SEED STORAGE PROTEINS OF *HELIANTHUS (ASTERACEAE)*

BIOCHEMICAL AND CHARGE POLYMORPHISM

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Abstract
Biochemical heterogeneity was studied on the seeds of 19 sunflower species which were compared on the basis of their protein contents and the relative proportions of their protein fractions. The globulin content varied from 50% to about 70% and the albumin content from 18% to 35% according to the species. The level of intermediate *Mr* polypeptides showed a great variability (9.6 to 24.3%).
Charge polymorphism was studied using isoelectric focusing (IEF) and IEF-PAGE in mono and bidimensional procedures in the presence or absence of 2-mercaptoethanol (2-ME). Detailed nomenclature of the α, α' and β, β' polypeptides constituting the different helianthinin globulin subunits is given via the results of pI and *Mr* analyses. Monodimensional IEF patterns of the more basic albumins (pI >8.0) appear to provide a more valuable approach to identifying specific protein markers.

Introduction

.The genus *Helianthus* contains 50 species representing a large genetic diversity. Thus, as for agronomic characteristics (DREHER & al. 1983) or for oil production (SEILER 1985) one way of improving the protein fraction of cultivated sunflower seeds is to examine the genetic resources of wild sunflower species. In this study the protein contents as the relative proportions of the protein fractions from nineteen wild species (including three *H. petiolaris* subspecies) were determined. *Mr* and charge polymorphism of the polypeptide constituents were then analyzed using mono and bidimensional SDS-PAGE and IEF techniques.

Materials and methods

Plant material. Seeds of *Helianthus* spp. (wild, cultivated and hybrids) were provided by the Institut National de la Recherche Scientifique (INRA, Montpellier, France).
Extraction of proteins. Dehulled seeds of each species (70 mg) were ground in a mortar and extracted with 1.5 ml of the extraction buffer (4% (w/w) SDS, 50 mM Tris-HCl buffer pH 6.8 containing 30% (v/v) glycerol) at room temperature during 30 min under magnetic stirring. An aliquot was stored and the remaining slurry centrifuged (4 400 g, 10 min, 4°C). The supernatant (crude extract) was either directly analyzed or fractionated into albumins and globulins by dialysis (DALGALARRONDO & al. 1984) and stored at -20°C. In reducing conditions, 3% (v/v) of 2-ME

were added and the extracts were heated at 100°C for 2 min. For IEF, SDS in the extraction buffer was replaced by 7M urea.

Electrophoretic procedures..

IEF under non-reducing or reducing conditions was carried out according to the procedure of TRIEU & GRIPON (1981) on a L.K.B. multiphor 2117 apparatus. Slab gel (1 mm) contained 5% (w/v) polyacrylamide, 7.0 M urea, and 5% ampholines (Serva) of pH range 4.0 to 9.0 with omission of nonidet P40. Prefocusing (1h) and focusing (2h 30 min) were performed at constant power (7W). Bands were coloured according to BLAKESLEY & BOEZI (1977) using coomassie blue G250 (Serva).

For the two-dimensionnal electrophoresis, stained bands of the first run (1D) were excised and incubated (2 x15 min) with the buffer suitable for the next electrophoresis.

Densitometric analyses. After electrophoresis, the position of the polypeptidic bands and their intensity were measured with a LKB 2202 Ultroscan laser densitometer and a LKB 2220 integrator. The relative proportions of the bands (albumin, globulin and intermediate *Mr* proteins) were determined. The densitometer response was linear in the concentration range tested..

Results

The relative proportions of globulin, albumin and proteins of intermediate *Mr* determined by densitometry with respect to total stained proteins are reported in Table 1. The globulin content (polypeptide of *Mr* >52 kD) varied from 50% (*H. ciliaris*) to about 70% (*H. maximiliani*) with an average value of 60%. The albumin fraction (*Mr* <15 kD) represented about 25% of the stained polypeptides. In some species, such as *H. exilis* or *H. ciliaris*, the increase of the albumin fraction seemed to be related to a lower globulin content. *H. maximiliani* displayed the highest globulin and the lowest albumin content. Levels of intermediary polypeptides(18 kD<*Mr*<50 kD) showed great variability (9.6 to 24.3%).

IEF analyses in non reducing conditions

Monodimensionnal separation of total globulin and albumin extracts. As shown on Fig. 1 considerable heterogeneity is observed. DALGALARRONDO & al. (1985) have demonstrated that the helianthinin A, B and C subunits focused in the 5.4-6.8 pHi range. Previous studies on purified albumin fraction (DECHERF-HAMEY & al. 1986) have shown numerous proteins focusing in a neutral and mainly basic pH range. The albumin fraction, particularly in the basic pH range (pH >8.0) appears to present a more easily interpreted measure of interspecific variability.

Bidimensionnal separation. IEF/SDS-PAGE analyses are shown on fig.2 and were detailed for *H. annuus* (cv. Mirasol) used as a stradard and *H. petiolaris*. The pattern of *H. annuus* (Fig. 2, left panels) shows that the helianthinin fraction focused between pH 5.4 and 6.8 with two 6.3-6.8 groups and two groups focusing in the range 5.7-6.0 and 5.4-5.7. In the pH range of 6.3-6.8 only the intermediary subunits A and B (*Mr* 56 and 55 kD) were present. The major B subunits also focus in the pH 5.7-6.0 range. The minor C subunits focussed in a more acidic pH range (5.4-5.7). In our conditions, helianthinin intermediary subunits A, B and C are focussed in the upper part of the gel, where traces of subunits of Mr 53-54 kD are also visible. In this part of the gel the 44kD doublet of the albumin fraction is easily detected (Fig. 2, left diagramm, lower black arrow). As in our previous *Mr* polymorphism studies (RAYMOND & al. 1994), the main differences in the helianthinin fraction concern the three *H. petiolaris* accessions where the 53-54 kD subunits are strongly represented (Fig. 2, right pannels, thick arrows) and whose pI's are in the 5.4-6.0 range whereas a part of the polypeptides (pI's 5.7-6.0) of the B subunit diseappear. All the other species present a bidimensionnal pattern very close to *H. annuus* despite the fact that minor quantitative differences may be observed (results not shown).

Albumins can be easily separated in 2D (non reducing media) as they possess a very low *Mr* (12 to 15 kD) (Fig. 2, upper right panel, marked by a box). Among the more basic albumins some spots are oleosin (thin arrow).

IEF in reducing conditions

Monodimensionnal analyses show (Fig. 3A) the IEF patterns observed in the presence of 2-ME. Numerous α, α' and β, β' polypeptides are vizualized but only the IEF/PAGE under reducing conditions enables a determination of the apparent molecular weight of the focussed polypeptides. In Fig. 3B we observe that the polypeptides could be separated into (1) *Mr* 38-39 kD focussing into a 5.6-5.8 pH range (α1), or in a pH 6.3-7.0 range (α2 and α3), (2) *Mr* 32 kD focussing in a 5.2-5.4 pH range (α'1, α'2 and α'3) (the intermediary *Mr* α4 polypeptides mainly represented in *H. petiolaris* is the most acidic of them), (3) β and β' polypeptides of *Mr* 22-24 kD focussing into a more basic pH range (6.8-8.0).

Discussion

In our present knowledge, the total protein extract as well as the relative proportions of the main proteins (albumin and globulin) or minor ones (intermediate *Mr* proteins) is not obviously variable within the genus *Helianthus*. From a nutritional point of view, the globulin fraction is characterized by a low content in Lys and sulfur containing amino acids (MOSSE & BAUDET 1972) which is not the case for the albumin fraction In this respect the species *H. exilis* and *H. ciliaris* are of particular interest as they possess a large proportion of albumin (30%). Breeding programmes may take into account these data with the aim of improving the nutritional value of sunflower hybrids. For the characterization of *Helianthus* species the use of the albumin/globulin ratio may be of interest but further investigations are needed to determine the exact intraspecific variability and the influence of environmental as well as cultural conditions.
Monodimensional IEF of albumins seems to be the most promising avenue for obtaining useful results.

References

BLAKESLEY. R. W.. BOEZI. J. A.. 1977: A new staining technique for proteins in polyacrylamide gels using Coomassie brillant blue G250. - Anal. Biochem. 82: 580-582.

DALGALARRONDO. M.. RAYMOND. J.. AZANZA. J. L.. 1984: Sunflower seed proteins. Characterization and subunit composition of the globulin fraction. - J. Exp. Botany 35: 1618-1628.

DALGALARRONDO. M.. RAYMOND. J.. AZANZA. J. L.. 1985: Sunflower seed proteins: size and charge heterogeneity in subunits of the globulin fraction. - Biochimie 67. 629-632.

DECHERF-HAMEF. S.. MIMOUNI. B.. RAYMOND. J.. AZANZA. J. L.. 1986: Partial characterization of polypeptide components of sunflower (*H. annuus* L.) seed albumin fraction. - Die Nahrung 34: 387-398.

DREHER. M. L.. ROATH. W. W.. HOLM. E. T.. D'APPOLONIA. B.. 1983: Yield. characteristics and composition of oil-type hybrid sunflower seed grown in North Dakota. - JAOCS 60: 1876-1877.

MOSSE. J.. BAUDET. J. 1972: Aspects biochimiques de la qualité de la richesse en lysine des proteines de la graine de tournesol. Proc. 5th Int. Sunflower Conf.. Clermont Ferrand. 437-440.

RAYMOND. J.. MIMOUNI. B.. AZANZA. J. L.. 1994: Variability in the 11S globulin fraction of seed storage proteins of *Helianthus* (*Asteraceae*). - Pl. Sys. Evol. 193: 69-79

SEILER. G.J. 1983: Effect of genotype. flowering date. and environment on oil seed content and oil quality of wild sunflower seed. - Crop Sci. 25: 1063-1068.

TRIEU. P.. GRIPON. J. C.. 1981: Electrofocusing and two-dimensional electrophoresis of bovine caseins. - J. Dairy Research 48: 303-310.

Table 1 Total protein content and relative proportions of protein fractions in the seeds of 19 wild sunflower species.

Species (n°)	Total protein (%)	Gl + HMw*	I	Alb
1	16.1	59.9	16.6	23.6
2	8.6	57.0	14.7	28.3
3	8.3	57.0	18.3	24.7
4	8.8	60.2	14.8	24.9
5	10.0	66.1	10.5	23.4
6	12.8	54.5	14.4	31.2
7	12.4	62.6	16.3	21.1
8	11.8	56.2	18.4	25.3
9	10.5	63.0	13.7	23.4
10	11.5	50.1	15.2	34.7
11	11.7	65.0	9.6	25.4
12	13.5	59.3	16.8	23.9
13	17.3	61.3	14.4	24.3
14	16.1	64.1	13.8	22.1
15	13.4	65.5	15.9	18.7
16	12.7	62.0	10.5	27.5
17	14.1	69.9	13.9	16.2
18	14.0	58.3	24.3	17.5
19	14.0	63.9	11.0	25.1
M	10.3	51.9	26.4	21.6

M: *H. annuus* cv mirasol used as a standard

* GL + Hmw; globulin plus high *Mr* fractions; I: intermediate *Mr* proteins; Alb: albumin fraction. Protein % are on dry basis and fractions in % of the total densitometric area.

1: H. petiolaris petiolaris. 2: H. argophyllus. 3: H. niveus canescens. 4: H. petiolaris fallax. 5. H. petiolaris. 6: H. exilis. 7: H. debilis. 8: H. anomalus. 9: H. praecox hirtis. 10: H. ciliaris. 11: H. hirsutus. 12: H. tuberosus. 13: H. occidentalis occidentalis. 14: H. mollis. 15: H. rigidus subrhomboides. 16: H. eggertii. 17: H. maximiliani. 18: H. nuttallii nuttallii. 19: H. siphioides.

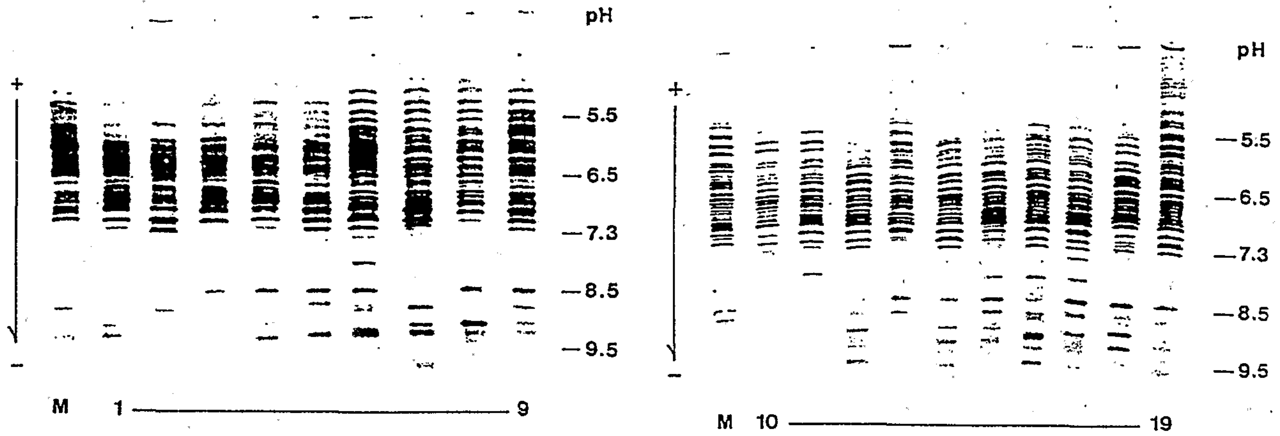

Fig.1 . IEF analysis of sunflower seed storage proteins (ca. 70 μg protein). Numbers correspond to the species described in Table 1 *Helianthus annuus* (cv. Mirasol) is used as a standard

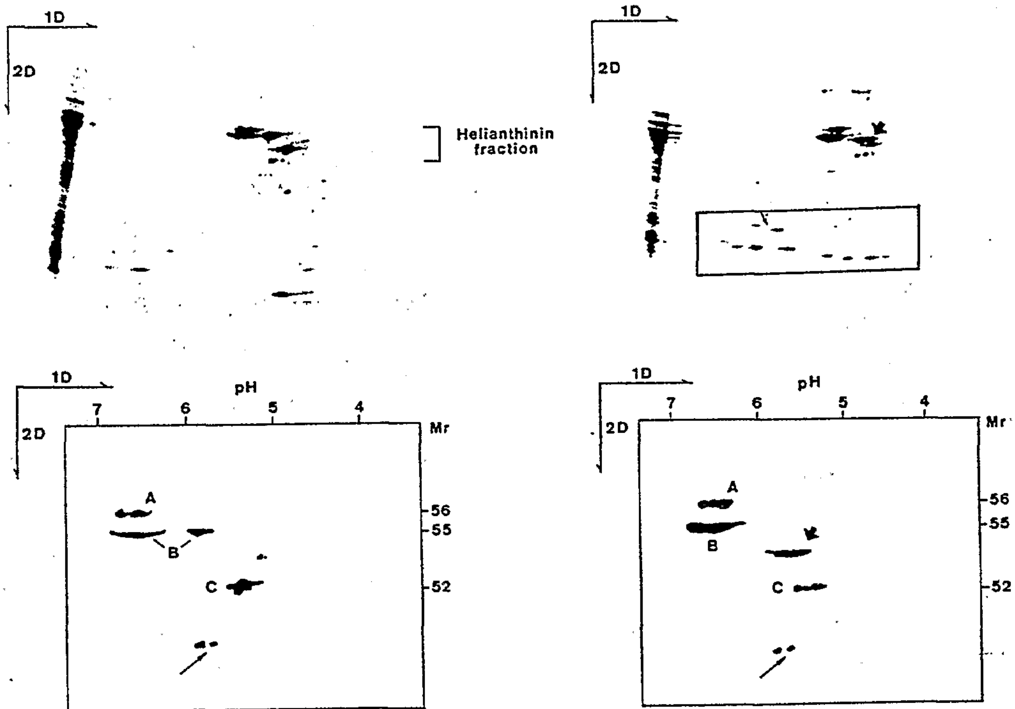

Fig.2 . Bidimensional IEF/SDS-PAGE analysis of the different *Helianthus* species. Proteins (ca. 100 μg) were separated by IEF (1D, pHs are indicated) and SDS-PAGE (2D, numbers are $Mr \times 10^{-3}$). Monodimensional SDS-PAGE is shown at the left of the 2D patterns: *H. annuus* pattern (upper left panel) and diagram of the globulin fraction (lower left panel); *H. petiolaris* pattern (upper right panel) and diagram of the globulin fraction (lower right panel); the main helianthinin subunits (A, B, and C) are indicated in the lower panels; the lowest arrow shows the 44 kD albumin doublet. In the right panels, the 53–54 kD subunits are indicated (thick arrows); albumins are marked by a box (including some oil body membrane proteins: arrow)

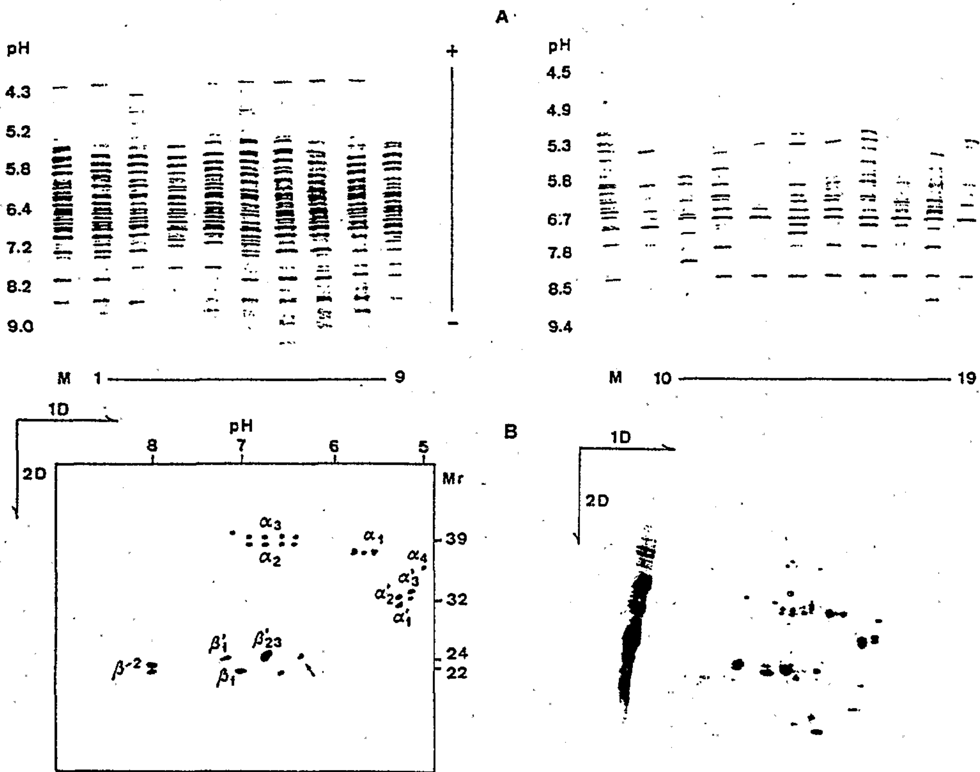

Fig.3. *A* IEF analysis of reduced seed storage proteins (ca. 70 μg protein). Numbers and letters correspond to species described in the legend of Fig. 1; pHs are indicated. *B* IEF (+ 2ME)/SDS-PAGE (+ 2ME) analysis of *Helianthus annuus* (right panel) and diagram of the globulin fraction (left panel) (1D, pHs are indicated; 2D, numbers are $Mr \times 10^{-3}$). The different $\alpha\beta$ and $\alpha'\beta'$ polypeptides separated after reduction are shown