11S GLOBULIN FRACTION OF *HELIANTHUS (ASTERACEAE)* *Mr* POLYMORPHISM ANALYSES

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Key Words : *Helianthus*.- Compositae, sunflower, storage proteins, M_r polymorphism.

Abstract

The seed storage globulins from six *Helianthus* and four hybrids were studied using mono and bidimensional gel SDS electrophoresis (+ 2 mercaptoethanol). The αβ polypeptide composition of each subunit was determined. Different αβ pairs are specifically expressed according to the species studied. Three typical patterns were discriminated. All the studied species exhibit five subunits : two of them are expressed in all the species ($\alpha'_1\beta'_1$ and $\alpha'_2\beta'_2$). The subunit corresponding to the $\alpha_1\beta_1$ pair is present in *H. petiolaris* and in the three populations of *H. annuus* studied. The $\alpha_{2b}\beta_2$ pair is common to *H. annuus* and *H. argophyllus*. *H. petiolaris* presents two specific $\alpha_{2a}\beta_2$ and $\alpha_4\beta'_4$ pairs and *H. annuus* a specific $\alpha_3\beta'_3$ pair. In *H. argophyllus* $\alpha_1\beta_1$, $\alpha_3\beta'_3$ or $\alpha_4\beta'_4$ are never observed but are replaced by $\alpha_1\beta'_3$ and $\alpha_3\beta_1$ pairs. Some globulins, poorly represented, are of αβ' forms but present α chains of higher molecular weights (in the range 54-56 kDa).

Introduction

The 11S globulins of, *H. annuus* designated helianthinin (SCHWENKE & al. 1978), is structurally similar to the legumin-like seed proteins of other plant species and is represented in plants by an approximately 300 000 molecular weights (M_r) hexameric holoprotein which constitutes the bulk of seed proteins (SCHWENKE & al. 1979 , DALGALARRONDO & al. 1984, ALLEN & al. 1985, THIS & al. 1988, PLIETZ & al. 1983). Each subunit of the holoprotein (A, B and C subunits) consists of two either α or α' polypeptides (M_r 38 000-32 000) linked by disulfide bonds to either β or β' polypeptides (M_r 22 000-24 000) (DALGALARRONDO & al. 1984) and some studies on the 11s globulins are available (KORTT & CALDWELL 1990, SRIPAD & NARASINGA RAO 1987). The α, α' as well as the β, β' polypeptides are generated proteolytically from larger precursor polypeptides (HIGGINS 1984), each pair representing the expression of a single gene. We have shown that both M_r as well as charge polymorphism were similar in commercial cultivated *H. annuus* varieties (RAYMOND & al. 1991). The aim of the present study was to compare a number of ancestral wild and old cultivated *Helianthus* coming from various distant geographical areas with the hope finding specific protein markers.We gave precisions concerning the αβ composition between *H. annuus*, *H. petiolaris* and *H. argophyllus*. M_r polymorphism in the seed globulin fraction was also investigated on hybrids derived from wild *Helianthus* and *H. annuus*.

Materials and Methods

Plant material. Seeds of Helianthus spp.(wild, cultivated species and hybrids) were provided by the Institut National de la Recherche Agronomique (Clermont-Ferrand, France). The characteristics of the *Helianthus* studied were reported in Table 1 as the origin of the hybrids.

Extraction of proteins. Total proteins were extracted from non-defatted seeds (pooled samples (approximately 20 seeds) : 100mg/1ml) with the extraction buffer (4% SDS, 0.05 M Tris-HCl pH 6.8 containing 30% glycerol) at room temperature during 30 min under magnetic stirring. The remaining slurry was centrifuged (4 400 x g) in order to discard the non soluble material. The supernatant was directly analyzed. In reducing conditions 3% (v/v) of 2-mercaptoethanol (2ME) were added and the extracts were heated at 110°C for 2 min.

Electrophoresis. Monodimensional SDS-PAGE and bidimensional electrophoresis were performed as previously described (RAYMOND & al. 1991).

Results and Discussion

Monodimensional analysis. The protein patterns of the samples studied and those of different crosses with *H. annuus* are shown in fig. 1A. For *H. annuus* (RAYMOND & al. 1991) the M_r 56, 55 and 52 000 correspond respectively to the A, B and C subunits of helianthinin, M_r 12 to 16.5 are albumins and M_r ca. 19 000 oil body membrane proteins. Little information can be obtained from these monodimensional analyses exept for the presence of a 78-80 000 M_r doublet which is present only in the *H. petiolaris* species while the other species have only one 79 000 M_r. The hybrids show more homogeneity in the 1D banding patterns (for example all hybrids show only the 79 000 M_r).

The monodimensional analysis in the presence of 2-ME is shown in fig 1B. Large M_r polymorphism is observed for the α polypeptides including also a qualitative variability between species. The β (M_r 22 000) and β' (M_r 24 000) polypeptides are difficult to analyse in 1D as their M_r are very close to those of oil bodies membrane proteins (OBMP) and to some albumins whose M_r is increased after reduction (DECHERF-HAMEY & al. 1986). Nevertheless the two α' bands are clearly observed in all the samples studied.

Bidimensional analysis. 2D SDS-PAGE are shown on fig. 2. The spots analyzed were restricted to the major A, B and C subunits (M_r 56, 55 and 52 000) and to some minor subunits of higher M_r. The 2D analyses enable a more accurate comparison of the studied species since α α' and β β' may be analyzed separately as pairs. As a consequence each species may be characterized by the number and the nature of the αβ pairs composing the A, B and C subunits. For this purpose all the αβ pairs detected are shown in fig. 3 with their respective nomenclature. The αβ composition of the different species is shown in Table 2.

It appears that the pairs of lower M_r $\alpha'_2\beta'_2$ and $\alpha'_1\beta'_1$ are common to all the studied samples.The $\alpha_1\beta_1$ pair is always present except for *H. argophyllus*. The three populations of *H. annuus* possess $\alpha_3\beta'_3$ in addition. *H. petiolaris* 1398 and *H. petiolaris* 1303 are identical with two characteristic and easily detected $\alpha_{2a}\beta_2$ and $\alpha_4\beta'_4$ pairs. *H. argophyllus* possess $\alpha_1\beta'_3$ and $\alpha_3\beta_1$ pairs plus a common pair $\alpha_{2b}\beta_2$ with *H. annuus*. So all the *Helianthus* studied appears to express 5 main αβ subunits on a M_r basis and a good discrimination is possible between *H. annuus*, *H. argophyllus* and *H. petiolaris* on the base of M_r polymorphism in their globulin fraction. All these results are summarized in Fig. 3.

Fig. 4 shows the 2D SDS-PAGE from two of the crosses described in Fig. 1. It is apparent from this analysis that the minor α_0 54-56 000 *Mr* doublet of *H. petiolaris* is no longer

observed in the progeny. In this case the biochemical phenotype of F1 seeds is not completely additive with respect to the banding patterns of the parental lines. In contrast the $\alpha_4\beta'_4$ pair, which is always present, is expressed in a codominant manner along with the others $\alpha\beta$ pairs and represents, among all the specific pairs studied, a useful (easy to detect) biochemical phenotypic marker of *H. petiolaris*.

Helianthus seeds were all found to contain five major globulins while some contained one or two minor globulins in addition. Specimens have been found to differ in their content in $\alpha\beta$ pairs in the 11S globulin fraction. Analysis of the globulin fraction provide in *Helianthus* varieties an indication of storage protein make up. Nevertheless multiple crosses quickly lead to an homogenization of the storage protein patterns. In spite of this the α_{2a} and α_4 polypeptides of *H. petiolaris* remain the most valuable specific markers of this species (black arrows on Fig. 4). It now appears that *H. petiolaris*, *H. argophyllus* and *H. annuus* present *Mr* polymorphism in their globulin fraction but that variation in this polymorphism is not very large. Except in some cases (*H. petiolaris* in our study) and for wild and geographically-separated samples, the pattern of seed storage globulins enables to discriminate between species.

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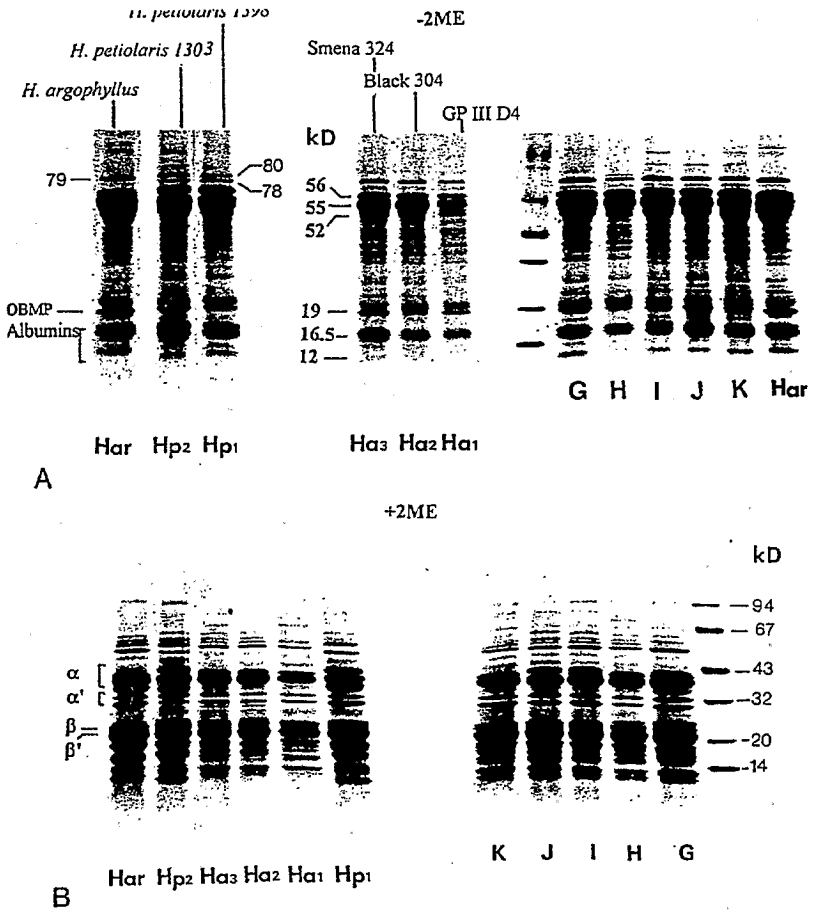

Fig. 1. Monodimensional electrophoresis (SDS-PAGE) of sunflower seed storage proteins in non-reducing conditions (*A*, − 2-ME) and reducing conditions (*B*, + 2-ME). Hp 1 and Hp 2 *Helianthus petiolaris*; Ha 1, Ha 2, and Ha 3 *H. annuus*; Har *H. argophyllus*; G–K hybrids. Details on the material sources are given in Table 1

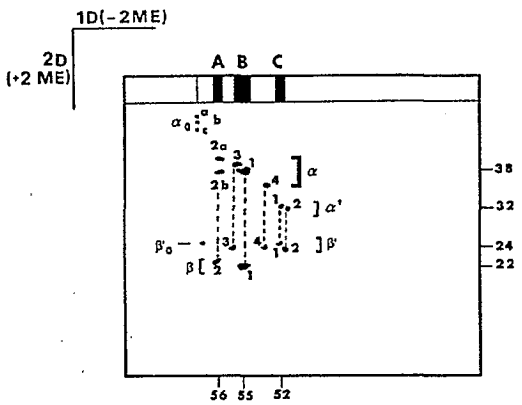

Fig. 3. Diagram of the polypeptide pairs separated by two-dimensional SDS polyacrylamide gel electrophoresis (first dimension, non-reducing conditions; second dimension, reducing conditions). αβ, αβ′ and α′β′ pairs are major subunit pairs, $\alpha_0\beta'_0$ represent minor subunit pairs. The diagram summarizes all the subunit pairs found in the *Helianthus* spp. studied

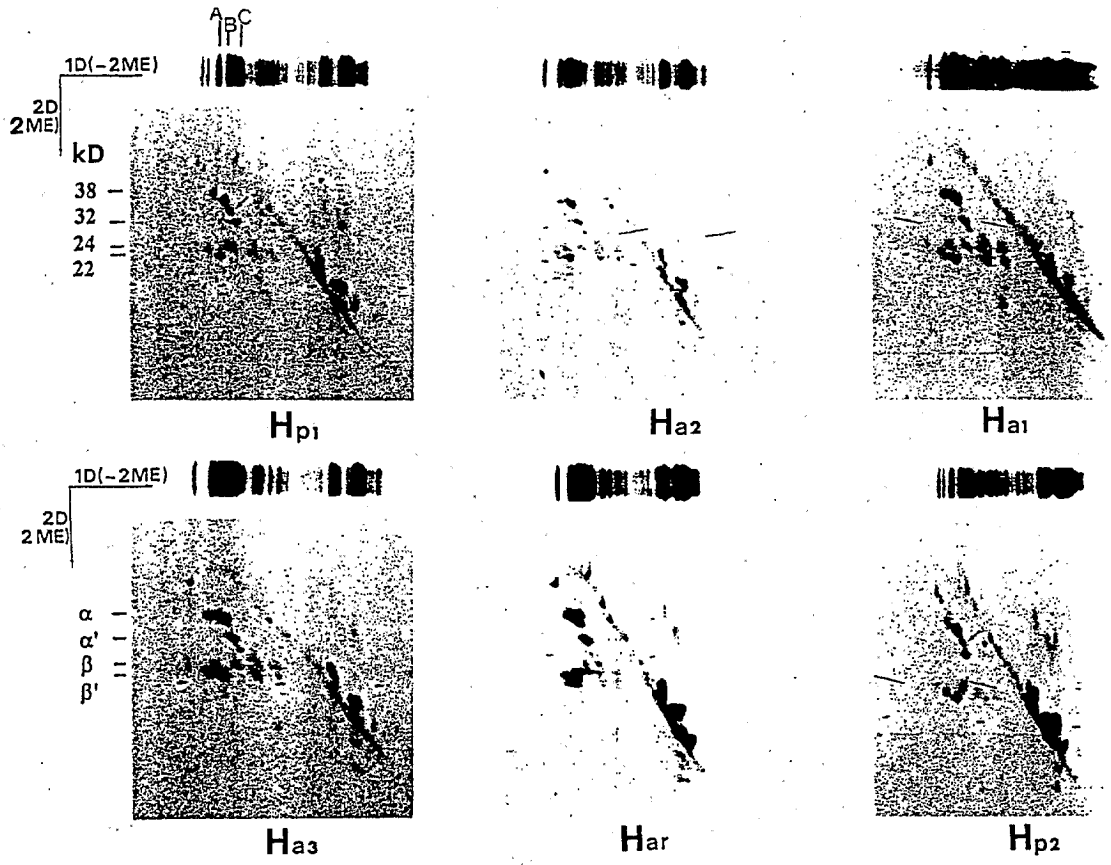

Fig. 2. SDS-PAGE bidimensional analysis of sunflower seed storage proteins in non-reducing (1D) and reducing (2D) conditior Hp 1 and Hp 2 *Helianthus petiolaris*; Ha 1, Ha 2, and Ha 3 *H. annuus*; Har *H. argophyllus*. Details of the material sources are giv in Table 1

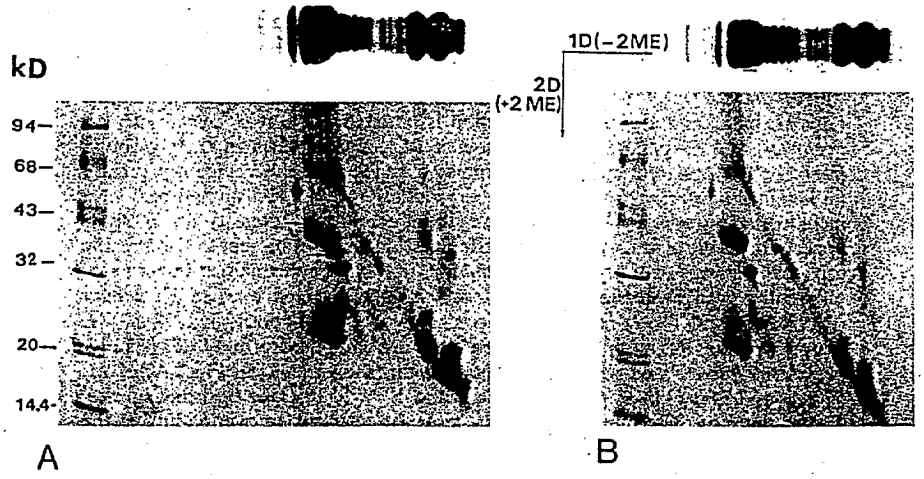

Fig.4 Bidimensional analysis of hybrids. *A Helianthus annuus* × *H. petiolaris; B H. annuus* × *H. debilis*. Black arrows indicate the specific α_{2n} and α_4 polypeptides from *H. petiolaris*

Table 1. List of *Helianthus* seed samples examined

Taxon	Source and line code	Abbreviation
H. annuus L.	G. P. III D4 (pool wild × cultivated) Davis (California)	Ha 1
H. annuus	Black 304 (cultivated for birds feeding) Kenya	Ha 2
H. annuus	Smena 324 Russian population (cultivated around 1960)	Ha 3
H. argophyllus TORREY & GRAY	North America (collected in Nebraska)	Har
H. petiolaris NUTT.	1398 (sib cross) North America	Hp 1
H. petiolaris	1303 F1 (Free fecondation) North American population	Hp 2
Hybrids		
H. annuus × *H. petiolaris*		G
H. annuus × *H. debilis* HEISER		H
progeny of cross		I
H. argophyllus × *H. annuus*		
cross *H. annuus* × I		J
cross *H. annuus* × J		K

Table 2. Composition of the polypeptide pairs in the globulin fraction. Hp 1 *H. petiolaris* (1394), Ha 1 *H. annuus* (G. P. III D4), Ha 2 *H. annuus* (Black 304), Ha 3 *H. annuus* (Smena 324), Hp 2 *H. petiolaris* (1203 F1), Har *H. argophyllus*. Details on the material sources are given in Table 1. αβ, αβ′, and α′β′ pairs are major subunit pairs. $\alpha_0\beta'_0$ represent minor subunit pairs. Indices correspond to M_r polymorphism of the polypeptides

Taxa	Globulin subunits					
	Major					Minor
Hp 1	$\alpha_{2a}\beta_2$	$\alpha'_1\beta'_1$	$\alpha'_2\beta'_2$	$\alpha_1\beta_1$	$\alpha_4\beta'_4$	$\alpha_{0a}\beta'_0$ $\alpha_{0c}\beta'_0$
Ha 1	$\alpha_{2a}\beta_2$	$\alpha'_1\beta'_1$	$\alpha'_2\beta'_2$	$\alpha_1\beta_1$	$\alpha_4\beta'_4$	$\alpha_{0a}\beta'_0$ $\alpha_{0c}\beta'_0$
Ha 2	$\alpha_{2b}\beta_2$	$\alpha'_1\beta'_1$	$\alpha'_2\beta'_2$	$\alpha_1\beta_1$	$\alpha_3\beta'_3$	$\alpha_{0b}\beta'_0$
Ha 3	$\alpha_{2b}\beta_2$	$\alpha'_1\beta'_1$	$\alpha'_2\beta'_2$	$\alpha_1\beta_1$	$\alpha_3\beta'_3$	$\alpha_{0b}\beta'_0$
Hp 2	$\alpha_{2b}\beta_2$	$\alpha'_1\beta'_1$	$\alpha'_2\beta'_2$	$\alpha_1\beta_1$	$\alpha_3\beta'_3$	$\alpha_{0b}\beta'_0$
Har	$\alpha_{2b}\beta_2$	$\alpha'_1\beta'_1$	$\alpha'_2\beta'_2$	$\alpha_3\beta_1$	$\alpha_1\beta'_3$	$\alpha_{0b}\beta'_0$