Quantitative variability in *Pisum* seed globulins: its assessment and significance

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Abstract. Five different techniques have been used with seven *Pisum* (pea) genotypes to demonstrate the existence of extensive genotypic variability in the proportions of the major pea seed globulins, legumin, vicilin and convicilin. The accuracy and reproducibility of each method have been assessed and the significance of the observed variability discussed both in relation to studies of the control of globulin synthesis and to breeding for pea seed protein with higher nutritional quality.

Introduction

During the development of the pea embryo, large amounts of the storage globulins, legumin and vicilin, are produced and deposited in the cotyledon. This synthesis represents the activity of probably only a few genes, or gene families, at a particular stage of development and as such is of fundamental interest to the plant molecular biologist. It is also of some practical consequence; the globulins, which constitute 70% or more of the protein of this high-protein crop, are the cause of the nutritionally inferior status (low methionine and cysteine contents) of pea (and other legume) seed protein.

Although some information has accumulated on the structure [1, 3, 5, 6, 9, 12, 16, 18, 29, 32], genetics [2, 30], cell-free biosynthesis [7, 8, 14, 15, 20] and post-translational processing [21, 27, 28] of pea seed storage proteins, little is known about the molecular basis of seed protein genetics or of the regulation of their biosynthesis during the developmental process. It would therefore be highly desirable to have variant forms of *Pisum* in which such phenomena could be studied.

Genotypic variability in the amounts and proportions of pea seed proteins – which could reflect variation in the regulation of protein synthesis during seed development – has been reported by a number of authors who have used several different methods [3, 10, 11, 17, 19, 25, 29, 31]. Of particular interest is the relationship between legumin synthesis and the round-seeded/wrinkled-seeded phenotypes [11], an understanding of which could lead to a

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Table 1. Genotypes used in the measurement of legumin contents

Genotype	Description
JI 15	Very tall, purple-flowered plant; round seeds, yellow cotyledons
JI 181	Kheeran pea; short plant, purple flowers; very small speckled seeds; yellow cotyledons
JI 184	Selection from primitive cultivar Khadra, from Sudan; very tall, white flowers; round seed, yellow cotyledons
JI 224	*Pisum fulvum* from Israel. Small, pink-flowered plant. Very small, brown seeds, yellow cotyledons
JI 227	*Pisum abyssinicum* from Ethiopia. Tall, purple-flowered plant. Dark, round seed, yellow cotyledons
JI 407	cv. Victory Freezer. Large wrinkled seeds, green cotyledons
JI 583	cv. Minarette. Small wrinkled seeds, green cotyledons
cv. Filby	Leafless plant. Medium-sized round seeds, yellow cotyledons

better appreciation of the control of legumin synthesis. It is axiomatic that if variant genotypes are used to study the biochemical basis of quantitative variability, the latter should be assessed as accurately as possible and should clearly be shown to be genotypically defined and be independent of the environment.

This report describes the extent of variability in the levels of the major seed globulins which exists within the genus *Pisum* and assesses the suitability and accuracy of a number of methods for measuring seed protein proportions.

Materials and Methods

Pea genotypes

Eight *Pisum* genotypes, covering a wide range of morphological characters and encompassing both *P. fulvum* and primitive and modern forms of *P. sativum* were used for analysis of protein composition. The origin and a brief description of each genotype are shown in Table 1. Five of these genotypes have previously been analysed in terms of the subunit structure of legumin [2]. Dry, mature seeds harvested from plants grown in the field and greenhouse were used.

Extraction of protein for cellulose acetate electrophoresis, analytical ultracentrifugation and immunological measurements

Testas and embryonic axes were removed and the cotyledons finely ground to pass a 60-mesh sieve. The ground meal was blended with 10 vol 0.5 *M* NaCl/0.05 *M* Tris–HCl pH 7.5/0.1 m*M* dithiothreitol and the homogenate vigorously stirred at 4°C for 2 h. After centrifugation (20 000 *g*, 10 min, 4°C); the supernatant was removed and the pellet re-extracted for 1 h with half the original volume of buffer. The homogenate was centrifuged and the re-extraction of the insoluble material repeated once more. After a final centrifugation, the three supernatants were combined and these represented the 'total extract' used for subsequent analyses. The protein concentration of total extracts was

between 8.0 and 12.7 mg/ml depending on the genotype, which represented greater than 90% extraction for all genotypes. Every extraction of each genotype was repeated at least once on a separate occasion; all measurements on each total extract were made at least in triplicate.

Sample preparation for differential scanning calorimetry (DSC) analysis

Pea meals were suspended in a small volume of 0.2 *M* NaCl, 0.05 *M* NaH_2PO_4 pH 7.0, and then dialysed overnight against the same buffer. The resulting dispersions (approx. 20 mg) were weighed directly into Perkin–Elmer volatile sample pans for DSC analysis. For meals of low globulin content, it was found necessary to extract proteins from the meal using the above buffer and then concentrate the resulting solution by dialysis against a suspension of Sephadex G-75 (Pharmacia, Ltd., Hounslow, England).

Estimation of protein concentrations

Protein concentrations in solution were measured by dye-binding assay or ninhydrin assay after acid hydrolysis, as previously described [1]. The latter method was also used to measure the protein content of dry pea meal.

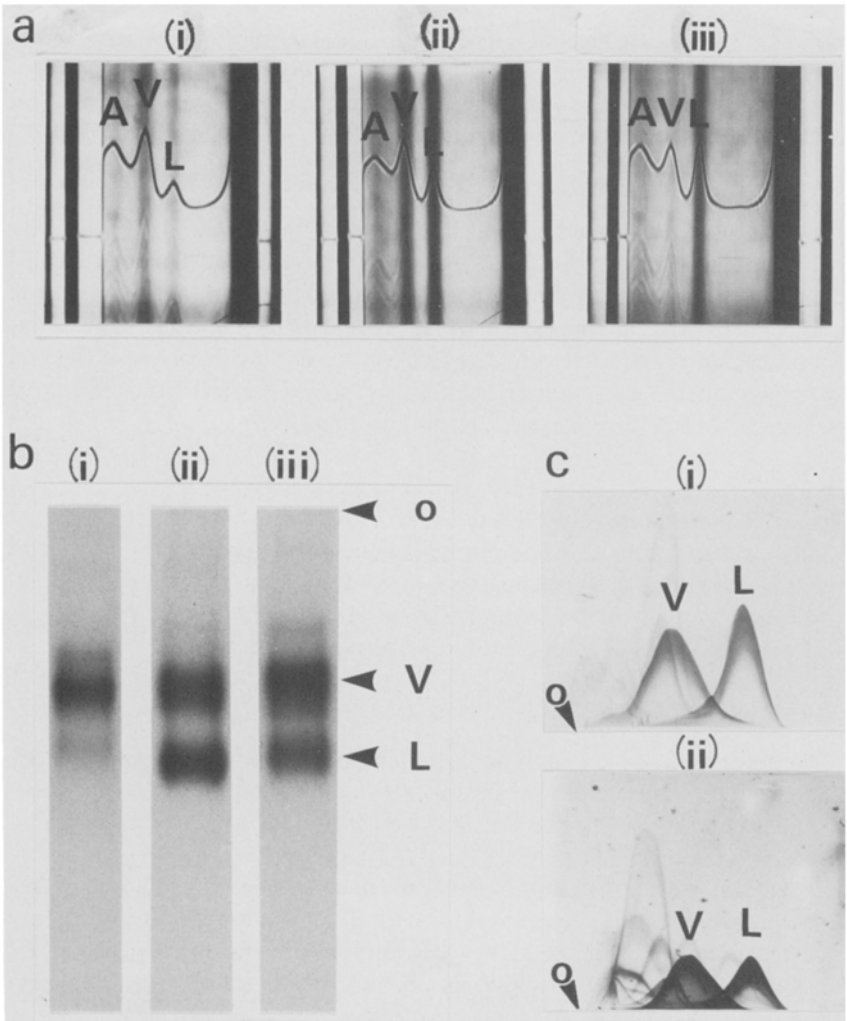
Protein contents of DSC samples were determined after the DSC analysis using a Carlo Erba ANA 1400 automated nitrogen analyser.

Measurement of relative protein compositions

Cellulose acetate electrophoresis. Legumin–vicilin ratios were measured by cellulose acetate membrane electrophoresis of total extracts and subsequent staining and densitometry, as described elsewhere [10, 11].

Analytical ultracentrifugation. Total extracts were subjected to analytical ultracentrifugation as described by Casey [1]. An appropriate frame was selected from the Schlieren plate and a photographic image produced at × 7 enlargement. The position of the baselines was estimated by eye, the peaks traced onto graph paper and the areas of the peaks corresponding to 12S (legumin), 7S (vicilin) and 2–4S (presumed to be mostly albumin) were calculated by weighing. Although the sedimentation coefficients of the major species are similar, no account was taken of possible Johnston–Ogston effects [22]. In two genotypes (JI 181 and 227), traces (1%–2%) of 17S material (aggregated legumin – see Casey [1]) were observed and were included in the legumin peak area.

Crossed immunoelectrophoresis. Approximate legumin–vicilin ratios were estimated by crossed immunoelectrophoresis as described [19]: 5 µl of total extract was separated first in 1% agarose gel at pH 8.6 (7 V/cm, 45 min) and then electroimmunodiffusion was carried out at right angles (2.5 V/cm for 18 h) into a 1% agarose gel (pH 8.6) containing 5% (vol/vol) antiserum to



whole pea extract. The antiserum was raised, as described [1], in response to a total extract of ground dry seeds from the eight different *Pisum* genotypes listed in Table 1.

After staining and destaining, the dried plates were either used directly to measure the arc areas for legumin and vicilin, or the measurements were made from photographic enlargements.

Although peas contain several vicilin species, only one *major* vicilin arc – that corresponding to vicilin-4 (see Millerd et al. [25]) – is obvious on crossed immunoelectrophoresis (Fig. 1c and see Guldager [19]); it is this arc which was measured to calculate legumin–vicilin ratios. The vicilin-4 arc also contains convicilin (see *Discussion* and Millerd et al. [25]).

d

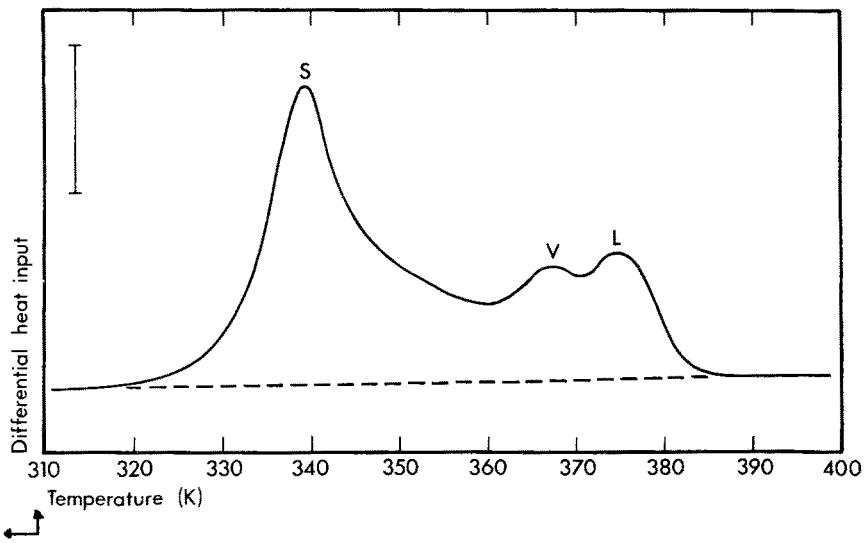


Figure 1a–d. Some of the methods used for the measurement of *Pisum* seed globulin ratios. (a) Analytical ultracentrifugation patterns of total extracts of (i) JI 224, (ii) JI 15, and (iii) JI 181. Photographs taken 66 min after attaining a speed of 52 000 rpm. L, legumin; V, vicilin; A, albumins.

(b) Cellulose acetate electrophoresis pattern of total extracts of (i) JI 224, (ii) JI 15, and (iii) JI 184. Migration is from top to bottom. L, legumin; V, vicilin; O, origin.

(c) Crossed immunoelectrophoresis of total extracts of (i) JI 227 and (ii) JI 15. L, legumin; V, vicilin-4; O, origin.

(d) DSC thermogram of JI 227 dialysed against 0.2 *M* NaCl, 0.05 *M* NaH_2 PO_4 pH 7.0. Heating rate 10 K min^{-1}. Bar = 0.1 mcal s^{-1}. S, starch; V, vicilin; L, legumin.

Rocket immunoelectrophoresis. The absolute amounts of legumin in total extracts were estimated by rocket immunoelectrophoresis [24] using 1% agarose in barbital buffer, pH 8.6, as described by Weeke [33]. Monospecific rabbit IgG from antiserum raised in response to legumin from *Pisum sativum* cv. Dark Skinned Perfection was purified as described [13] and used at a concentration of 400 ng/cm^{-2} of gel. Total extracts were diluted 1 in 50 and 1 in 100 prior to application of 2 μl of sample. Standards (2 μl) containing 100 and 200 ng of purified legumin were included in duplicate in all plates. The protein content of each sample was measured by dye-binding.

Differential scanning calorimetry. DSC analyses of meals and extracts were performed using a Perkin–Elmer DSC-2B as described [35]. In order to quantify the thermograms obtained, samples of purified legumin and total vicilin from pea were also subjected to DSC analysis and their denaturation

enthalpies (ΔH) determined. Under the standard solvent conditions chosen for the study, the values of ΔH were found to be 5.4 and 4.6 cal g^{-1} for legumin and vicilin, respectively. The identity of transitions in the DSC thermograms of pea samples (see Fig. 1d) was based on comparison with thermograms of the purified components and also on previous data [34].

Purification of legumin, vicilin and convicilin

Legumin (for use as a standard in rocket immunoelectrophoresis and differential scanning calorimetry was purified from cv. Filby by hydroxylapatite chromatography (D.J. Wright and V.K. Newby, unpublished). Convicilin was purified from JI 224 as described [3].

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS) was carried out using the Laemmli system [23] as described [1].

Results and Discussion

Figure 1 shows some typical patterns observed with selected genotypes using four of the methods described above, to illustrate the extent of variability.

Table 2 shows the mean percentage of legumin, within the total globulins ('legumin proportion'), of seven *Pisum* genotypes, determined using the five different methods. The data are presented to indicate (a) that the values from replicate extracts of the same genotype are reasonably reproducible, (b) that the variation between individual measurements of a given extract is insignificant for most methods, and (c) that there are significant differences between genotypes with respect to legumin proportions relative to vicilin.

We have examined the legumin–vicilin ratio of the above seven genotypes grown under different conditions and could find no evidence for an effect of the environment on legumin–vicilin ratios; we therefore consider it likely that most of the observed quantitative variability is genotypically determined. Analysis, however, of the legumin–vicilin ratio of a single genotype (cv. Filby) grown in a range of different field situations showed some significant environment-dependent variability (data not shown) and it may be that certain genotypes are more susceptible to the influence of the environment on legumin proportion than are others.

It is possible that the observed variation in legumin proportions could be an artefact of selective extraction of a particular globulin species from a given genotype, although it is unlikely that such an artefact would be as consistent as the results of Table 2 suggest. Extraction of pea meals with boiling SDS solution leads to quantitative extraction of all globulin subunits and, as shown in Figure 2, the SDS gel electrophoresis patterns of such extracts from the selected genotypes broadly agree with the legumin proportions shown in Table 2. For instance, Figure 2 suggests that JI 227 has a high proportion of

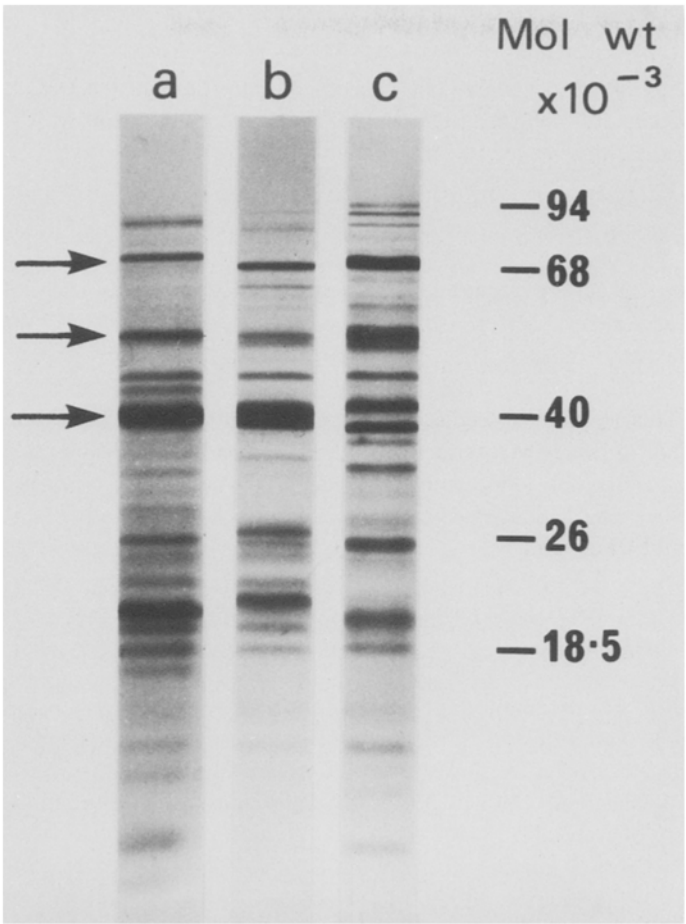


Figure 2. SDS–polyacrylamide gel electrophoresis of SDS extracts of whole seed meals from (a) JI 227, (b) JI 181, and (c) JI 224. For experimental details see the text. The regions corresponding to the α-subunits of legumin (40×10^3) the major subunit of vicilin (50×10^3) and that of convicilin (70×10^3) are indicated by arrows.

legumin (as seen from the relative intensity of the α-subunits) and that JI 224 has low levels of legumin but high levels of the 70×10^3 mol wt subunit of convicilin [3, 9]. Since these data substantiate the values obtained in Table 2, it seems unlikely that selective extraction by the normal extraction buffer is the basis for the differences in proportion of legumin seen in the different genotypes. Further confirmation of this conclusion comes from the DSC analysis of whole meal and isolated protein of JI 227 (Table 2).

Legumin proportions were usually estimated from fresh extracts and it was not the practice to store extracts before making measurements (although storage at 4°C overnight seemed to have no effect on legumin–vicilin ratio). Thus, the measurements of legumin proportion of a given genotype were usually

made with a different extract for each method. As a further check on the possibility of variation from one extraction to another, a single genotype (JI 224) was extracted and the legumin proportion measured on the one extract during a single day, using all the methods; the same legumin proportions were observed as in Table 2.

To illustrate the range of variability in the ratios of storage globulins, the results from each genotype are first discussed separately.

JI 15. In this variety, legumin comprises about 40%–50% of the globulins. There was some variability between methods; in particular, DSC was 10% under the overall average.

JI 181. This very small seeded genotype probably has the highest legumin proportion of the eight genotypes examined, at about 50%–60% of the globulins. There was good agreement between all methods for this genotype except for extract number 2 in crossed immunoelectrophoresis (12% below the overall average).

JI 184. Again, like JI 15, a legumin proportion of about 40%; good agreement between methods.

JI 224. A *P. fulvum* genotype, this is of considerable interest in terms of storage protein composition. The legumin is less negatively charged than most and migrates sufficiently slowly on cellulose acetate electrophoresis at pH 7 [2] that it is difficult to distinguish from the vicilins. In JI 224 the latter consist largely of convicilin [3, 9] which has a subunit molecular weight of about 70×10^3. Crossed immunoelectrophoresis of salt extracts of JI 224 which had been supplemented with purified convicilin showed that the apparent major vicilin arc was actually convicilin; we could not distinguish convicilin from vicilin-4 by crossed immunoelectrophoresis, which confirms its immunological similarity to vicilin [9]. Thus a large percentage of the 7S peak observed in Figure 1a is convicilin. No legumin could be detected by DSC.

JI 227. With the exception of analytical ultracentrifugation, all methods suggested that this genotype has a legumin proportion of 40%–50%. Analytical ultracentrifugation gave a value of about 60% using either whole extract or isolated globulins. We find this discrepancy surprising in view of the generally good agreement between DSC and analytical ultracentrifugation, but have been unable to explain it. If nothing else, the disagreement illustrates the wisdom of using more than one method for measuring legumin proportion.

JI 407. This is a wrinkled-seeded genotype with a low legumin proportion (about 20%–30%); rocket immunoelectrophoresis seems to have given an overestimate of legumin in this genotype.

JI 583. Another wrinkled-seeded type, with a low (20%–25%) legumin proportion, which showed no starch transition on DSC, suggesting that an unusual form of starch was present; normal starch grains were observed.

From the above and from Table 2 it can be seen that, for a given genotype, similar legumin proportions are obtained by the different methods, with some exceptions. The fact that these exceptions occur suggests that a number of methods should be used to measure the legumin proportion of a particular genotype.

Each method, however, has its particular advantages and disadvantages.

Cellulose acetate electrophoresis is only moderately reproducible, but is rapid and sensitive enough to be suited to mass screening of portions of single seeds. The method can encounter difficulty when faced with variant globulins with altered electrophoretic mobilities, such as in JI 224; this particular genotype was subjected to electrophoresis for three times the normal length of time to obtain separation of legumin and the 7S globulins. The method could also be affected by variant proteins showing different degrees of staining as a result of mutation of surface amino acid residues; it is not known whether this is likely to be a phenomenon worth serious consideration.

Rocket immunoelectrophoresis is also rapid and sensitive enough for screening and has reasonable reproducibility (although it is technically fairly demanding). Electrophoresis for extended periods showed that under our conditions, rocket formation was complete in the normal 4-h running time. The method requires that each sample be assayed for protein and, since it is prudent to perform this assay on the sample placed in the immunoelectrophoresis well, a sensitive method (such as dye-binding) is needed. A monospecific antibody is required, the production of which is time-consuming. The method measures only the antigen to which antibody is available, which in our case was legumin, so legumin–vicilin ratios were estimated by using the albumin contents obtained from analytical ultracentrifugation.

Crossed immunoelectrophoresis is more time-consuming than rocket immunoelectrophoresis, but has the same sensitivity and similar reproducibility. Crossed immunoelectrophoresis (and rocket immunoelectrophoresis) has the theoretical advantage over the other methods that the relative concentrations of legumin and vicilin can be measured without any possible error due to contaminating proteins.

There are, however, a number of potential problems associated with the immunoelectrophoretic measurement of genetic variants of proteins by using a calibration based on one or a few protein varieties, as in rocket immunoelectrophoresis; genetic variants might theoretically differ in the nature and number of antigenic sites, in charge, in affinities for other macromolecules, etc., all of which could affect their mobility during immunoelectrophoresis. This, however, does not seem to have greatly influenced the results reported

Table 2. 12S seed globulin (legumin) proportions of seven *Pisum* genotypes

Method	Analytical ultracentrifugation			Crossed immunoelectrophoresis		Rocket immunoelectrophoresis		Cellulose acetate electrophoresis		Differential scanning calorimetry	
	Extract number									1	2
Genotype	1	2	3	1	2	1	2	1	2	(Whole meal)	(Protein extract)
JI 15	44.2 ± 0.3	44.0 ± 0.6	–	55.7 ± 1.7	52.8 ± 1.4	51.6 ± 3.2	55.9 ± 2.7	44.4 ± 1.2	38.5 ± 1.1	35.9 ± 0.5	–
JI 181	62.0 ± 0.6	59.7 ± 2.4	–	53.8 ± 0.9	42.9 ± 1.4	57.8 ± 1.4	50.8 ± 2.4	49.8 ± 4.3	49.2 ± 0.8	59.9 ± 0.8	–
JI 184	41.3 ± 2.1	37.5 ± 0.1	–	47.1 ± 1.6	42.2 ± 0.9	40.2 ± 0.9	34.4 ± 3.3	42.5 ± 0.4	38.2 ± 2.1	42.5 ± 1.4	–
JI 224	22.7 ± 0.2	24.2 ± 0.2	23.8 ± 0.5	25.2 ± 1.9	29.7 ± 1.0	32.9 ± 1.6	27.4 ± 2.2	28.4 ± 0.2	28.5 ± 2.8	a	a
JI 227	61.0 ± 0.4	60.5 ± 0.2	58.8 ± 0.6	43.7 ± 5.0	49.2 ± 0.7	45.9 ± 0.5	51.0 ± 2.5	40.0 ± 0.1	50.7 ± 0.7	49.1 ± 0.5	51.9 ± 0.3
JI 407	21.2 ± 0.4	20.6 ± 0.6	–	22.2 ± 0.7	21.5 ± 1.3	32.1 ± 2.0	36.9 ± 2.8	23.3 ± 0.9	24.5 ± 0.5	–	17.3 ± 1.0
JI 583	20.7 ± 1.3	25.0 ± 1.2	–	30.1 ± 0.1	30.9 ± 0.3	26.3 ± 1.0	28.3 ± 1.0	21.7 ± 0.4	17.3 ± 0.8	–	26.2 ± 0.9

Values are given as percentage of 12S (legumin) component in the total globulins (12S + 7S); the percentage of 7S globulin is therefore 100 minus the above. Values are given as mean ± SE

The rocket immunoelectrophoresis measurements were of legumin content only; thus, to obtain legumin – vicilin ratios, the figures were adjusted for albumin proportions (obtained by analytical ultracentrifugation)

[a] No legumin transition could be detected in JI 224 (see text)

here, since much the same values were obtained for any given genotype, using all the different methods. In addition, the antibody composition of the antiserum used for crossed immunoelectrophoresis, even though raised against protein from several pea lines, may affect the results when genetic variants are analysed. This was noticeable in our studies in the instance of JI 224 which on crossed immunoelectrophoresis using Guldager's [19] antiserum (which latter would not contain antibody specifically to JI 224 legumin) gave very low legumin–vicilin ratios (data not shown).

Analytical ultracentrifugation was extremely reproducible but very slow, a maximum of four runs being possible per centrifuge per working day. Although the method has certain potential artefacts when used for the analysis of mixtures of macromolecules with similar sedimentation coefficients [22], the values obtained, with the exception of JI 227, were closely similar to those from DSC and thus there does not seem to have been any serious interference from such effects.

Theoretically, DSC should be one of the more accurate methods of quantitating the storage proteins, since in most cases its use obviates the necessity for extraction and therefore avoids any of its possible artefacts [34]. It has the further advantage that it can also measure starch by monitoring the gelatinisation transition. A single DSC run takes about 20 min, but with a minimum of six replicates required, the experimental time is increased to 2–3 h. The measured peak areas have to corrected for differences in the values of the denaturation enthalpies for legumin and vicilin, and this therefore entails the purification of the two globulins. For vicilin, which is known to consist of more than one protein [3, 9, 25, 30, 32], preliminary DSC studies revealed similar transition temperatures for the component proteins (Wright and Bacon, unpublished results). As a first approximation, therefore, vicilin was assumed to be an homogeneous entity.

No legumin transition could be discerned unambiguously in the thermograms of meals of JI 224; either the amount of legumin is so small as to be beyond the detection limits of the DSC, or the legumin present possesses an uncharacteristically low denaturation temperature, resulting in overlapping transitions for vicilin and legumin. The first explanation appears unlikely in view of the levels of legumin detected by the other techniques. Evidence for the presence of an atypical form of legumin, however, is provided by electrophoretic analysis, in which the legumin migrated anomalously; further substantiation of this interpretation requires the purification of the variant legumin.

DSC is a useful alternative to ultracentrifugation as it yields similar results in a shorter time; it is not an appropriate technique for the mass screening of pea varieties. One possible disadvantage of DSC is its inability to provide any information on very heterogeneous protein classes, such as the 2–4S (albumin) fraction in peas, but this is probably offset by its capacity to measure variations in starch and starch content.

Taken together, the results show that there is a range of legumin–vicilin ratios, over the *Pisum* genotypes examined, of about 0.2–1.5, similar to those reported by Davies [11], but differing noticeably from those reported by Gatehouse et al. [17]. All the methods used achieved a similar ranking of genotypes with respect to legumin–vicilin ratio.

Although our results have not shown a detectable effect of the environment on the legumin proportions in the varieties listed in Table 2, it does not necessarily follow that all cultivars will be similarly insensitive, since cv. Filby showed some evidence of environmentally induced variability in legumin–vicilin ratio; extremes of environment clearly can affect legumin synthesis relative to that of vicilin [26].

As well as variability in legumin proportion, we also observed variation in starch content, using DSC, and in albumin levels, using analytical ultracentrifugation (data not shown); albumins (2–4S protein) seemed to range from about 13% to slightly more than 30% of the total extractable protein.

What, then, is the significance of variability in legumin–vicilin ratios? Analysis of variants with widely varying ratios of legumin to vicilin is likely to give useful clues as to the control of storage protein synthesis; do, for example, high legumin types have more legumin structural genes, more legumin mRNA, an mRNA which is more stable, increased translation/transcription rates, etc.? In this context, near-isogenic round and wrinked lines [11] may prove very helpful, although it is likely that the control system existing to reduce the legumin proportion of wrinkled-seeded, relative to round-seeded, peas may be not generally applicable to all genotypes.

The practical significance of variability in legumin–vicilin ratio is still not clear. Vicilin is devoid of cysteine and methionine [9], which are the nutritionally limiting amino acids in pea seed proteins, whilst legumin has a small to moderate amount of these amino acids, depending on genotype [4]. Increasing the legumin–vicilin ratio thus effects a small, but significant, improvement in seed protein quality, which can probably be enhanced by selection of a legumin variant with a more desirable methionine–cysteine content; increases in methionine–cysteine content to the level required by FAO (ca. 3.5%), however, will not be achieved by simple manipulation of legumin–vicilin ratios.

Summary

Measurements of legumin–vicilin ratios in the storage proteins of *Pisum* seeds were made using crossed immunoelectrophoresis, rocket immunoelectrophoresis, cellulose acetate membrane electrophoresis, analytical ultracentrifugation and differential scanning calorimetry. All five methods were found to be reasonably reproducible and accurate, although only rocket immunoelectrophoresis and cellulose acetate electrophoresis were suited to extensive screening of seed material. The results showed considerable variability in the

legumin–vicilin ratio, from a value of about 0.2 up to about 1.5; this variability did not appear to be an effect of the plants' growth enhancement, but seemed to be genotype-dependent. The implications of such variability for improvement of the nutritional status of pea seed proteins and for studies of the control of the synthesis of pea seed storage protein was discussed.

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References

1. Casey R (1979) Immunoaffinity chromatography as a means of purifying legumin from *Pisum* (pea) seeds. Biochem J 177:509–520
2. Casey R (1979) Genetic variability in the structure of the α-subunits of legumin from *Pisum* – a two-dimensional gel electrophoresis study. Heredity 43:265–272
3. Casey R, Sanger E (1980) Purification and some properties of a 7S seed storage protein from *Pisum* (pea). Biochem Soc Trans 8:658
4. Casey R, Short MN (1981) Variation in amino acid composition of legumin from *Pisum*. Phytochemistry 20:21–23
5. Casey R, March JF, Sanger E (1981) N-terminal amino acid sequence of β-subunits of legumin from *Pisum sativum*. Phytochemistry 20:161–163
6. Croy RRD, Derbyshire E, Krishna TG, Boulter D (1979) Legumin of *Pisum sativum* and *Vicia faba*. New Phytol 83:29–35
7. Croy RRD, Gatehouse JA, Evans IM, Boulter D (1980) Characterisation of the storage protein subunits synthesised in vitro by poly-ribosomes and RNA from developing pea (*Pisum sativum* L.) I. Legumin. Planta 148:49–56
8. Croy RRD, Gatehouse JA, Evans IM, Boulter D (1980) Characterisation of the storage protein subunit synthesised in vitro by polyribosomes and RNA from developing pea (*Pisum sativum* L.) II. Vicilin. Planta 148:57–63
9. Croy RRD, Gatehouse JA, Tyler M, Boulter D (1980) The purification and characterisation of a third storage protein (convicilin) from the seeds of pea (*Pisum sativum* L.). Biochem J 191:509–516
10. Davies DR (1976) Variation in the storage proteins of peas in relation to sulphur amino-acid content. Euphytica 25:717–724
11. Davies DR (1980) The r_a locus and legumin synthesis in *Pisum sativum*. Biochem Genet 18:1207–1219
12. Derbyshire E, Wright DJ, Boulter D (1976) Legumin and vicilin, storage proteins of legume seeds. Phytochemistry 15:3–24
13. Domoney C, Davies DR, Casey R (1980) The initation of legumin synthesis in immature embryos of *Pisum sativum* L. grown in vivo and in vitro. Planta 149: 454–460
14. Evans IM, Croy RRD, Hutchinson P, Boulter D, Payne PI, Gordon ME (1979) Cell free synthesis of some storage protein subunits by polyribosomes and RNA isolated from developing seeds of peas (*Pisum sativum* L.). Planta 144:455–462
15. Evans IM, Croy RRD, Brown P, Boulter D (1980) Synthesis of complementary DNAs to partially purified mRNAs coding for the storage proteins of *Pisum sativum* (L). Biochim Biophys Acta 610:81–95
16. Gatehouse JA, Croy RRD, Boulter D (1980) Isoelectric-focusing properties and carbohydrate content of pea (*Pisum sativum*) legumin. Biochem J 185:497–503
17. Gatehouse JA, Croy RRD, McIntosh R, Paul C, Boulter D (1980) Quantitative and qualitative variation in the storage proteins of material from the EEC joint field bean test. In: Bond DA (ed) *Vicia faba*; feeding value, processing and viruses. Brussels: EEC, pp 173–190
18. Grant DR, Lawrence JM (1964) Effects of sodium dodecyl sulfate and other dissociating reagents on the globulins of peas. Arch Biochem Biophys 108:552–561

19. Guldager P (1978) Immunoelectrophoretic analysis of seed proteins from *Pisum sativum* L. Theor Appl Genet 53:241–250
20. Higgins TJV, Spencer D (1977) Cell free synthesis of pea seed proteins. Plant Physiol 60:655–661
21. Higgins TJV, Spencer D (1981) Precursor forms of pea vicilin subunits. Modification by microsomal membranes during cell-free translation. Plant Physiol 67:205–211
22. Johnson PJ, Ogston AG (1946) A boundary anomaly found in the ultracentrifugal sedimentation of mixtures. Trans Farad Soc 4:789–799
23. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 277:680–685
24. Laurell CB (1967) Quantitative estimation of proteins by electrophoresis in antibody-containing agarose gels. In: Peeters H (ed) Proteins in biological fluids, vol 14. Amsterdam: Elsevier, pp 499–502
25. Millerd A, Thomson JA, Schroeder HE (1978) Cotyledonary storage proteins in *Pisum sativum*. III. Patterns of accumulation during development. Aust J Plant Physiol 5:519–534
26. Randall PJ, Thomson JA, Schroeder HE (1979) Cotyledonary storage proteins in *Pisum sativum*. IV. Effects of sulfur, phosphorus, potassium and magnesium deficiencies. Aust J Plant Physiol 6:11–24
27. Spencer D, Higgins TJV (1980) The biosynthesis of legumin in maturing pea seeds. Biochem Int 1:501–509
28. Spencer D, Higgins TJV, Button SC, Davey RA (1980) Pulse-labelling studies on protein synthesis in developing pea seeds and evidence of a precursor form of legumin small subunit. Plant Physiol 66:510–515
29. Thomson JA, Schroeder HE, Dudman WF (1978) Cotyledonary storage proteins in *Pisum sativum*. I. Molecular heterogeneity. Aust J Plant Physiol 5:263–279
30. Thomson JA, Schroeder HE (1978) Cotyledonary storage proteins in *Pisum sativum*. II. Hereditary variation in components of the legumin and vicilin fractions. Aust J Plant Physiol 5:281–294
31. Thomson JA, Millerd A, Schroeder HE (1979) Genotype-dependent patterns of accumulation of seed storage proteins in *Pisum*. In: Seed proteins improvement in cereal and grain legumes, vol 1. STI/PUB/496. Vienna: IAEA, pp. 231–240
32. Thomson JA, Schroeder HE, Tassie AM (1980) Cotyledonary storage proteins in *Pisum sativum*. V. Further studies on molecular heterogeneity in the vicilin series of holoproteins. Aust J Plant Physiol 7:271–282
33. Weeke B (1973) Rocket immunoelectrophoresis. Scand J Immunol [Suppl 1] 2: 37–46
34. Wright DJ, Boulter D (1980) Differential scanning calorimetric study of meals and constituents of some food grain legumes. J Sci Food Agric 31:1231–1241
35. Wright DJ, Leach IB, Wilding P (1977) Differential scanning calorimetric studies of muscle and its constituent proteins. J Sci Food Agric 28:557–564