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Molecular heterogeneity and genetics of *Vicia faba* seed storage proteins

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Summary. Legumin and vicilin were purified from seeds of *Vicia faba* L. var. *Scuro*, characterized in different electrophoretic systems, and used to produce polyclonal antibodies in rabbits. Two-dimensional electrophoretic studies showed a wide range of heterogeneity in the subunits of both legumin and vicilin. Legumin was found to be composed of 29 disulphide-linked subunit pairs with different molecular weight and/or isoelectric point. Western blot analysis of legumin of several mutants revealed molecular polymorphism based on a corresponding gene family. Three different α-major legumin patterns were found, and inheritance studies showed that the 34.3-kD legumin polypeptide is the product of one locus, *Lg-1α*, which is the first legumin genetic locus described in *Vicia faba*. Vicilin was found to be composed of as many as 59 subunits distributed in a molecular weight range of 65.7 to 42.8 kD (major polypeptides) and 37.2 to 15.2 kD (minor polypeptides), with different isoelectric points. A model is proposed that explains the possible formation of the minor subunits and the major subunits of 48.2 and 46 kD molecular weight (MW) from proteolytic cleavages and/or glycosilation of precursor polypeptides. Ten different vicilin electrophoretic patterns were observed among the analyzed accessions, which showed large molecular polymorphism that proved to be under genetic control.

Key words: *Vicia faba* – Legumin – Vicilin – Structure – Genetics

Introduction

Grain legume seeds contain large amounts of salt-soluble proteins, which are designated as 7 S globulin, vicilin, and 11 S globulin, legumin, according to their sedimentation coefficient. Both classes of proteins consist of several polypeptides (Derbyshire et al. 1976; Casey et al. 1986). Data are available on the genetic localization, number, and inheritance of the genes coding for these proteins in species of *Pisum*, *Phaseolus*, and *Glycine* (reviewed in Casey et al. 1986).

In *Vicia faba* the isolation of a vicilin gene (Weschke et al. 1988) and of four legumin genes, some of which are certainly expressed during seed development (Wobus et al. 1986), has been recently reported. Data are, however, scanty both for the genetics of the legumin subunits (De Pace et al. 1985) and for the subunit composition of the vicilin proteins, due to their complex electrophoretic patterns and to the outbreeding nature of *Faba* bean cultivars (Porceddu et al. 1980).

In this paper we provide detailed results on the subunit composition and genetic control of the two major seed storage protein classes of *Vicia faba*.

Materials and methods

Plant material considered is listed in Table 1. Seeds of the chromosome mutant accessions were kindly supplied by P. Perrino, Institute of Germoplasm, Bari (Italy); the mutant B10 was obtained from M. H. Poulsen (Denmark). Plants were grown and crossed under greenhouse conditions to prevent outbreeding. In inheritance and segregation studies, plants were tested for their biochemical phenotype, extracting the proteins from a cotyledon, leaving the latter in place attached to the embryo axis.

Protein extraction and SDS gel electrophoresis

Single, dehulled cotyledons were milled and the flour was defatted with 70% (v/v) ethanol (200 ml/g). All subsequent operations

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Table 1. List of plant material

Mutant lines	Description
B-10	Closed flower
A	Tr (I–III)
C	Tr (I–IV)
D	Tr (I–III)
E	Tr (IV–V)
I	Tr (I–V)
K	Tr (I–VI)
M	In (I)
N	In (I)
BK	In (V), Tr (I–VI)
Cultivars	
Trois fois blanche (T.f.b.)	*V. faba* var. *equina*
Scuro Torre Lama	*V. faba* var. *minor*
Primus	*V. faba* var. *minor*
Kleine-Thuringer	*V. faba* var. *minor*

Chromosomes involved in the rearrangements are indicated with roman numbers. Tr = translocation; In = inversion

were carried out at 4 °C. Total seed proteins were extracted with 0.2 *M* TRIS/HCl buffer, 2% sodium dodecyl sulfate (SDS), 10% sucrose, pH 6.8 (sample buffer), reduced when required with 1% 2-mercaptoethanol (2-ME), and immediately submitted to SDS polyacrylamide gel electrophoresis (SDS-PAGE) on 15% or 17% polyacrylamide (PAA), according to Laemmli (1970), modified by Matta et al. (1981). Each gel was calibrated with molecular weight markers (Pharmacia).

Legumin and vicilin purification

Total proteins were extracted from defatted flour (0.1 g/ml) with 20 m*M* Na-borate buffer, 0.5 *M* NaCl, 1 m*M* EDTA, pH 8.9, containing 0.1 m*M* phenyl-methyl-sulphonil-fluoride, 10 m*M* phenantroline, and 10 m*M* iodoacetamide as proteolitic cleavage inhibitors. Globulins were prepared and submitted to zone precipitation as described by Rao (1984), modified by Scholz et al. (1974). Legumin- and vicilin-containing fractions were pooled, dialyzed against cold, distilled water, and freeze-dried (crude proteins).

A column (3.5 × 16 cm) of hydroxylapatite (LKB) was equilibrated with 50 m*M* potassium phosphate buffer, pH 8.0, and loaded with 25 ml of crude legumin dissolved in the same buffer. After elution of the unbound material with the same buffer, a linear gradient, 50–500 m*M* potassium phosphate (600 + 600 ml), was applied to the column. Legumin fractions were pooled, dialyzed against distilled water, and freeze-dried.

Crude vicilin was purified by immunoaffinity chromatography. Immunoglobulin G, (IgG) purified according to Goding (1978) from anti-*Vicia* legumin serum, was coupled to CnBr-activated Sepharose 4B (Pharmacia), following the manufacturer's instructions, and packed in a syringe. Crude vicilin dissolved in PBS (138 m*M* NaCl, 2.68 m*M* KCl, 8.1 m*M* Na_2HPO_4, 1.47 m*M* KH_2PO_4, pH 7.2) was applied to the column and incubated for 30 min to allow immobilization of contaminating legumin. Pure vicilin was then eluted with PBS.

Vicilin used in the immunization experiments was isolated from SDS-PAGE: the bands corresponding to the major polypeptides (about 43 kD) were cut out after visualization with ice-cold KCl (Hager and Burgess 1980). The gel slices were finely ground and extracted with 0.5 *M* TRIS/HCl buffer, 0.2 *M* NaCl, 6 *M* urea, 0.1% SDS, pH 7.5. Vicilin was dialyzed against PBS and adjusted to a final concentration of 0.1 mg/ml by ultrafiltration.

Two-dimensional (2D) electrophoresis

Non-reducing SDS-PAGE/reducing SDS-PAGE. Dissociated proteins were run on 12.5% PAA in the first dimension; strips were cut, incubated with sample buffer containing 1% 2-ME, and run on 15% PAA in the second dimension.

IEF/reducing SDS-PAGE. Isoelectric focusing (IEF) was performed under dissociating, non-reducing conditions according to Trieu-Cuot and Grifon (1981) using 23 × 11.5 × 0.7 cm slab gels containing ampholines (LKB) with a pH range of 3.5 to 10 (2 parts) and 4 to 6 (1 part), to a final concentration of 7%. Strips from the first dimension were cut and incubated for 2 h in sample buffer containing 1% 2-ME, and then submitted to SDS-PAGE in the second dimension on 15% PAA.

PAGE/SDS-PAGE. Seven and a half percent PAA gels were run under non-dissociating conditions at pH 4.0 according to Gabriel (1971). Cut gel strips were incubated for 2 h in sample buffer containing 2-ME and run on SDS-PAGE.

Immunochemical techniques

Antibodies against legumin and vicilin were raised in rabbits. One milligram legumin or 0.1 mg vicilin major polypeptides in 1 ml PBS and 1 ml Freund's complete adjuvant were injected intramuscularly. Similar booster injections, except for the absence of adjuvant, were given 2 weeks later and then every week. Animals were bled at weekly intervals starting 3 weeks after the first immunization. Before the injection series, the rabbits were bled to obtain pre-immune sera. Double immunodiffusion was carried out in 1% (w/v) agarose gel in PBS. Immunoelectrophoresis was performed according to the method of Hirshfeld (1960).

For Western blotting, proteins were submitted to SDS-PAGE and then transferred onto nitrocellulose filters by electroblotting (Towbin et al. 1979). The filters were allowed to react with anti-vicilin and -legumin antibodies and then with peroxidase-coupled goat anti-rabbit IgG (Biorad), before staining with diaminobenzidine (Johnson et al. 1984; Graham and Karnovsky 1966).

For vicilin and legumin purification control, filters were processed with anti-pea vicilin and legumin (kindly supplied by R. Casey, John Innes Institute, Norwich) as primary antibodies, which are known to cross-react with *Vicia faba* storage proteins (Croy et al. 1979).

Enzyme-linked immunosorbent assay (ELISA) was carried out as described (Capparelli and Iannelli 1989).

Results

Total *Vicia faba* seed proteins, analyzed by means of SDS-PAGE, displayed a complex pattern with great heterogeneity and large variability (Fig. 1). In the attempt to identify which proteins are responsible for such polymorphism, the major components of the globulin fraction, legumin and vicilin, were purified from seeds of the variety Scuro, characterized under different electrophoretic conditions, and used to produce specific polyclonal antibodies. The antisera obtained proved to be protein-class-monospecific, as no cross-reaction between vicilin and legumin was detected by double immunodiffusion, ELISA, or Western blot (Fig. 2).

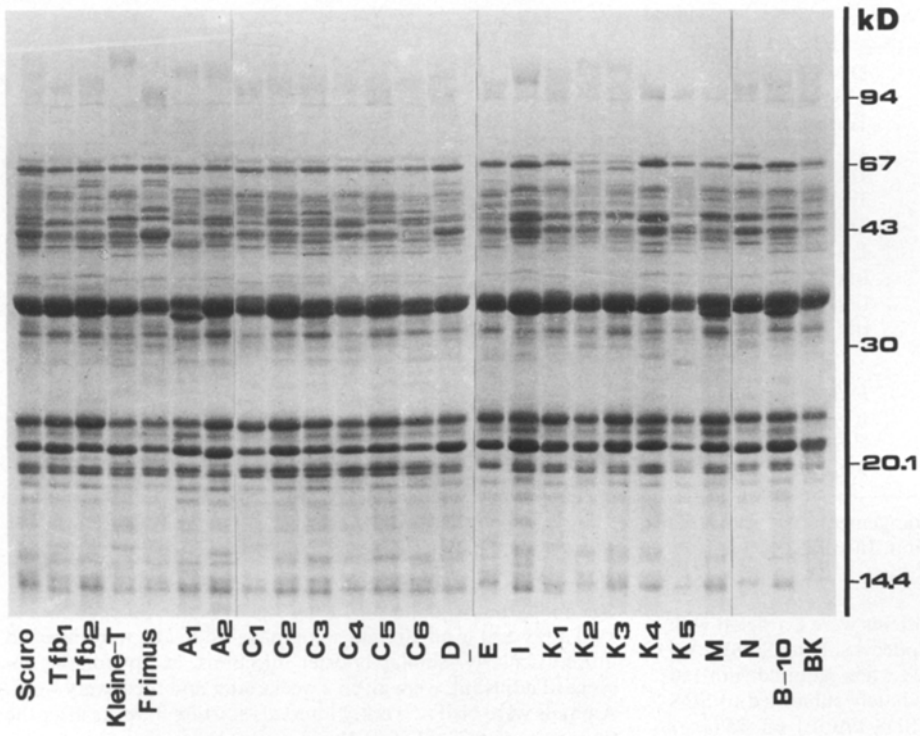

Fig. 1. SDS-PAGE profile of total proteins extracted from cotyledons of different *Vicia faba* genotypes. Genotypes indicated with the same letter represent different plants of the same accession

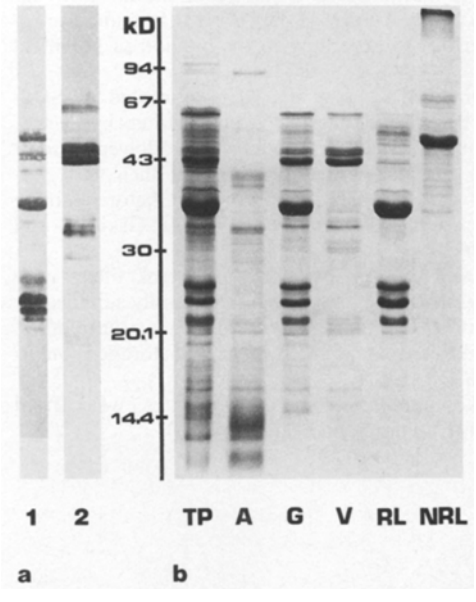

Fig. 2. **a** Western blot of total proteins separated by SDS-PAGE, processed with anti-legumin (*1*) and anti-vicilin (*2*) IgG and peroxidase-stained. **b** SDS-PAGE of total proteins (*TP*), albumins (*A*), globulins (*G*), vicilin (*V*), reduced legumin (*RL*), and non-reduced legumin (*NRL*) from cv Scuro

In total extracts most of the observed electrophoretic heterogeneity is due to globulins, vicilin and legumin (see G, V, RL, and NRL in Fig. 2), while a pattern represented by proteins quantitatively poorly expressed characterizes the water-soluble proteins (albumin fraction). The few relevant albumin bands consist of several polypeptides, the majority of which have a MW of about 14 kD or lower.

Legumin: molecular and subunit composition

Legumin purified by hydroxylapatite chromatography and analyzed under dissociating, nonreducing conditions (Fig. 3 a) showed the typical 11 S protein structure with two major subunit pairs of 55,400 and 53,700 daltons apparent MW (H and I in the figure) and a number of minor bands (A-G1, J) distributed over a MW range of 82,000 to 39,000 daltons. Figure 3 shows also the relationship of subunit pairs before reduction to their respective polypeptides after reduction. This was investigated through 2D-PAGE employing separation in the first dimension under nonreducing conditions (both SDS and IEF gels), followed by separation of the polypeptides of each pair, after reduction, on a second dimension, SDS-PAGE. Legumin analyzed with these techniques was shown to be composed of 29 subunit pairs – listed in Table 2 – differing in molecular weight and/or isoelectric point (IP). A high complexity was found for legumin type J, which showed several $\alpha\,\beta$-pairs. In fact, the polypeptide of about 39 kD apparent MW proved to consist of two pairs of subunits, which may be paired in two different combinations. However, as the α- and β-polypeptides forming the pair are equimolar (Casey et al. 1986), the most plausible combination should be 25.2–20.1 kD and 23.8–22.5 kD. Owing to the selected concentration of samples loaded on IEF gel, in Fig. 3 b it was not possible to detect legumin type J.

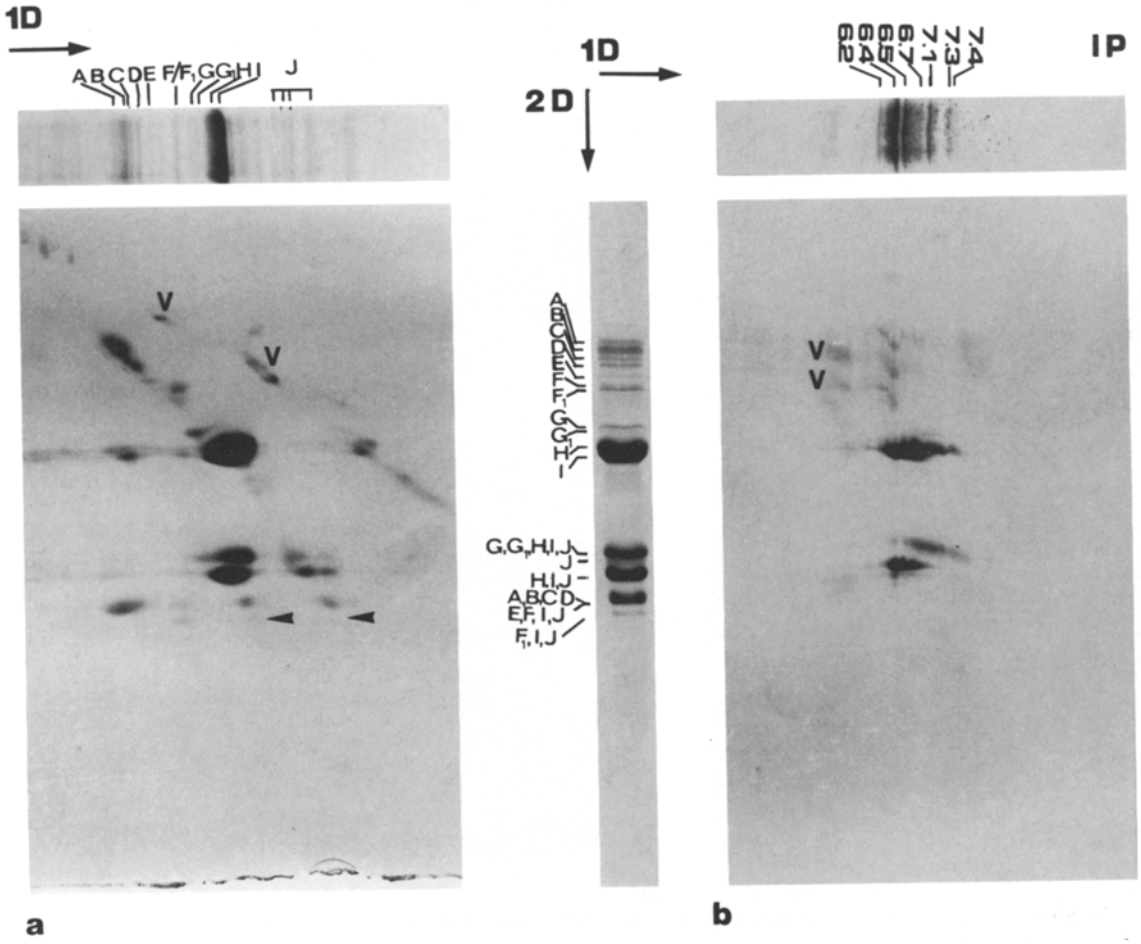

Fig. 3 a and b. Two-dimensional analyses of crude legumin of cv Scuro. **a** *Upper*: 1D, SDS-PAGE under non-reducing conditions; *lower*: 2D, SDS-PAGE under reducing conditions. *Arrows* indicates 20.1-kD β-polypeptide. **b** *Upper*: 1D, IEF under nonreducing conditions; *lower*: 2D, SDS-PAGE under reducing conditions. *A–J*: legumin subunit pairs as described in the text. *V*: vicilin

Vicilin: molecular and subunit composition

Vicilin purified by immunoaffinity chromatography (Fig. 2) exhibits, on SDS-PAGE under reducing or nonreducing conditions, four major bands with an apparent MW of about 65,700, 48,200, 46,000, and 42,800 daltons and a number of minor bands (E, Q) distributed over a MW range of 37,000 to 15,000 daltons. The vicilin polypeptide of about 66 kD has also been referred to as convicilin (Croy et al. 1980).

The unreduced and the reduced vicilin electrophoretic patterns are identical, as this protein does not contain any disulphide-linked polypeptides. The observed vicilin heterogeneity was confirmed by analyzing the protein both on denaturing IEF gel and on 2D gel, which used IEF in the first dimension and SDS gel in the second dimension (Fig. 4). The resulting vicilin composition is listed in Table 3. At least 17 bands could be detected on IEF gel, focalizing in the pH range of 5.2 to 7.5. Most of the bands focalizing in the pH range of 5.5 to 6.0 are formed by three out of the four major vicilin polypeptides with MW of 48,200, 46,000, and 42,800 daltons, while the bands focalizing in the pH range of 6.1 to 7.5 were found to be composed of all the vicilin major polypeptides. The minor polypeptides are distributed along the whole pH interval (Fig. 4a). When vicilin was analyzed on a nondissociating PAGE, in addition to some bands generated by globulins association (see also Schlesier et al. 1984), four main bands were detected, which probably correspond to different trimers forming the native molecules. In fact, those bands showed in the second dimension, SDS-PAGE, a different polypeptide composition (Fig. 5). The band with higher mobility (band n 4) was found to be composed of the main vicilin polypeptides – types B, C, and D – besides all the minor polypeptides – types E–Q – while all the others contained also the vicilin type A, even if in different amounts.

Vicilin and legumin variability and genetics

Total seed extracts of at least three seeds of each *Vicia faba* accession were separated by SDS-PAGE, blotted

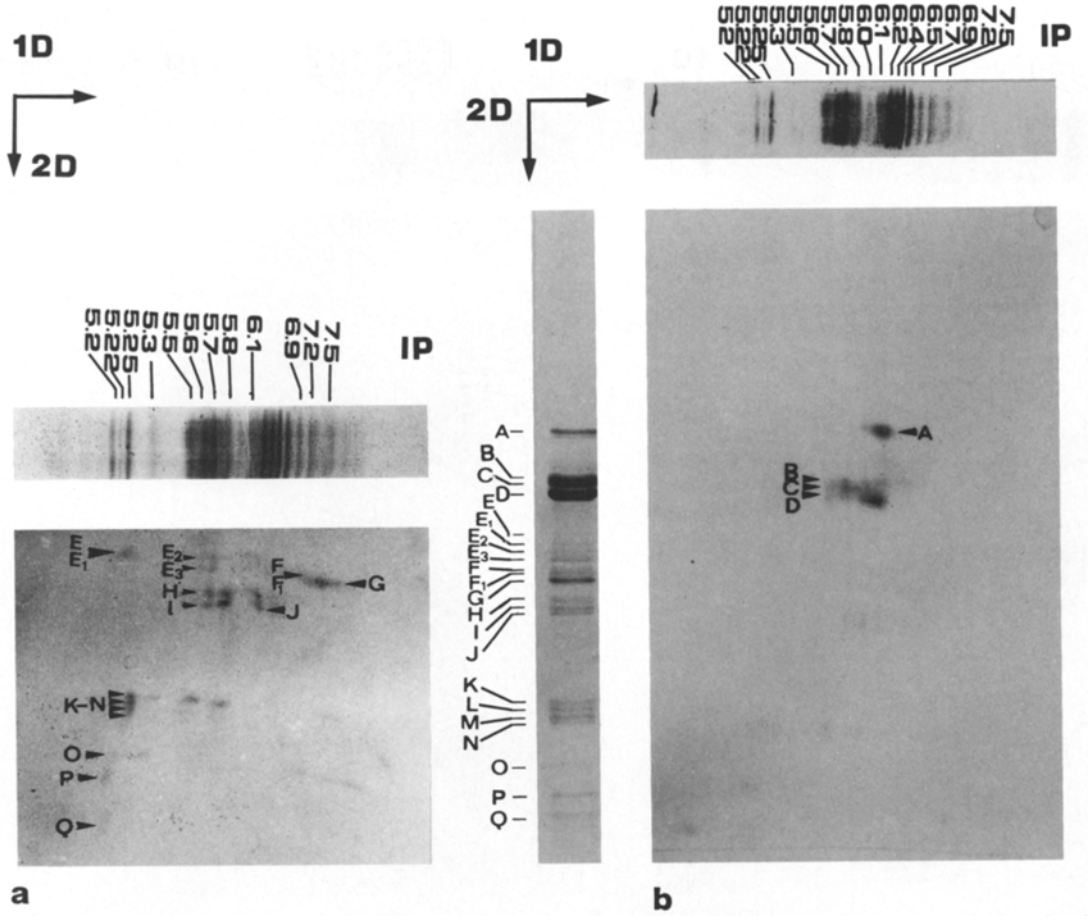

Fig. 4 a and b. Two-dimensional analysis of vicilin of cv Scuro. (1D: IEF; 2D: SDS-PAGE). *A–Q*: vicilin subunits as described in the text. **a** Minor vicilin subunits. **b** Major vicilin subunits

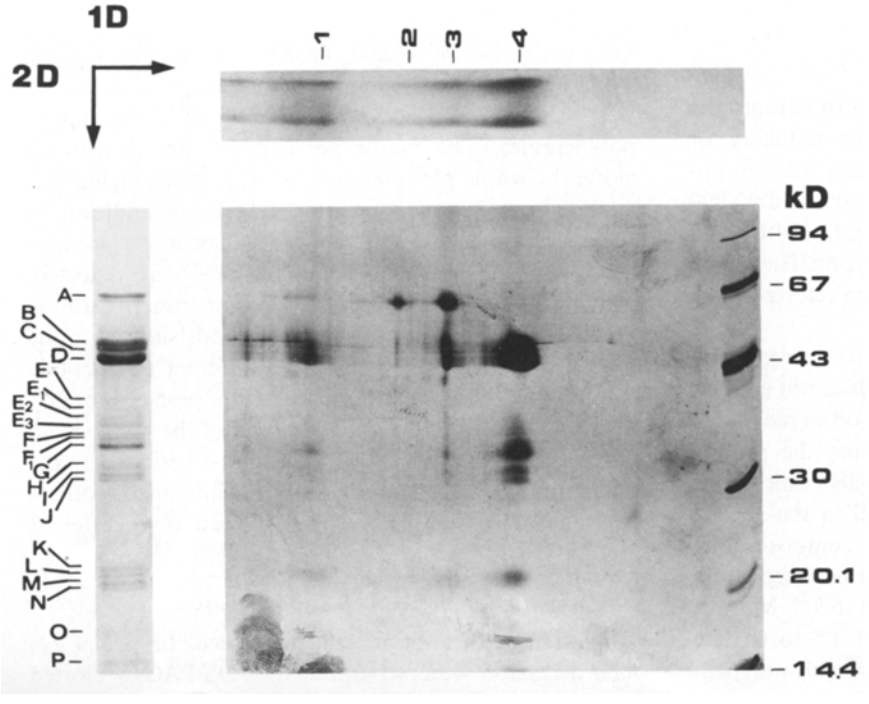

Fig. 5. Two-dimensional analysis of vicilin of cv Scuro 1D: non-dissociating PAGE at acidic pH; 2D: SDS-PAGE with MW markers. *A–P*: vicilin subunits as described in the text

Table 2. Apparent molecular weight (MW) and isoelectric point (IP) of unreduced and reduced legumin subunits

Type[a]	MW × 10^{-3}			IP	No. of pairs for each MW-IP combination
	αβ pairs	α	β	αβ pairs	
A	81.6	59.9	22.5	6.2	1
B	79.0	56.3	22.5	6.4	1
C	76.7	53.7	22.5	6.2	1
D	74.4	51.3	22.5	6.4	1
E	71.0	43.2	22.5	–	1
F	66.7	43.9	22.5	6.2	1
F_1	65.7	43.6	20.1	6.4	1
G	61.7	39.0	25.5	7.1; 7.2	2
G_1	59.9	38.4	25.5	7.3; 7.4	2
H	55.4	36.1	25.5	6.7; 7.1; 7.3; 7.4	4
			23.8	6.7; 7.1	2
I	53.7	35.2	23.8	6.2; 6.4; 6.5; 6.7	4
			25.5	6.4	1
			22.5	6.4	1
			20.1	6.4	1
J	43.2	25.5	25.0	–	1
	41.9	25.5	23.8	–	1
	40.6	23.8	22.5	–	1
		25.5	22.5		1
			or	–	
			20.1		1
	39.0				
		23.8	22.5		1
			or	–	
			20.1	–	1
					29

[a] 1D-SDS nomenclature (see Fig. 3a)

Table 3. Apparent molecular weight (MW) and isoelectric point (IP) of vicilin subunits

Type[a]	MW × 10^{-3}	IP	No. of polypeptides for each MW-IP combination
A	65.7	6.1; 6.2; 6.4; 6.5; 6.7	5
B	48.2	5.5; 5.6; 5.7; 5.8; 6.1; 6.5; 6.7; 6.9; 7.2; 7.5	10
C	46	5.5; 5.6; 5.8; 6.0; 6.1; 6.2; 6.4; 6.7; 6.9; 7.2; 7.5	11
D	42.8	5.5; 5.6; 5.8; 6.1; 6.2; 6.4	6
E	37.2	5.25	1
E_1	36.4	5.25	1
E_2	35.8	5.6; 5.7	2
E_3	35.2	5.6; 5.7	2
F	34.2	6.9	1
F_1	33.6	6.9	1
G	33.1	7.2; 7.5	2
H	31.1	5.7; 5.8; 6.1	3
I	30.1	5.7; 5.8; 6.1	3
J	29.7	6.1	1
K	22.3	5.25; 5.3	2
L	21.7	5.25; 5.5	2
M	21.0	5.25; 5.8	2
N	20.7	5.25	1
O	18.1	5.22	1
P	16.3	5.2	1
Q	15.2	5.2	1
			59

[a] 1D-SDS nomenclature (see Fig. 4a)

onto nitrocellulose, and processed with anti-legumin and anti-vicilin antibodies. Legumin and vicilin banding variants were observed among different accessions as well as within the same accession.

Ten patterns of vicilin were identified, differing from each other either in the number and/or in the electrophoretic mobility of the major polypeptides (types A, B, C, and D_1; Fig. 6a). This variability is mainly related with the polypeptides of types B and C, which differred in almost every sample, while only three variants were detected for type A. All genotypes showed the 42.8-kD band, while an additional polypeptide of about 41.4 kD (D_1) was noted in several samples.

Large variability was also found among vicilin polypeptides of types F–J (Fig. 6b), while with the selected amounts of protein it was not possible to follow the variability of the vicilin polypeptides types K–Q.

Anti-legumin immunoblots (not shown) confirmed that the α-major legumins have three electrophoretic variants (cf. Fig. 1); some genotypes, like the variety Scuro, have the α-major polypeptides formed by doublet with MW of about 36.1–35.2 kD (pattern 1), while others have one more α-major polypeptide with a MW of either 34.8 kD (pattern 2) or 34.3 kD (pattern 3).

Consistent variability was also detected for the high-molecular-weight legumin. No variability was detected for β-legumin subunits. SDS-PAGE analysis of legumin and vicilin from different seeds of the same accession and comparison of the parental patterns with those of their progenies gave the following results: for both protein classes different patterns were found among seeds of the same accession. As far as the legumin loci are concerned, the progenies of these genotypes did not segregate, indicating that the accessions we received were formed by different homozygous genotypes. Some of the progenies, however, segregated at vicilin loci while others were stable, indicating that only a fraction of the lines were homozygous for vicilin alleles.

Genotypes having legumin patterns 1 and 3 were selected to follow the inheritance of one legumin α-major polypeptide (Fig. 7). Reciprocal crosses gave an F_1 that exhibits a three-band, α-major legumin polypeptide pat-

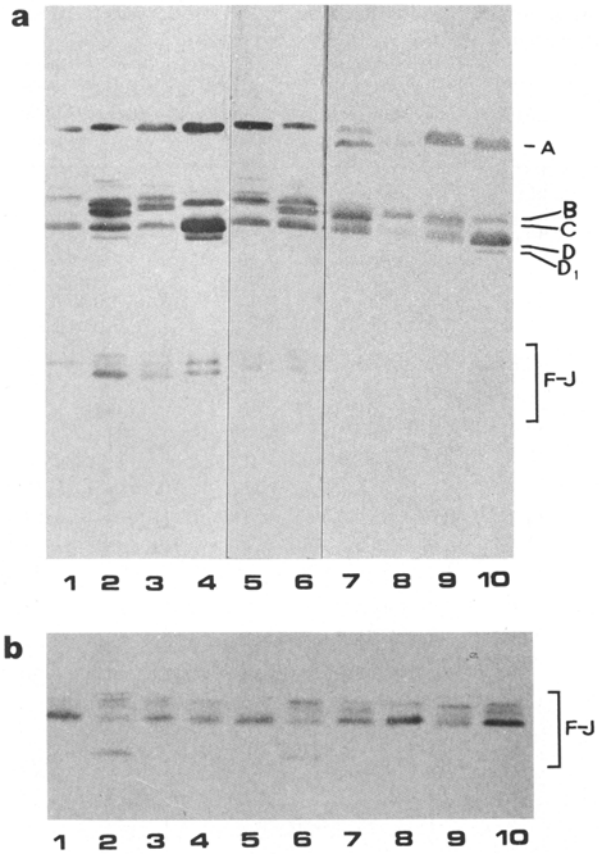

Fig. 6 a and b. Western blot of total proteins from different genotypes of *Vicia faba,* separated by SDS-PAGE, processed with anti-vicilin IgG, and peroxidase-stained. **a** Major vicilin polypeptides. Genotypes: *1*: C_1; *2*: Kleine T., A_1, C_3, C_6, N_1, I; *3*: A1; *4*: Primus, K_1; *5*: C_5; *6*: Scuro, T.f.b. BK, B-10, N_2, M, C_2, C_4, D, E; *7*: K_2; *8*: K_3; *9*: K_4; *10*: K_5. **b** Minor vicilin polypeptides. Genotypes: *1*: E; *2*: I; *3*: K_1; *4*: K_2; *5*: K_3; *6*: K_4; *7*: K_5; *8*: M_1; *9*: M_2; *10*: N_1

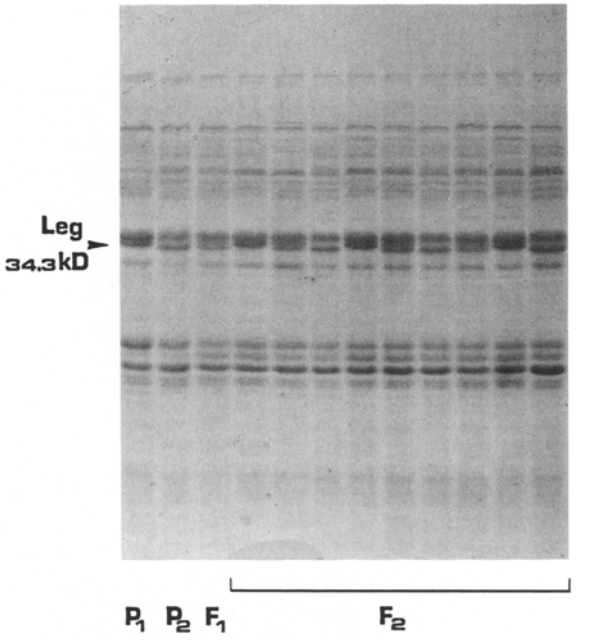

Fig. 7. SDS-PAGE of total proteins from single F_1, F_2 and parental seeds obtained from the crosses between genotypes of T.f.b. and M. *Arrow* indicates the α-major legumin polypeptide, which segregates

tern, and the segregation ratio observed on 100 F_2 seeds showed that the α-legumin polypeptide studied behaves as the product of single Mendelian genes ($\chi^2 = 0.66$ for three-to-one segregation).

Discussion

Legumin: molecular and subunit composition

The use of 2D non-reducing/reducing SDS-PAGE in conjunction with IEF proved to be of considerable value in identifying legumin disulphide-linked subunit pairs, demonstrating that *Vicia faba* legumin has a higher heterogeneity compared to the description given by others (Utsumi et al. 1980; Matta et al. 1981; Horstmann 1983).

Twenty-nine disulphide-linked subunit pairs were detected, some of which have not been described before (some subunits of the J type, subunits F_1 and G_1). This could partially be due to the genotypes used in our study. The discrepancy between the MW of unreduced α β-pairs and the sum of their polypeptides has been related to the presence of inter- and intra-chain disulphide bonds which affect the unwinding of the unreduced molecules (Matta et al. 1981).

The presence of differently charged forms of α β-pairs with very close IP and almost identical MW suggests the existence of small differences in the amino acid sequence of the protein subunits, reflecting sequence heterogeneity among members of a gene family. Like other 11 S storage proteins, *Vicia faba* legumins are synthesized as precursor molecules containing both the acidic and the basic polypeptides, which are cleaved by post-translational modifications leading to the mature polypeptides (reviewed in Casey et al. 1986). This indicates that each legumin gene codes for a subunit pair. Moreover, it is known that *Vicia faba* legumin is controlled by two main subfamilies of genes (Wobus et al. 1986). Considering all the evidence available, it can be concluded that most of the heterogeneity reported here is likely to be a direct expression of genetic heterogeneity. Other modifications, such as deamidation of glutamine or asparagine to generate charge heterogeneity, cannot however be totally ruled out.

Vicilin: molecular and subunit composition

Vicilin is the most heterogeneous protein among *Vicia faba* globulins. It is characterized by four main polypep-

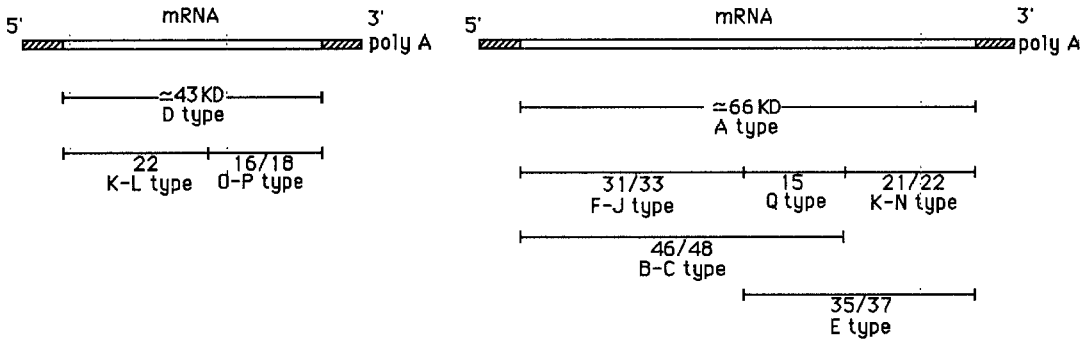

Fig. 8. Diagrammatic representation of the possible proteolytic processing of *Vicia faba* vicilin precursors. The sizes of the polypeptides produced by cleavage as well as of precursors are indicated

tides – types A to D – and a large number of minor polypeptides – types E to Q – which are the products of post-translational proteolytic cleavage of vicilin precursor polypeptides (Scholz et al. 1983; Gatehouse et al. 1984). This explains the identical immunological properties shown by all the vicilin polypeptides (i.e., high and low MW polypeptides).

The general accepted structure of the native protein is that of a trimer composed of either equal (Croy et al. 1980) or different subunits (Schleiser 1984). No 7 S protein composed of subunits of the same size (the convicilin type, formed only by 70-kD polypeptides) was found in the cv Scuro, thus supporting the model of Schleiser (1984), who described *Vicia faba* vicilin as a protein family of trimeric isomers composed of different ratios of different subunits. Some of the subunits will in due course be proteolytically split at one or two positions, after the assembly of the 7 S oligomers, and possibly such "nicking" contributes to native protein heterogeneity (Gatehouse et al. 1981).

Each of the major polypeptides, vicilin types A–D, is composed of several charge forms reflecting the sequence heterogeneity within members of a gene family. In fact, vicilin represents the product of a polymorphic gene family, which has diverged sufficiently to produce different combinations of processing sites, some members having no such sites and constituting the uncleaved polypeptides. Consequently, the low MW vicilin polypeptides correspond to a mixture of cleavage products from different vicilin gene products (Gatehouse et al. 1984; Spencer et al. 1983; Casey et al. 1986).

We suggest a possible model (Fig. 8) in which the large number of minor vicilin types plus two of the major types, B and C, are produced by cleavage of two different precursor polypeptide families, one of ca. 43 kD MW and the other of ca. 66 kD MW. The "nicking" sites would differ among members of the precursor families and would also be genotype-specific. Moreover, part of the observed heterogeneity could be a consequence of different patterns of glycosylation of the resulting polypeptides, as occurs in *Phaseolus vulgaris* and in *Pisum sativum* (Bollini et al. 1983; Davey and Dudman 1979).

Legumin and vicilin: variability and genetics

The use of specific antibodies raised against legumin and vicilin proved to be a powerful tool for the identification of the variability found among SDS-PAGE seed protein patterns of different *Vicia faba* genotypes. A limited variability was found for the legumin major subunits such as types H and I. Only three electrophoretic patterns were identified: pattern 1, which is the most common, pattern 2, which is rare, and pattern 3, which is less unusual and has already been reported by Muntz et al. (1986) in 20 out of 158 analyzed inbred lines. Since the genotypes with different α-major legumin banding patterns were not segregating for this character, it was possible to use the observed genetic variation for inheritance studies. It was then demonstrated that one of the α-major legumin polypeptides is the product of one locus we named *Lg-1α*, which is the first legumin genetic locus described for *Vicia faba* and which may contain a closely related group of genes belonging to a subfamily, as occurs in *Pisum* (Domoney and Casey 1985; Domoney et al. 1986).

The large variability found in vicilin subunits has been proved to the under genetic control through comparison between single plants and their relative progeny, but a true genetic study has yet not been performed. The different variants observed for the vicilin subunit types B and C could be the result of sequence variability along the cleavage sites of the family precursor polypeptides, as a consequence of the mutations characterizing the accessions we have analyzed, possibly in combination with different levels of glycosylation (Bollini et al. 1983). The vicilin polypeptide of about 43 kD MW (D) appears to be strongly conserved as it does not show any detectable polymorphism through SDS-PAGE analysis. Presumably the amino acid sequence of this polypeptide is essential and it is maintained unaltered through selection.

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