Preliminary characterization of ssps (Seed Storage Proteins) IN *Argania spinosa* L.

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Abstract

SSPs (Seed Storage Proteins) of the 11S type have been preliminary characterized in the seeds of the argan tree, an endemic species from Morocco. Protein extracts from mature seeds were prepared by using different solutions in order to assess the solubility of the major protein forms. SSPs of the 11S type were classified as albumins according to the further SDS-PAGE analysis of these extracts. The combination of both reducing- and non-reducing conditions for the SDS-PAGE analysis, together with immunoblot experiments allowed us to determine the presence of three precursor forms of these proteins (pro1, pro2 and pro3), which are composed of six individual peptides (p1 to p6) in different combinations.

Key words: *Argania spinosa*, protein bodies, seed, SSP, 11S.

Introduction

The argan tree (*Argania spinosa* L. -syn. *Argania syderoxylon* L., *Sideroxylon spinosum* L. and *Elaerandron argan* Retz-) is an oleaginous tree endemic to Morocco. The appearance of argan trees dates from the tertiary era (1). The plant is distributed throughout the Atlas mountain chain, as well as in the northern regions of the country (Berkane-Chouihiya). The fruits display different forms (spindle-shaped, oval, drupe, round or globular) (2). They are green when unripe, and turn bright yellow at maturity. The pericarp comprises three layers, the exocarp (skin), mesocarp (outer pulp) and the endocarp (an ovate hard-shelled nut, which encloses 1, 2 or 3 fleshy albumen or argan kernels -the endosperm-). Endosperm is very rich in oils, which are up to date the components of major economical interest, although they also contain a large proportion of proteins. In higher plants, the amount of protein present in seeds varies from ~10% (in cereals) to ~40% (in certain legumes and oilseeds) of the dry weight, forming a major source of dietary protein. A vast majority of these proteins (named seed storage proteins: SSPs) serve to provide amino acids which are used during germination and seedling growth. They are of particular importance because they also determine the quality of seeds for various uses. Storage proteins are formed during seed maturation and set down predominantly in specialized storage tissues (i.e. cotyledon or endosperm), in most cases in the form of protein bodies. Detailed study of seed storage proteins was initiated last century, when Osborne (1924) (3) classified them on the basis of their extraction and solubility into albumins, globulins, prolamins and glutelins. Globulins are the most widely distributed group of SSPs. They have been studied mainly in legumes. Legumins are the major storage proteins in many other dicots and some cereals. They are stored as large complexes (hexameric structures) in protein bodies. Each subunits in the hexamer is itself composed of a large acidic α- and a small basic β- polypeptide, derived from a single precursor (prolegumin) and linked by a disulphide bond (4-7). In a previous work, we have analyzed the composition and distribution of SSPs of the 11S-type, similar to legumins in the seeds of the olive tree (*Olea europaea* L.) (8). In this paper, we have attempted to perform a preliminary characterization of SSPs in the seed of the argan tree.

Materials and methods

In order to assess solubility characteristics of SSPs, proteins from ground mature seeds were extracted using the following solutions: a) distilled water, b) 0.5 M NaCl, c) 70% (v/v) 2-propanol, d) 60% (v/v) acetic acid, e) 0.1M sodium hydroxide, and f) 0.1 M sodium borate, 1% SDS and 50 mM dithithreitol (DTT).

For SDS-PAGE analytical purposes, crude protein extracts were resolved under denaturing, non-reducing conditions (protein extraction performed using 125 mM Tris-HCl, 0.2% sodium dodecyl sulphate -SDS-) or denaturing, reducing conditions (the same as above, plus 1% 2-β-mercaptoethanol). Identical gels were transferred into PVDF membranes and probed with an antibody to 11S-type SSPs from olive (*Olea europaea* L.) (8). An Alexa 488-conjugated anti-rabbit IgG (Molecular Probes) diluted 1:10.000 served as the secondary antibody and the

detection reaction was performed in a Pharox FX high-resolution fluorescence scanner (Bio-Rad).

All bands reactive to the antibody, corresponding to either complex forms or individual polypeptides, were individually purified as follows: bands were cut out from stained gels, and homogenated in 100 mMTris-HCL, O.5% (w/v) SDS, pH8.2. After centrifugation, the proteins in the supernatant were recovered by cold acetone precipitation, and then electrophoretically separated. Each individual component of 11S proteins was cut out from the stained gels and recovered as above.

Results and discussion

Solubility of SSPs

Figure 1 illustrates the solubility of the major protein forms of both the cotyledon and the endosperm of the argan seed after using different extraction buffers. Solubility experiments determined that the majority of these argania seed proteins were extracted with water and dilute salt solution. Therefore they could be classified as albumins. No major differences were detected in both tissues.

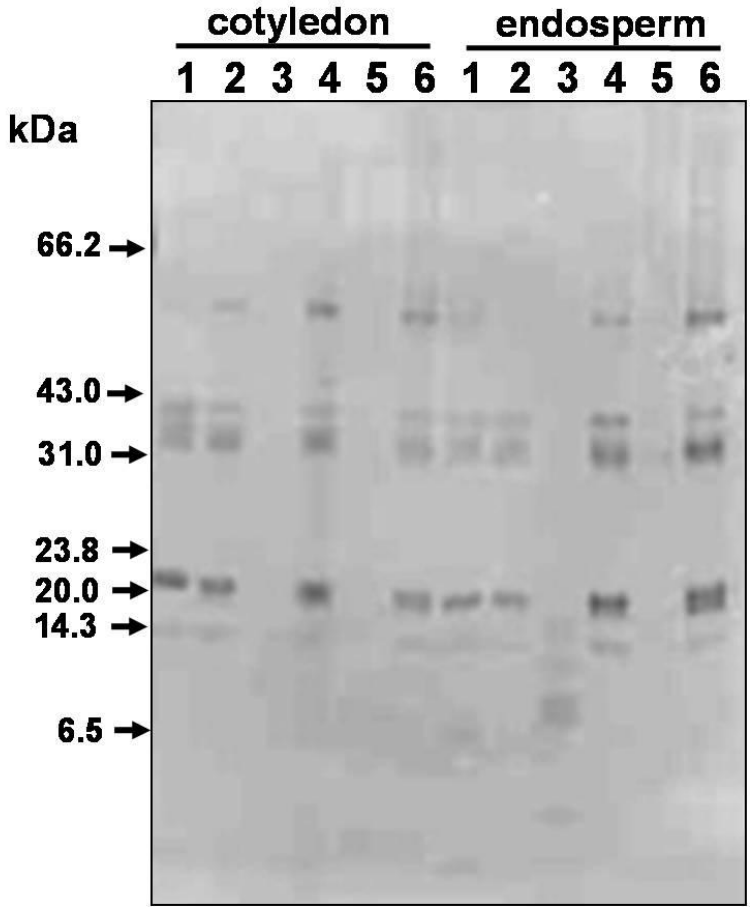

Fig. 1. Solubility of argan seed proteins in different solutions. The proteins were extracted with distilled water (1), 0.5 M sodium chloride (2), 2-isopropanol (3), 70% (v/v) acetic acid (4), 0.1 M sodium hydroxide (5) and a buffered solution (pH10) containing 0.1 M sodium borate, 1% (w/v) SDS and 50 DTT (6). SDS-PAGE gel run under reducing conditions and stained with Coomassie blue.

Peptide composition of the 11S proteins

Precursor forms of these proteins were resolved by SDS-PAGE using non reducing conditions. These forms occurred as 3 polypeptides named pro1, pro2 and pro3 (Figure 2 panel A, lane 1). The analysis of these precursors using denaturing, reducing conditions yielded 6 peptides, which were named p1 to p6 (Figure 2 panel A, lane 2). No major differences were detected when individualised endosperms, cotyledons or whole seeds were used.

All the above mentioned protein forms were recognized by an antibody raised to SSP- peptides of the 11S type from olive when used in immunoblotting experiments, although some of them (p5 and p6) very weakly.

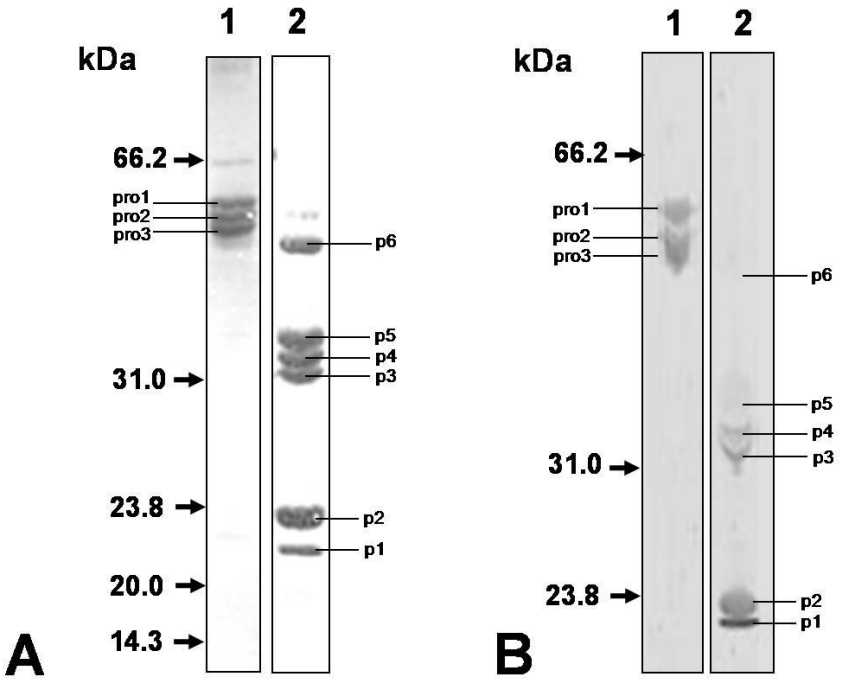

Fig. 2. Panel A: SDS-PAGE gels run under non-reducing (lane 1) and reducing (lane 2) conditions, and stained with Coomassie blue. Panel B, equivalent immunoblots probed with the anti olive SSP antibody.

Our results suggests that the 11S proteins of argania seeds may accumulate as hexameric complexes, the monomers of which consisting of a larger, acidic alpha polypeptide linked via disulphide bridges to a smaller, basic beta polypeptide. A model of the putative composition of the different forms is presented in Table 1.

Table 1: Peptides likely integrating each one of the precursor forms of the 11S SSPs in the argan tree.

Precursor form	Integrating Peptides
Pro 1 (62.18 kDa)	p1 (20.21 kDa)
	p5 (40.67 kDa)
Pro 2 (57.40 kDa)	p2 (22.83 kDa)
	p3 (34.12 kDa)
Pro 3 (52.76 kDa)	p2 (22.83 kDa)
	p4 (36.74 kDa)

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