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Genetic Variation in the Subunits of Globulin-1 Storage Protein of French Bean

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Summary. Charge and molecular weight heterogeneity of globulin-1 (G1) polypeptides of the bean, *Phaseolus vulgaris* L., were revealed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Different bean cultivars were classified into three groups: 'Tendergreen', 'Sanilac', and 'Contender' on the basis of their protein subunit composition. Nine distinct major bands: $\alpha 51, \alpha 49$, $\alpha 48.5, \beta 48^T, \beta 48^S, \beta 47, \gamma 45.5, \gamma 45^S$, and $\gamma 45^C$, and two minor bands: $\gamma 46^T$ and $\gamma 46^S$ were found to account for the three profiles seen on one-dimensional SDS-PAGE. Two-dimensional analysis revealed these eleven protein bands to be composed of a minimum of fourteen distinct protein subunits. The 'Tendergreen' and 'Sanilac' types differ in their G1 polypeptide composition. The protein patterns of the 'Contender' types are intermediate, containing many protein subunits found in the patterns of the 'Tendergreen' and 'Sanilac' types suggesting a genetic and evolutionary relationship.

Key words: *Phaseolus vulgaris* – Storage proteins – Electrophoresis – Genetic variation – Banding types

Introduction

Heritable variation in the subunit composition of G1 storage protein from several *P. vulgaris* cultivars has been shown by single dimension SDS-PAGE (Romero et al. 1975; Hall et al. 1977). Electrophoretic screening of ninety-five pure lines of *P. vulgaris* revealed only three different banding types (Romero unpublished data; Ma 1977) suggesting that only limited variation existed. The observed variation is not due simply to post-translational modifications (Hall et al. 1980). Subsequently, charge microheterogeneity has been observed within G1 polypeptides migrating as uniform molecular weight species in the cultivar 'Tendergreen' (Brown et al. 1980). Zein, the major storage protein of *Zea mays* exhibits large charge heterogeneity allowing the identification of single varieties on the basis of their isoelectrofocusing (IEF) banding patterns (Gianazza et al. 1976). Two-dimensional electrophoresis of legumin from pea (*Pisum sativum*) and broad bean (*Vicia faba*) has shown heterogeneity of charge and molecular weight for both the acidic and basic subunit groups of these proteins (Casey 1979a, b; Croy et al. 1979; Gatehouse et al. 1980). Catsimpoolas and Wang (1971) showed charge heterogeneity of the acidic and basic subunits of glycinin, the major storage protein from soybean (*Glycine max*) using IEF in urea-dithiothreitol media.

In this paper the polypeptide composition and the molecular weights of G1 polypeptides are re-evaluated for the three different banding types. Two-dimensional electrophoresis allowed the close examination of the varietal variation for charge and molecular weight heterogeneity of G1 polypeptides, and on this basis, relationships between the banding types were observed.

Materials and Methods

Plant materials

The cultivars of *P. vulgaris* studied were 'Sanilac', 'Pinto 111', 'Mecosta', 'PI 229815', 'Tendergreen', 'PI 302542', 'BBL 240', 'Contender', 'Romano', 'Gina', and 'Nikos' and were obtained from stocks held by Dr. F.A. Bliss.

G1 Purification and Preparation for Electrophoresis

The seed coat and testa were removed from the dry seeds and G1 protein extracted from the ground cotyledons as described by Sun and Hall (1975). The G1 protein was resuspended in 0.5 M NaCl and stored at −27°C. Before electrophoresis, the G1 protein in 0.5 M NaCl was dissociated by heating to 100°C for 3 min in an equal volume of a buffer containing 0.625 M Tris-HCl, pH 6.8, 2 mM EDTA, 2% (w/v) SDS, 40% (w/v) sucrose, 1% (v/v) 2-mercaptoethanol, and 0.01% (w/v) bromophenol blue marker dye. For sin-

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gle seed analysis, the non-germ end was dissected (Romero et al. 1975) and ground in a mortar and pestle. The G1 was extracted from the flour with 1 ml of 0.5 M NaCl (adjusted to pH 2.4 with HCl) for 30 min. 100 µl of the supernatant fraction was removed and dissociated as described above.

SDS-polyacrylamide Gel Electrophoresis

Proteins were separated according to molecular weight using the SDS-PAGE system of Laemmli (1970) as modified by Ma and Bliss (1978). Electrophoresis of the extracts in 0.75 mm thick, 13% (w/v) polyacrylamide slab gels was carried out at 30 mA/gel for 3 to 4 h.

Molecular Weight Determinations

Apparent molecular weights for the G1 polypeptides were calculated according to the method of Weber and Osborn (1969). The molecular weight standards used were: phosphorylase B (94,000 daltons), bovine serum albumin (68,000), catalase (58,000), fumarase (49,000), aldolase (40,000), malate dehydrogenase (34,000), and soybean trypsin inhibitor (21,000).

Two-dimensional Electrophoresis

Proteins were separated by charge in the first dimension and by molecular weight in the second dimension by combining IEF (O'Farrell 1975) and the SDS-polyacrylamide gel system described above. The ampholine mixture used in the IEF dimension was a 2 : 1 mixture of pH 5.0-8.0 : pH 3.5-10.0 ampholines (LKB). Isoelectrofcusing was at 200v for 16 h followed by 400v for 1 h. After equilibration (O'Farrell 1975), the IEF rod gels were laid along the top of the SDS slab gel and overlaid with a solution containing 1% (w/v) agarose (electrophoretic purity, BIO-RAD), 0.35 M Tris, 0.1% (w/v) SDS, 0.02 M EDTA-Na_2, and 0.01% (w/v) bromophenol blue marker dye.

Results

Varietal Variation in Molecular Weight of G1 Polypeptides

The SDS-PAGE patterns of G1 from the eleven varieties studied fell into three different banding types (Fig. 1)

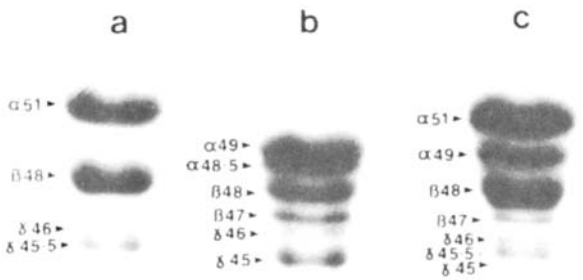

Fig. 1. a-c. SDS-PAGE of G1 proteins from bean cultivars. a 'Tendergreen' types: 'Tendergreen', 'PI 302542', 'BBL 240'; b 'Sanilac' types: 'Sanilac', 'Mecosta', 'Pinto 111', 'PI 229815'; c 'Contender' types: 'Contender', 'Romano', 'Nikos', 'Gina'. 40 µg G1 protein loaded per slot

corresponding to those described previously (Romero et al. 1975; Ma 1977; Hall et al. 1977). The patterns of G1 from 'Tendergreen', 'BBL 240', and 'PI 302542' were identical, having three major and one minor band (Fig. 1). G1 samples from 'Sanilac', 'Pinto 111', 'Mecosta' and 'PI 229815' had five major and one minor band, and those from 'Contender', 'Nikos', 'Romano' and 'Gina' had six major and one minor band (Fig. 1). The molecular weights and terminology of the component polypeptides of the 'Tendergreen', 'Sanilac', and 'Contender' types are given in Table 1.

It is expedient to classify the component G1 polypeptides, and the system of Hall et al. (1977) where the three major bands of 'Tendergreen' were called α, β, and γ will be applied. In order to differentiate between protein bands which have the same molecular weights but different isoelectric points, the superscripts 'S', 'T', and 'C' are used to identify the three different banding types. For example, the 48,000 dalton band from 'Tendergreen' and 'Sanilac' types are referred to as $\beta 48^T$ and $\beta 48^S$ respectively (Table 1).

Table 1. Classification of the SDS-PAGE banding patterns of G1 polypeptides according to molecular weight and isoelectric point

Molecular weight	Subunit group	'Tendergreen' type	'Sanilac' type	'Contender' type
51,000	α	α51 (1, 2)[a]		α51 (1A, 2A)
49,000	α		α49 (6)	α49 (6A)
48,500	α		α48.5 (7)	
48,000	β	β48^T (3)	β48^S (8, 9)	β48^T (3A)
47,000	β		β47 (10)	β47 (10A)
46,000	γ	γ46^T (4)	γ46^S (11)	γ46^T (4A)
45,500	γ	γ45.5(5)		γ45.4 (5A)
45,000	γ		γ45^S (12, 13)	γ45^C (14)

[a]Numbers in parentheses correspond to the IEF variants shown in Fig. 2 which compose the SDS-PAGE bands. The superscripts 'S', 'T', and 'C' are used to distinguish proteins with identical molecular weight but different isoelectrofocusing properties

Two-dimensional Analysis of G1 Polypeptides

The two-dimensional gel electrophoretic patterns of the cultivars are shown in Figure 2. These patterns occupy only a small area of the total gel (see Brown et al. 1980). The G1 of 'Tendergreen', 'BBL 240' and 'PI 302542' (i.e. the 'Tendergreen' types) showed four major protein subunits (Fig. 2a, nos. 1-3 and 5) and at least one minor subunit (Fig. 2a, no. 4). There were two very faintly staining subunits, one slightly to the acidic (right) side of subunit 2 and the other to the acidic side of subunit 3, corresponding to subunits 3 and 5 described by Brown et al. (1980). Subunits 1 and 2 correspond to the α51 band, subunit 3 to the $\beta 48^T$ band, subunit 5 to the γ45.5 band, and subunit 4 to the minor $\gamma 46^T$ band (Table 1).

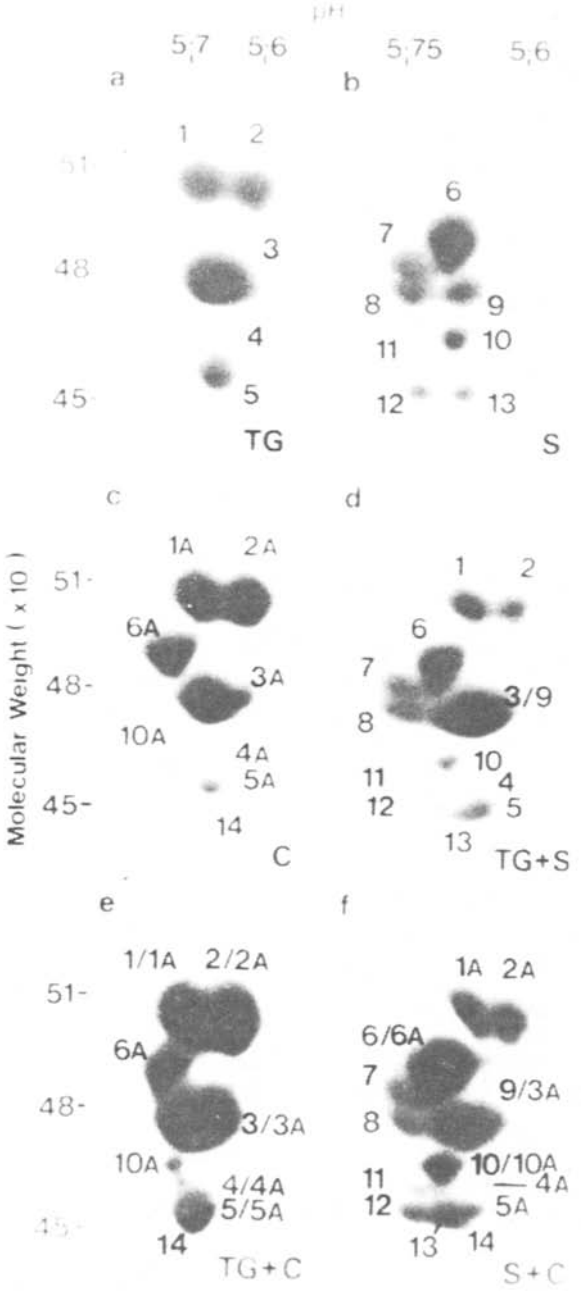

Fig. 2. a-f. Two-dimensional electrophoretic patterns of bean cultivars. a 'Tendergreen' types; b 'Sanilac' types; c 'Contender' types; d mixture of 'Tendergreen' and 'Sanilac' types; e mixture of 'Tendergreen' and 'Contender' types; f mixture of 'Sanilac' and 'Contender' types. 40 μg of G1 protein was loaded in a-c; 30 μg of each G1 protein was mixed and loaded in d-f

'Sanilac', 'Pinto 111', 'Mecosta' and 'PI 229815' (the 'Sanilac' types) showed a two-dimensional pattern consisting of seven major subunits (Fig. 2b, nos. 6-10, 12 and 13) and one minor subunit (no. 11). A faintly staining subunit to the acidic side (right) of subunit 6 (Fig. 2b) could be discerned. Subunits 6 and 7 give rise to the α49 and α48.5 bands respectively, while subunits 8 and 9 combine to produce the $\beta 48^S$ band. Subunit 10 gives the β47 band, subunits 12 and 13 form the γ45 band, and subunit 11 gives the minor $\gamma 46^S$ band (Table 1).

The two-dimensional pattern of the 'Contender' types (i.e. 'Contender', 'Nikos', 'Romano' and 'Gina') shows seven major subunits (Fig. 2c, nos. 1A-3A, 5A, 6A, 10A, and 14) and one minor subunit (no. 4A).

When samples of G1 from a 'Tendergreen' and a 'Sanilac' type are mixed, two-dimensional electrophoresis yielded the positional relationships shown in Figure 2d. Of the thirteen numbered protein subunits (Fig. 2a and b), only two (subunits 3 and 9) were not distinct in terms of molecular weight and isoelectric point (i.e. they were superimposed). These will be treated as different subunits because subunit 3 is consistently more heavily stained and because it alone forms the $\beta 48^T$ band while both 8 and 9 form the $\beta 48^S$ band.

A mixture of G1 samples from 'Tendergreen' and 'Contender' types (Fig. 2e) showed the superimposition of subunits 1-5 (Fig. 2a) by subunits 1A-5A (Fig. 2c), thus indicating similarity on the basis of both molecular weight and pI. In the same way, a mixture of G1 from 'Sanilac' and 'Contender' types (Fig. 2f) revealed the subunit pairs 6 and 6A, 9 and 3A, and 10 and 10A had identical molecular weights and isoelectric points. However, on the basis of intensity of staining, the 'Contender' types appear to contain subunit 3 of 'Tendergreen' rather than subunit 9 of 'Sanilac'. G1 from a single seed extraction yielded the same two-dimensional electrophoretic profile as did G1 purified from a batch of seeds.

Discussion

Molecular Weight Heterogeneity of G1 Polypeptides

The greater resolution of the SDS-PAGE system used in this study and the use of protein markers similar in molecular weight to G1 has enabled a more accurate evalua-

tion of the number of component polypeptides of G1 and their molecular weights than was described previously (McLeester et al. 1973; Romero et al. 1975; Hall et al. 1977). Thus, the 53,000, 50,500, 47,000, and 43,000 dalton bands of Hall et al. (1977) have been recalculated here as being 51,000, 49,000, 48,000, and 45,500 daltons respectively. Hall et al. (1977) called the three major bands of the 'Tendergreen' pattern α, β, and γ. The γ band has less glycosylation than the α of β bands (Hall et al. 1977), and all three differ slightly in their primary structure, especially with the α polypeptide containing one more methionine residue than the β polypeptide (Ma et al. 1980). On the basis of these differences, the α, β, and γ nomenclature of Hall et al. (1977) has been retained. The G1 polypeptide bands of the 'Sanilac' types have been called 'α', 'β', and 'γ' on the basis of their molecular weight alone, and therefore no particular physical or genetical relationships are presumed between bands designated by the same symbol.

Charge Heterogeneity of G1 Polypeptides

Charge microheterogeneity for the G1 polypeptides of the cultivar 'Tendergreen' has already been demonstrated (Brown et al. 1980). Separation by IEF alone gave sharp resolution clearly showing minor G1 variants towards the acidic end of the IEF gels (Brown et al. 1980). Separation in the second (SDS) dimension yields disperse protein spots, and therefore, these minor variants were only observed clearly when high concentrations of G1 protein were loaded ($>$ 40 μg) (Brown et al. 1980). Thus, the number of variants shown in Figures 2a-c represents only the major G1 protein subunits.

Although the observed charge variations are entirely reproducible for a given seed line, it has not been excluded that they may reflect artifacts of either extraction procedure or IEF and, therefore, may not resemble the in vivo state of the protein. G1 is a glycoprotein, and its carbohydrate residues may be the source of the observed heterogeneity due to loss of some residues during purification (Goldstone and Koenig 1974). However, identical two-dimensional patterns are obtained with G1 from a short, acidic 0.5M NaCl extraction and with G1 from the extraction/purification method of Sun and Hall (1975) so that the isoelectrofocusing variants are not due to the purification procedure. Also, initial two-dimensional studies of the protein products of in vitro protein synthesis from G1 mRNA (where no glycosylation occurs) reflect the in vivo situation both in their isoelectric points and in the similarities and differences in protein composition between the three banding types (Brown unpublished data). Thus, glycosylation does not appear to cause the observed charge heterogeneity. In a detailed study of zein, Righetti et al. (1977) concluded that the heterogeneity was due to a combination of in vivo deamidation of glutamine and asparagine residues and point mutations in zein genes.

However, if the charge variation seen in G1 protein is not artifactual, nor due to non-specific post-transcriptional modifications of the products of one or a few genes, then it is likely that there are at least fourteen G1 genes differing slightly in their primary base sequence. Regardless of the molecular basis of the charge heterogeneity, the observed banding patterns consistently show the similarities and differences between individual G1 polypeptides and the three banding types, and are, therefore, valuable for studying inter-varietal relationships and the inheritance of G1 protein polypeptide expression.

Varietal Variation of G1 in P. vulgaris

The two-dimensional analyses support and extend the findings of Ma (1977) and unpublished observations (J. Romero and F.A. Bliss) that only three different varietal banding types of G1 exist. It is clear that the 'Tendergreen' and 'Sanilac' types are different in their G1 composition. The former is composed of $\alpha 51$, $\beta 48^T$, and $\gamma 45.5$ polypeptides (Fig. 1, 2a, subunits 1-5) and the latter of $\alpha 49$, $\alpha 48.5$, $\beta 48^S$, $\beta 47^S$, $\gamma 46^S$, and $\gamma 45$ polypeptides (Fig. 1, 2b, subunits 6-13). The mixture of G1 from 'Tendergreen' and 'Sanilac' G1 shows only subunits 3 and 9 to be similar (Fig. 2d). The 'Contender' types are intermediate containing bands from both the 'Tendergreen' types [$\alpha 51$, $\beta 48^T$, $\gamma 46^T$, and $\gamma 45.5$ (Fig 1, 2a, subunits 1-5)] and the 'Sanilac' types [$\alpha 49$ and $\beta 47$ (Fig. 1, 2b, subunits 6 and 10)]. The only polypeptide which is apparently unique to the 'Contender' types is $\gamma 45^C$ (Fig. 2c, subunit 14). Thus, the variation of G1 of *P. vulgaris* appears to consist of only nine major bands: $\alpha 51, \alpha 49, \alpha 48.5, \beta 48^T, \beta 48^S, \beta 47, \gamma 45.5, \gamma 45^S$, and $\gamma 45^C$, and two minor bands, $\gamma 46^T$ and $\gamma 46^S$. Based on two-dimensional analyses, these eleven bands are made up of a minimum of fourteen distinct protein subunits.

Only four bean lines of the 'Contender' type are known to us at present: 'Contender', 'Nikos', 'Romano' and 'Gina'. These were examined and found to have an identical G1 composition. 'Mecosta' was reported previously as a 'Contender' type (Ma 1977), but two-dimensional electrophoresis showed it to be a 'Sanilac' type. The low frequency of appearance of 'Contender' types and the intermediate nature of their G1 composition suggests that these lines have arisen from lines with the other two banding types. As far as is known, only 'Romano' and 'Gina' are related lines so the 'Contender' types may have arisen by three separate events. The mechanism responsible for the production of these lines may have involved a trans-

location or an unequal cross-over event following a cross between two lines of the major banding types. At present, such mechanisms are speculative since our only evidence for any evolutionary relationship between the bean lines is the electrophoretic banding patterns presented here. Electrophoresis of storage proteins has been widely used in the study of the evolution of hexaploid wheat (Caldwell and Kasarda 1978; Johnson 1975) but has mainly added to more classical findings involving cytological and morphological observations.

On the basis of molecular weights and isoelectric points, the fourteen subunits of G1 appear to be similar (Fig. 2a-c). They all lie in the molecular weight range of 45,000 to 51,000 daltons and in the pH range of 5.6 to 5.8. This variation, covering only small ranges in molecular weight and pH, is similar to that found for the storage proteins of other legumes, although the specific values of the molecular weight and pH ranges differ (Casey 1979a; Gatehouse et al. 1980).

The subunits of α^m-legumin occupy a similar pH range to G1 but their molecular weights lie between 40,000 and 43,000 daltons (Casey 1979b). Two-dimensional analyses of legumin from pea show small differences for molecular weight and isoelectric point within each of the acidic and the basic groups of subunits (Casey 1979a, b; Gatehouse et al. 1980). Similarly, legumin from broad bean exhibited heterogeneity for molecular weight and charge (Wright and Boulter 1974; Croy et al. 1979). Differences in electrophoretic banding patterns between pea lines and between broad bean lines have been observed with the main variation being in the ca. 40,000 dalton acidic subunits (Casey 1979a, b; Croy et al. 1979). The range of variation found for G1 protein is considerably less than that of the cereals where storage protein variation is used to identify individual cultivars (Autran and Bourdet 1975; Elton and Ewart 1962; Shewry et al. 1978; Soave et al. 1976).

The present evidence suggests strongly that there is small variation in G1 composition between bean varieties, with the differences falling into two major distinct groups: the 'Tendergreen' and 'Sanilac' types and one small group with intermediate composition: the 'Contender' type. This raises the question of the origin and evolution of, firstly, the two very different major banding types, and secondly, the intermediate 'Contender' types, and this may be studied by screening the seed proteins of primitive bean lines. Also, making use of the finding in this paper that two-dimensional patterns from a single seed extraction are identical to those of purified G1, single seed analysis can be used to provide a detailed study of the heritability of G1 polypeptides and thereby to increase our understanding of the genetic control of the production of G1 polypeptides.

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