicon Ultra-0.5 mL Centrifugal Filters. The amount of total proteins in the mixture was monitored by measuring the optical density of the sample by spectrophotometry at E_{280}. The concentration of

proteins in all samples were normalised at 4 mg/ml. Samples were stored at -20℃.

2.2.2 SDS-PAGE

Samples were mixed with equal volume of Laemmli sample buffer that contained 2% SDS, 5% glycerine and bromophenol blue. They were heated in a boiling-water bath and were analysed or stored no more than 3 months at -20℃. The molecular weights of proteins were determined by SDS-PAGE using 10% gels prepared accordingly to Laemmli [16]. Ready-to-Use Phos-tag™ Acrylamide Gels (10%) were used to determine the phosphorylation of studied proteins [17].

Protein samples were loaded on a gel (30 mkg/track) that was an optimum concentration for accurate detection of major proteins. All samples were normalized according to their optical density at E_{280}.

2.2.3 Densitometry

Electrophoresis gels were scanned with HP Photosmart C3100 and analysed using densitometry software TotalLab TL100 (Phoretix) [18], a tool that allows to identify molecular weights and amount of proteins separated by electrophoresis [19,20].

Molecular weight markers 0671 (Ferments) were used for the "pixel position"/"molecular weight" calibration.

2.2.4 Statistics

Statistical data analysis was performed using Microsoft Excel. All assays were performed in series of three independent experiments and the data were fitted with standard errors using "Statistica 7".

3. RESULTS AND DISCUSSION

SDS-PAGE is a simple and informative method that allows to separate proteins according to their molecular weight and detect them in comparison to molecular weight markers [21]. So it is widely used for the characterisation of biological objects providing the information of their protein compositions. The densitometry of obtained electrophoregrams allows estimating the amount of individual proteins and their ratio in the mixture [22].

Concerning plant objects SDS-PAGE was successfully used for the characterisation of seed storage proteins derived from different breeds [23], finding the common proteins in different species [24], estimation of the gall disease's effects on plant proteins [25], etc.

In our study we separated proteins of *O. vulgatum* leaves and roots samples collected at different stages of ontogenesis. According to SDS-PAGE the leaves and roots samples presented the same bands of major proteins in studied range from 10 to 110 kDa (Fig. 1). However the amounts of individual proteins differed between different stages of ontogenesis.

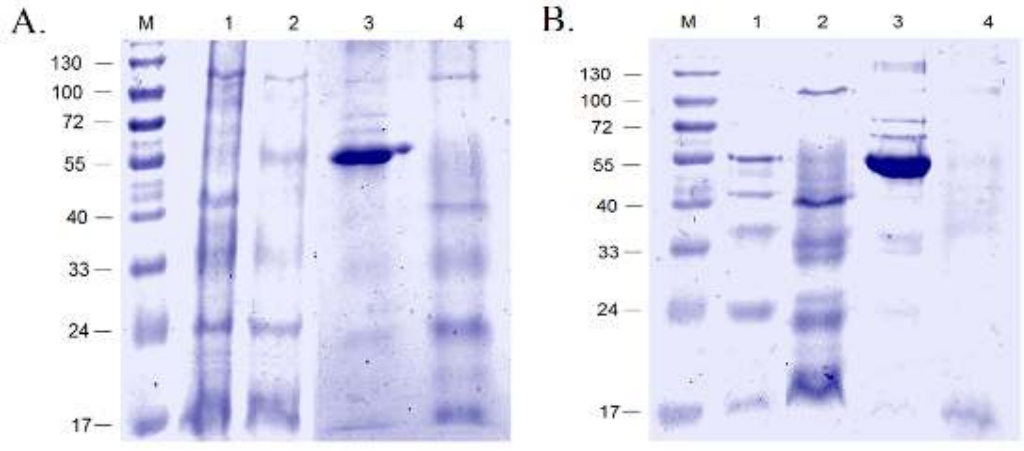

Fig. 1. SDS-PAGE of the protein extracts of *O. vulgatum* leaves (A) and roots (B); 1 – Young plant; 2 – Developing vegetative plant; 3 – Mature spore-bearing plant; 4 – Mature not spore-bearing plant. M – Molecular weight markers

For the estimation of the quantity of proteins in individual samples we used densitometry of electrophoregrams by TotalLab TL100. Densitograms corresponding to the tracks of protein samples separated by SDS-PAGE are shown on the Fig. 2. Protein fractions with the molecular weights of 112±1.3, 57±1.0, 45±1.2, 35.5±0.6, 25.8±0.5, 18±1.5 kDa (P = .05) were selected as the major protein bands and quantified using TotalLab TL100. The calculations of the series of three replicates are shown on the Fig. 3.

Here we have to point out a remarkable tendency in the protein composition of leaves and roots of *O. vulgatum*. First of all, protein band with molecular weight of 55±1.0 was shown to be the most variable fraction. It was distinctly increased on the stage of young plant (sample 1) leaf growth and highly increased when the plant started to bear spores (sample 3). From the other hand mature not spore-bearing plants (sample 4) showed the decreased amount of this protein fraction. This tendency was observed equally in the case of leaf's proteins as well as in the case of root's proteins.

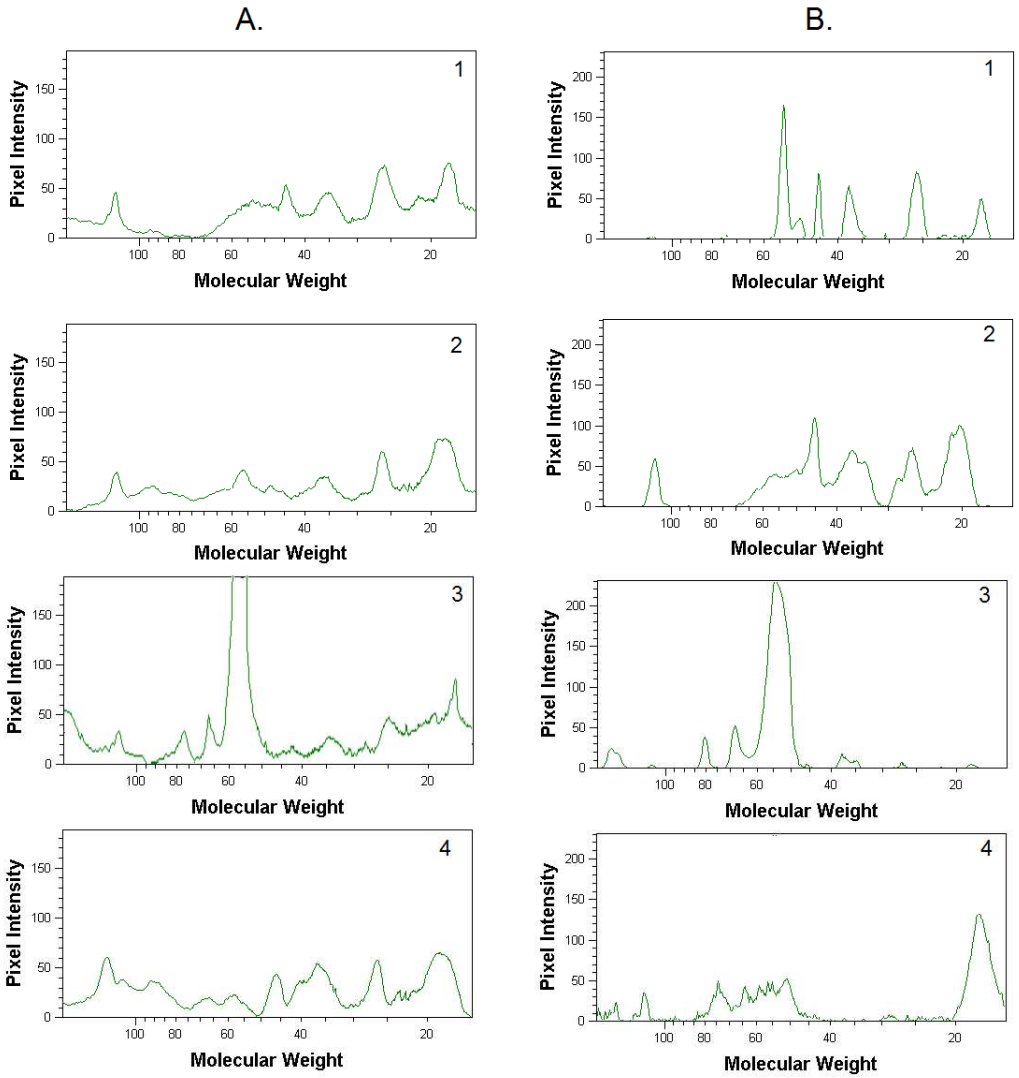

Fig. 2. Densitometry of SDS-PAGEs of the protein extracts of *O. vulgatum* leaves (A) and roots (B). 1 – Young plant; 2 – Developing vegetative plant; 3 – Mature spore-bearing plant; 4 – Mature not spore-bearing plant

According to the literature the major protein that makes up to 50% of the soluble protein of plant leaves is RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase) [26]. RuBisCO has a molecular mass of 560 kDa and consists of eight small (14 kDa each) and eight large (56 kDa each) subunits arranged as eight heterodimers [27].

It is known that the expression of RuBisCO protein is activated in the growing plants and parts of plant that grow intensely [6,28,29]. So we can conclude that 55±1.0 protein fraction detected in the samples of *O. vulgatum* corresponded to the large subunit of RuBisCO. We have shown that RuBisCO had similar dynamics in leaves as well as in roots and its expression was increased during plant initial growth and spore-bearing. It is interesting that the sporogenesis in the sporangium lead to activation of synthetic processes (coincident with detected RuBisCO expression increase) in leaves and even in roots of spore-bearing plants.

Studies of RuBisCO of different plant species showed that in some species it is a phosphoprotein by its nature and its enzyme activity is highly dependent on phosphorylation, when in others it is not [30]. It was also shown that RuBisCO large-chain fragments varied significantly in protein abundance and the level of phosphorylation among ontogenetic phases [31]. To check whether *O. vulgatum* RuBisCO was phosphorylated, we used Phos-Tag SDS-PAGE. It was shown that all RuBisCO large-chains that were detected using SDS-PAGE were phosphorylated (Fig. 4).

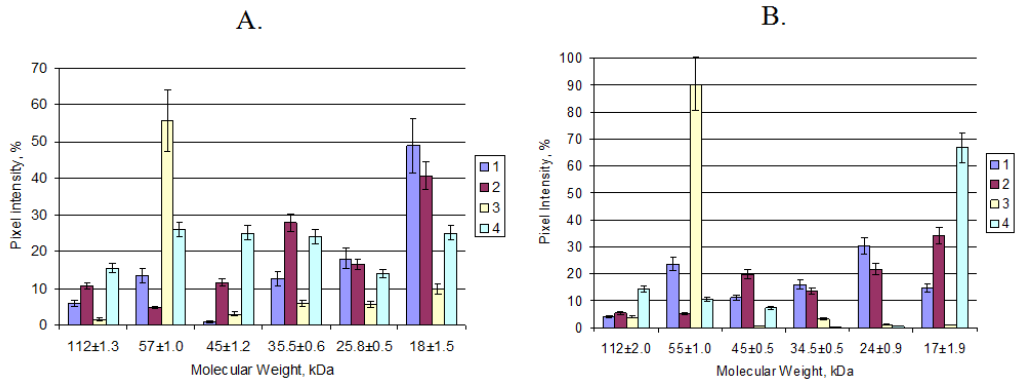

Fig. 3. The distribution of major protein fractions in the protein extracts of *O. vulgatum* leaves (A) and roots (B). 1 – Young vegetative plant; 2 – Developing vegetative plant: 3 – Spore-bearing plant; 4 – Not spore-bearing plant

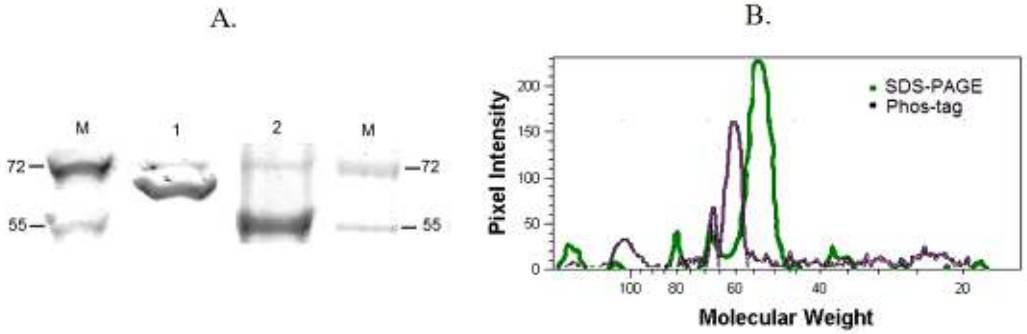

Fig. 4. SDS-PAGE (A) and corresponding densitogram (B) of RuBisCO derived from *O. vulgatum* leaves on the spore-bearing stage. 1 – Samples separated using Phos-tag electrophoresis; 2 - Samples separated using classic SDS-PAGE; M – Molecular weight markers

4. CONCLUSION

A simple and effective method of protein extraction from the leaves and roots of *O. vulgatum* was developed and applied. It allowed to obtain samples for SDS-PAGE excluding contaminants (DNA, pigments, etc) that influence proteins migration and worsen the quality of electrophoresis. SDS-PAGE analysis of *O. vulgatum* leaves and roots allowed us to reveal protein composition of this plant. Protein fractions with the molecular weights of 112±1.3, 57±1.0, 45±1.2, 35.5±0.6, 25.8±0.5, 18±1.5 kDa were shown as the major for both leaves and roots. The dynamic change of protein content was shown for different ontogenesis stages. Huge RuBisCO accumulation on the stages of plant initial growth and spore-bearing was shown. All large chains of detected RuBisCO were phosphorylated which is evidence that an active form of *O. vulgatum* RuBisCO is phosphoprotein. Presented results could be applied for further biochemical characterization of *O. vulgatum*. The comparison of data on plant hormones activation and proteins dynamics on different stages of ontogenesis could possibly help us to solve questions of the evolution of *Ophioglossaceae* and the nature of their mutualistic associations with fungi.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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