Theor. Appl. Genet. 62, 263–271 (1982)

Bean Lectins

Part 1: Relationships Between Agglutinating Activity and Electrophoretic Variation in the Lectin-containing G2/albumin Seed Proteins of French Bean (*Phaseolus vulgaris* L.)

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Summary. Single seeds of over 100 bean cultivars were analyzed by two-dimensional electrophoresis. The cultivars could be classified into eight groups by virtue of their G2/albumin electrophoretic patterns: T_{G2}, S_{G2}, V_{G2}, Pr_{G2}, B_{G2}, M_{G2}, P_{G2}, and Pi_{G2}. The polypeptide compositions of these types were largely inter-related having particular polypeptides in common. It was possible to correlate the G2/albumin patterns with agglutinating activity of cow and rabbit blood cells as measured by the agglutination ratio (minimum concentration of extract required to agglutinate cow blood cells: minimum concentration of extract required to agglutinate rabbit blood cells). The active lectin polypeptides were identified by extracting lectins from agglutinated erythrocytes and by comparing the qualitative similarities and differences of the G2/albumin patterns and their agglutination activities. A reference catalogue of over 100 bean cultivars giving their phaseolin and G2/albumin electrophoretic patterns, and agglutination ratios is presented.

Key words: *Phaseolus vulgaris* – Seed protein – Lectins – Electrophoresis – Agglutination

Introduction

The lectin proteins of *Phaseolus vulgaris* L. are of commercial importance due to their contribution to the poor nutritional value of raw bean flour (Jaffé 1969; Pusztai et al. 1979a, b). Purified "Navy" bean lectin was toxic when fed to quail chicks (Andrews and Jayne-Williams 1974) and the concentration of lectins in the diet of rats has been correlated to toxicity due to the severe disruption of the microvilli of rats' intestines (Pusztai et al. 1979a, b). Although these latter authors have shown quantitative variation for lectin content among bean cultivars, little information exists on the qualitative genetic variation for lectins of *P. vulgaris*.

Jaffé et al. (1972, 1974) were able to group bean cultivars into four different clases: A, B, C, and D, on the basis of variation in the degree of agglutination of blood cells from different animal species and in mitogenic activity. Seed extracts of type A cultivars agglutinated rabbit and trypsin-treated cow erythrocytes to a high degree and were mitogenic; type B cultivars agglutinated rabbit erythrocytes to the same extent as type A cultivars but agglutinated trypsin-treated cow erythrocytes to a lesser degree; type C cultivars agglutinated only cow erythrocytes and were mitogenic; and type D cultivars did not agglutinate either. Non-denaturing electrophoresis has shown the presence of different tetrameric forms of bean lectins and some variation for these electrophoretic patterns among bean cultivars has been observed (Manen 1978; Felsted et al. 1981).

The seed proteins of *P. vulgaris* have been purified according to solubility properties into three major fractions: phaseolin, globulin-2 (G2), and albumin (McLeester et al. 1973; Ma and Bliss 1978). The phaseolin fraction has been studied extensively and only three different types of phaseolin, distinguishable by polypeptide composition, have been found in cultivated bean accessions (Romero et al. 1975; Brown et al. 1981a). The G2 and albumin fractions have several polypeptides in common, in particular those which make up the G2/albumin group described by Brown et al. (1981b). The G2/albumin polypeptides have been correlated with haemagglutinating activity and therefore include lectin proteins (Brown et al. 1981b). The genes controlling the polypeptides of the lectin-containing G2/albumin groups are linked, co-dominant and are inherited in a block as a single Mendelian gene (Brown et al. 1981c). Previously, genetic variation has been reported for G2/albumin polypeptide composition of eleven cultivars with ten cultivars containing either the T_{G2} or S_{G2} electrophoretic patterns and one, 'Pinto UI 111' which contained no G2/albumin polypeptides (Brown et al. 1981b).

In the current study 107 different bean cultivars and plant introduction (PI) lines were screened for variation in the polypeptide composition of the lectin-containing G2/albumin protein group and in agglutination activity. The isolation of active lectin polypeptides from specific cultivars was consistent with observations of G2/albumin polypeptide composition and agglutination activities. By relating these results to the classification studies of Jaffé et al. (1972, 1974), it has been possible to identify the agglutinating polypeptides of different bean cultivars.

0040-5752/82/0062/0263/$ 1.80

Materials and Methods

Plant Materials

The cultivars and PI lines of *P. vulgaris* used in this study are listed in Table 1. Seeds of these cultivars and PI lines were obtained from stocks held by Dr. F. A. Bliss.

Protein Extraction and Electrophoresis

'Total' seed protein was extracted from single seeds and dissociated as described previously (Brown et al. 1981a). Dissociated extracts were separated by two-dimensional electrophoresis involving isoelectrofocusing followed by SDS-polyacrylamide gel electrophoresis as described by Brown et al. (1981a).

Haemagglutination Tests

Haemagglutination tests were performed on protein solutions extracted from flour of single seeds with phosphate buffered saline (PBS). PBS was also used to dissolve commercially available phytohaemagglutinin preparations. Dilution series of 1% (w/v) solutions were tested for haemagglutinating activity using erythrocytes from both rabbit and trypsin-treated cow blood. Haemagglutination tests using rabbit erythrocytes were carried out as previously described (Brown et al. 1981b). The same procedure was followed for cow erythrocytes except that the washed erythrocytes were first incubated at 38 °C in a 0.1% solution of trypsin in PBS for 30 min and then washed 3 times with PBS. In all experiments blood from the same cow source was used, and phytohaemagglutinin (Sigma) was included as a control.

Isolation of Active Agglutinating Polypeptides

Agglutinating polypeptides were isolated by two methods which differed in their initial extraction procedures. In the first procedure protein was extracted from the flour of 'Tendergreen', 'Sanilac', 'Bunsi', 'Protop P-1', 'M-1', 'Viva', 'Porrillo 70', and 'Pinto UI 111' in 5 ml of PBS to give 5% (w/v) solutions. After the solutions were shaken for 30 min, they were centrifuged at 30,000 × g for 20 min. The supernatant was divided into two aliquots and 25 µl of 10% (w/v) SDS were added to each. Upon the addition of SDS to the PBS extract a precipitate formed (this same precipitate formed with the addition of SDS to PBS alone and was therefore apparently due to salts coming out of solution). The aliquots were added to the same volume of either 3% rabbit erythrocytes or 3% trypsin-treated cow erythrocytes. Prior to centrifugation (30,000 × g, 20 min) the solutions were mixed thoroughly and allowed to stand for 30 min. The pellet was taken up in 50 µl PBS prior to dissociation and electrophoresis. In the second procedure, the flour was extracted with a solution of PBS to which 10% (w/v) SDS solution was added to a final concentration of 0.5% (v/v). Again, addition of the SDS solution to PBS caused a precipitation. After some 45 min of mixing, the salts went back into solution, and this cleared solution was used for the extraction of protein from the flour. After centrifugation, the supernatant was divided, added to erythrocyte solutions and dealt with a described above.

Results

Polypeptide Composition of G2/albumin Electrophoretic Patterns

Screening of the 107 *P. vulgaris* lines allowed the identification of eight classes of cultivars each with a different G2/albumin polypeptide group (Table 1). The two-dimensional electrophoretic pattern of total protein extracts of cultivars representing each of these groups are shown in Figs. 1a–h. These figures show the similarity in the positional relationship of the G2/albumin groups relative to the main protein group, phaseolin. The G2/albumin groups have more acidic isoelectric points (pH 5.0 to pH 5.5) and lower molecular weights (33,000 to 41,000 daltons) than phaseolin (pH 5.6 to 5.8; 45,000 to 51,000 daltons) (see also Brown et al. 1981b). The absence of any G2/albumin polypeptides in the same area of the gel in one of the classes of cultivars is clearly seen in the pattern of 'Pinto UI 111' (Fig. 1g) (Brown et al. 1981b).

The two-dimensional patterns of the different G2/albumin groups are shown in Figs. 2a–g and the polypeptide composition of each is summarized in Table 2. The polypeptides of the T_{G2} type (Fig. 2a, nos. 1–5) and the S_{G2} type (Fig. 2d, nos. 6–10) have been described previously (Brown et al. 1981b). Polypeptides 6–10 correspond to polypeptides 16, and 12–15 respectively, in the previous report. The electrophoretic pattern of the G2/albumin polypeptides of 'Viva' (V_{G2}) (Fig. 2e) was similar to S_{G2}, except for the absence of polypeptide 7 and presence of polypeptide 11 of apparent molecular weight 39,000 daltons and isoelectric point of pH 5.2. The G2/albumin pattern of 'Protop P-1' (Pr_{G2}) (Fig. 2f) was essentially the same as V_{G2} except that the concentration of polypeptide 9 was consietently greater. Similarly, a polypeptide with a more acidic pI, to the right of polypeptide 9 (Fig. 2f, no. 13), which was also observed as a faintly-staining polypeptide in other G2/albumin patterns containing polypeptide 9, was in greater concentration in the Pr_{G2} pattern. Both 'Protop' lines originated from a cross between 'BBL240' (T_{G2}) × 'PI 207227' (V_{G2}). The electrophoretic pattern of the G2/albumin polypeptides of 'Bunsi' (B_{G2}) (Fig. 2b) was similar to V_{G2} except for the addition of two polypeptides (Fig. 2b, nos. 2 and 12). That of 'M-1' (M_{G2}) (Fig. 2c) was similar to B_{G2} but lacked polypeptides 6 and 8. Polypeptide 12 had a molecular weight of 35,500 daltons and an isoelectric point of pH 5.3. The pattern of 'Porrillo 70' (P_{G2}) had five polypeptides (Fig. 2g, nos. 2, 8, 12, 14 and 15). Polypeptides 14 and 15 had isoelectric points of pH 5.0, and molecular weights of 42,000 and 39,000 daltons respectively. The pattern of 'Pinto UI 111' (Pi_{G2}) contained no G2/albumin polypeptides and is therefore not presented.

Table 1. Genetic variation for phaseolin type, G2/albumin type, and agglutination ratio in cultivated varieties of *Phaseolus vulgaris* L.

Cultivar	Phaseolin Type[a]	G2/albumin Type	Agglutination Ratio[b]
'Tendergreen'	T	T_{G2}	0.5
'BBL 240'	T	T_{G2}	1.0
'Greensleeves'	T	T_{G2}	1.0
'Mecosta'	S	T_{G2}	0.5
'PI 229815'	S	T_{G2}	1.0
'Contender'	C	T_{G2}	0.5
'Gina'	C	T_{G2}	0.5
'Nikos'	C	T_{G2}	1.0
'California Light Red Kidney'	T	T_{G2}	0.5
'Canario Divex'	T	T_{G2}	0.25
'Redkote'	T	T_{G2}	0.5
'Protop W-2'	S	T_{G2}	0.5
'Cornell 2101'	T	T_{G2}	0.5
'Triple R-41'	T	T_{G2}	0.5
'Saxa'[c]	T	T_{G2}	0.5
'S_3(COP 0-22)'	S	T_{G2}	0.5
'Hallados Grandes'[c]	T	T_{G2}	0.5
'Topnotch Golden Wax'	C	T_{G2}	0.5
'Earliwax'	T	T_{G2}	0.5
'Moongold'	T	T_{G2}	0.5
'Regal'	T	T_{G2}	1.0
'Goldcrop'	T	T_{G2}	1.0
'Round Pod Kidney Way'	T	T_{G2}	0.5
'Astro'	C	T_{G2}	1.0
'Pencil Pod Wax'	T	T_{G2}	0.5
'Resistant Kinghorn Wax'	T	T_{G2}	1.0
'Kinghorn Wax'	T	T_{G2}	0.5
'Resistant Cherokee Wax'	T	T_{G2}	1.0
'Black Valentine'	T	T_{G2}	1.0
'Midas'	T	T_{G2}	1.0
'Goldrush'	T	T_{G2}	0.5
'Bonanza'	T	T_{G2}	0.5
'Romano'	C	T_{G2}	0.5
'Sanilac'	S	S_{G2}	16
'PI 302542'	T	S_{G2}	32
'Bonita'	S	S_{G2}	32
'Veracruz 105'	S	S_{G2}	32
'PI 324584' ('Kaiser Wilhelm')[c]	S	S_{G2}	32
'PI 289426' ('Kaiser Wilhelm')[c]	S	S_{G2}	32
'Kentucky Wonder'	S	S_{G2}	16
'Gabriella'	S	S_{G2}	32
'Big Bend'	S	S_{G2}	32
'Rufus'	S	S_{G2}	32
'Red Mexican UI 34'	S	S_{G2}	32
'Red Mexican UI 36'	S	S_{G2}	32
'Triguito'[c]	S	S_{G2}	16
'30-L-50'	S	S_{G2}	16
'Gratiot'	S	S_{G2}	32
'Viva'	S	V_{G2}	16
'Puebla 152' (Br)	S	V_{G2}	16
'Black Turtle Soup'	S	V_{G2}	16
'Puebla 152' (Bl)	S	V_{G2}	16
'Honduras 46'	S	V_{G2}	16
'MSU 61371'	S	V_{G2}	16
'MSU 61834'	S	V_{G2}	16
'MSU 61065'	S	V_{G2}	16
'Chimbolo'	S	V_{G2}	16
'Carioca'	S	V_{G2}	16
'Cubagua'[c]	S	V_{G2}	16
'PI 313879'	S	V_{G2}	16
'Sutter Pink'	S	V_{G2}	32
'San Fernando'	S	V_{G2}	16
'Great Northern Nebraska # 1'[c]	S	V_{G2}	16
'Great Northern 31'[c]	S	V_{G2}	32
'PI 207227'	S	V_{G2}	16
'Protop P-1'	T	Pr_{G2}	8
'Protop W-1'	T	Pr_{G2}	8
'Bunsi'	S	B_{G2}	1.0
'Venezuala 36'	S	B_{G2}	0.5
'Chis 2193'	S	B_{G2}	0.5
'ICA Pijao'	S	B_{G2}	0.5
'PI 224730'	S	B_{G2}	0.5
'PI 345517' ('Porrillo # 1')[c]	S	B_{G2}	1.0
'Ex Rico 23'	S	B_{G2}	0.5
'Red Peanut Bean'	S	B_{G2}	0.25
'PR 7703-126'	S	B_{G2}	0.5
'15R-148'	S	B_{G2}	0.5
'Mexico 309'	S	B_{G2}	0.5
'Resel 3-33'	S	B_{G2}	1.0
'La Vega'	S	B_{G2}	1.0
'15R-55'	S	B_{G2}	1.0
'PI 313888'	S	B_{G2}	0.5
'PI 195401'	S	B_{G2}	0.5
'M-1'	S	M_{G2}	1.0
'Jamapa'	S	M_{G2}	1.0
'PR 7703-184'	S	M_{G2}	0.5
'PR 7703-106'	S	M_{G2}	1.0
'Cornell 49-242'	S	M_{G2}	1.0
'50600'	S	M_{G2}	0.5
'15R-87'	S	M_{G2}	1.0
'Porrillo 70'[c]	S	P_{G2}	< 0.016
'Porrillo Sintético'[c]	S	P_{G2}	< 0.016
'PI 297288 (Porrillo # 1)'[c]	S	P_{G2}	< 0.032
'PI 304111 (Porrillo # 1)'[c]	S	P_{G2}	< 0.008
'Pinto UI 111'	S	Pi_{G2}	–
'Pinto UI 114'	S	Pi_{G2}	–
'White Half Runner'	S	Pi_{G2}	–
'PI 368737'	S	Pi_{G2}	–
'Great Northern U.S. 1140'[c]	S	Pi_{G2}	–
'Great Northern 59'[c]	S	Pi_{G2}	–
'Juli'[c]	S	Pi_{G2}	–
'PI 310878'	S	S_{G2}, 12 and 12	0.5
'PI 312041'	S	V_{G2}, 2, 12 and 16	1.0
'California Small White 59'	S	S_{G2}, 11, 17	16
'Kerman'	S	S_{G2}, 11, 17	16
'California Dark Red Kidney'	T	3, 18–20	< 0.002

[a] T-'Tendergreen' type; S-'Sanilac' type; C-'Contender' type (Brown et al. 1981a)

[b] Agglutination Ratio = $\frac{\text{Minimum conc. to agglutinate trypsin-treated cow erythrocytes}}{\text{Minimum conc. to agglutinate rabbit erythrocytes}}$

[c] Lines comparable to those used by Jaffé et al. (1974)

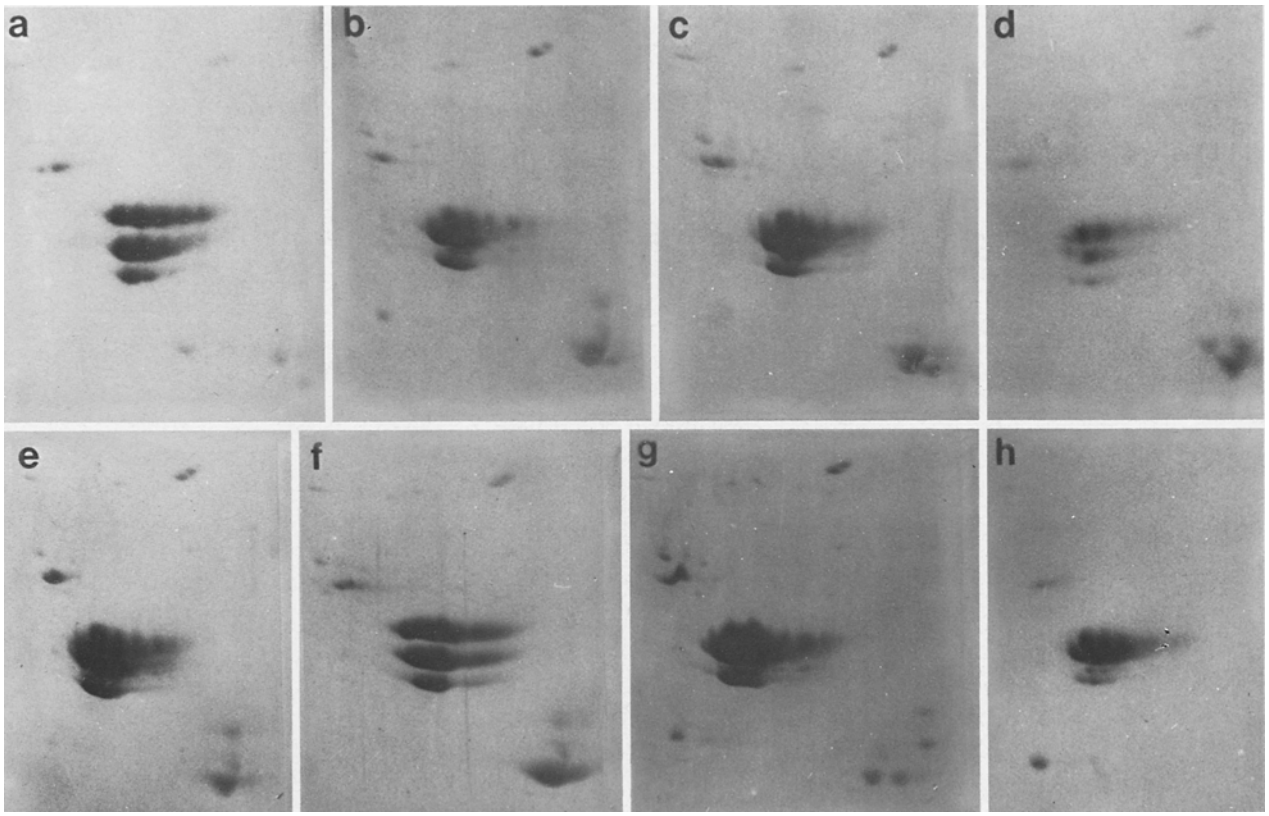

Fig. 1a–h. Two-dimensional electrophoretic patterns of protein extracts of bean cultivars. **a** 'Tendergreen'; **b** 'Bunsi'; **c** 'M-1'; **d** 'Sanilac'; **e** 'Viva'; **f** 'Protop P-1'; **g** 'Porrillo 70'; **h** 'Pinto UI 111'. The horizontal dimension represents separation by isoelectrofocusing with the left hand side being basic and the right hand side being acidic. The pH range covered in the gels is from approximately pH 5.0 to pH 6.3. The vertical dimension represents separation with regard to molecular weight by SDS-PAGE. The major protein group is phaseolin, and the lower molecular weight G2/albumin group is to the acidic (right) side of phaseolin. Further information on the two- dimensional separation of these protein groups is presented by Brown et al. (1981 a–c)

Of the 107 bean lines studied, six did not fall into one of the eight classes of G2/albumin electrophoretic patterns described above. The total protein profiles of five of these lines are shown in Figs. 3a–d, again showing the positional relationship of the G2/albumin groups to the phaseolin polypeptide group. The two-dimensional G2/albumin patterns of these lines are shown in Figs. 4a–d and their polypeptide compositions are summarized in Table 2. The pattern of 'PI 310878' (Fig. 4a) had the S_{G2} pattern with the addition of polypeptides 2 and 12 found in the B_{G2}, M_{G2}, and P_{G2} patterns. 'PI 312041' (Fig. 4b) contained the V_{G2} pattern plus polypeptides 2 and 12, and an additional polypeptide (Fig. 4b, no. 16) with a molecular weight of 35,500 daltons and an isoelectric point of approximately pH 5.4. The electrophoretic patterns of the G2/albumin groups of 'California Small White 59' and 'Kerman' were identical, (Fig. 4c), containing the S_{G2} pattern plus an additional polypeptide (Fig. 4c, no. 17) with a molecular weight of 38,000 daltons and an isoelectric point of pH 5.5. The pattern of 'California Dark Red Kidney' contained four polypeptides (Fig. 4d, nos. 3, 18, 19 and 20). Polypeptide 18 had an isoelectric point of pH 5.0 and a molecular weight of 34,500 daltons, while polypeptides 19 and 20 had the same isoelectric point, pH 5.1, and molecular weights of 34,000 and 33,000 daltons respectively. One line, 'Triple R-26' was found to be segregating for the T_{G2} and V_{G2} patterns with some seed containing both patterns (T_{G2}/V_{G2}). F_1 seed of eight crosses all had intermediate patterns containing all the polypeptides of the parental G2/albumin patterns (Table 3).

Relationships between the polypeptide composition of the different G2/albumin polypeptide patterns (Table 2) were investigated by two-dimensional electrophoresis of mixtures of protein extracts from the different varieties (gels not shown). Co-migration of polypeptides showed identity with regard to both molecular weight and isoelectric point.

Haemagglutinating Activity

The agglutination ratios (minimum concentration to agglutinate trypsin-treated cow erythrocytes: minimum

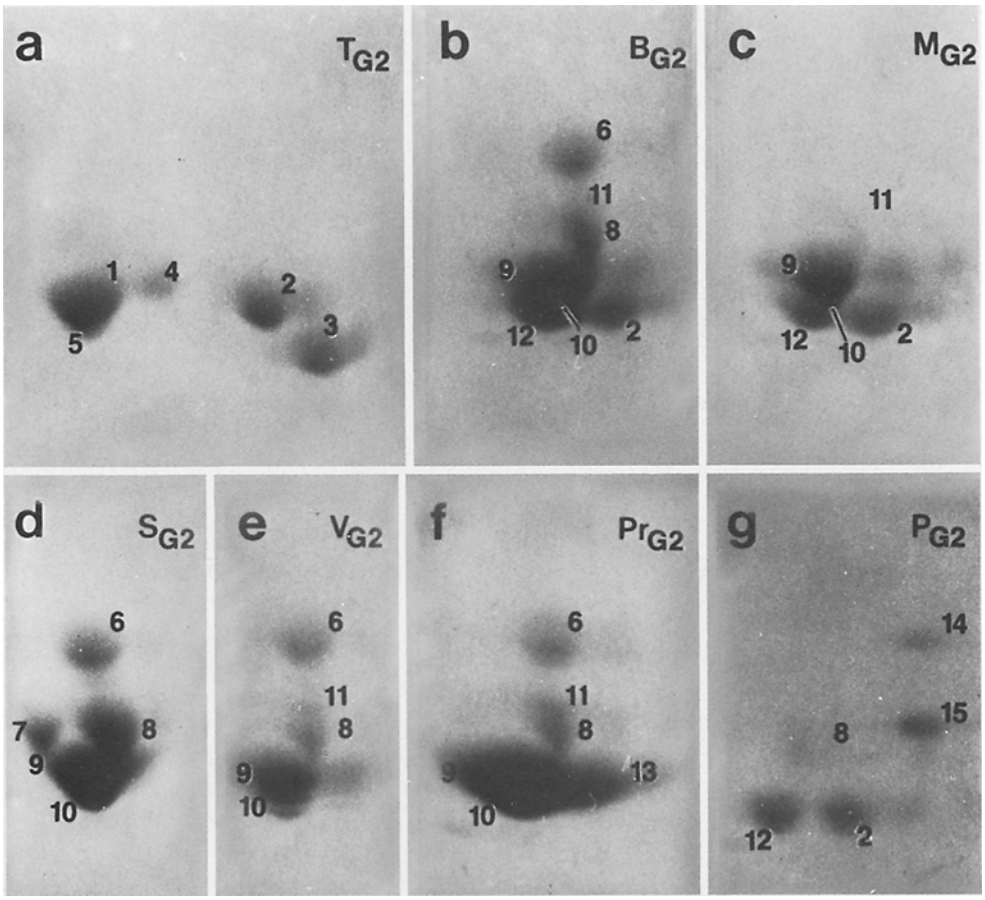

Fig. 2a – g. Enlargements of the two-dimensional electrophoretic patterns of G2/albumin types from different bean cultivars (Fig. 1). **a** T_{G2} ('Tendergreen'); **b** B_{G2} ('Bunsi') **c** M_{G2} ('M-1'); **d** S_{G2} ('Sanilac') **e** V_{G2} ('Viva') **f** Pr_{G2} ('Protop P-1'); **G** P_{G2} ('Porrillo 70')

Table 2. Correlation of G2/albumin types with agglutination types[a]

G2/albumin type	Polypeptide composition	Agglutination ratio	Agglutination type[a]	Lectin polypeptides
T_{G2}	1 – 5	0.25 – 1.0	A	1, 2, 3
B_{G2}	2, 6, 8 – 12	0.25 – 1.0	A	2, 9, 10, 12
M_{G2}	2, 9 – 12	0.25 – 1.0	A	2, 9, 10, 12
S_{G2}	6 – 10	16/32	B	9, 10
V_{G2}	6, 8 – 11	16/32	B	9, 10
Pr_{G2}	6, 8 – 11, 13	8	B	9, 10
P_{G2}	2, 8, 12, 14, 15	< 0.032	C	2, 12
Pi_{G2}	–	–	D	–
'PI 310878'	2, 6 – 10, 12	0.5	A	2, 9, 10, 12
'PI 312041'	2, 6, 8 – 12, 16	1.0	A	2, 9, 10, 12
'Kerman'	6 – 11, 17	16	B	9, 10
'California Sm. Wht. 59'	6 – 11, 17	16	B	9, 10
'California Dk. Rd. Kid.'	3, 18 – 20	< 0.002	C	3[b]

[a] Jaffé et al. (1972; 1974)
[b] Trypsin-treated cow erythrocyte agglutinating activity not determined for polypeptides 18 – 20

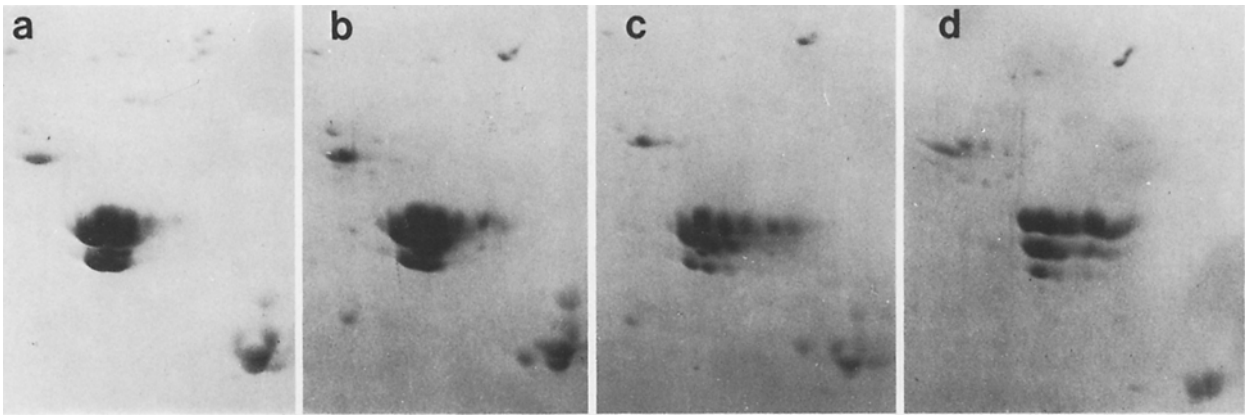

Fig. 3a – d. Two-dimensional electrophoretic patterns of protein extracts of bean cultivars with other G2/albumin groups. **a** 'PI 310878'; **b** 'PI 312041'; **c** 'California Small White 59' and 'Kerman'; **d** 'California Dark Red Kidney'

concentration to agglutinate rabbit erythrocytes) of the bean lines studied are given in Table 1 and summarized in Table 2. The titres of the minimum concentrations required to agglutinate erythrocytes were observed visually and are therefore liable to an error of + or – one dilution step. Thus, the agglutination ratios of cultivars with the T_{G2}, B_{G2}, and M_{G2} types and those with the S_{G2}, V_{G2}, and Pr_{G2} types lay in the ranges of 0.25 to 1.0 and 8 to 32 respectively. The cultivars with the P_{G2} pattern had agglutination ratios of < 0.032, while those with no G2/albumin polypeptides (Pi_{G2}) did not agglutinate either rabbit or trypsin-treated cow erythrocytes. The agglutination ratios of the five lines with other G2/albumin patterns, 'PI 310878', 'PI 312041', 'California Small White 59', 'Kerman', and 'California Dark Red Kidney' were 0.5, 1.0, 16, 16, and < 0.002 respectively. The three 'Triple R-26' segregants with the T_{G2}, V_{G2}, and T_{G2}/V_{G2} patterns had agglutination ratios of 1.0, 16, and 8 respectively (Table 3). The F_1 seed of the crosses between parents having different agglutination ratios, 'Porrillo 70' × 'Protop P-1', 'Porrillo 70' × 'Tendergreen', 'Porrillo 70' × 'Viva', 'Tendergreen' × 'Sanilac', and 'Viva' × 'Bunsi', had agglutination ratios intermediate between those of the parental cultivars (Table 3). In the reciprocal crosses between 'Pinto UI 111' and 'Protop P-1' for the F_1 seed had agglutination ratios similar to the 'Protop P-1' parent.

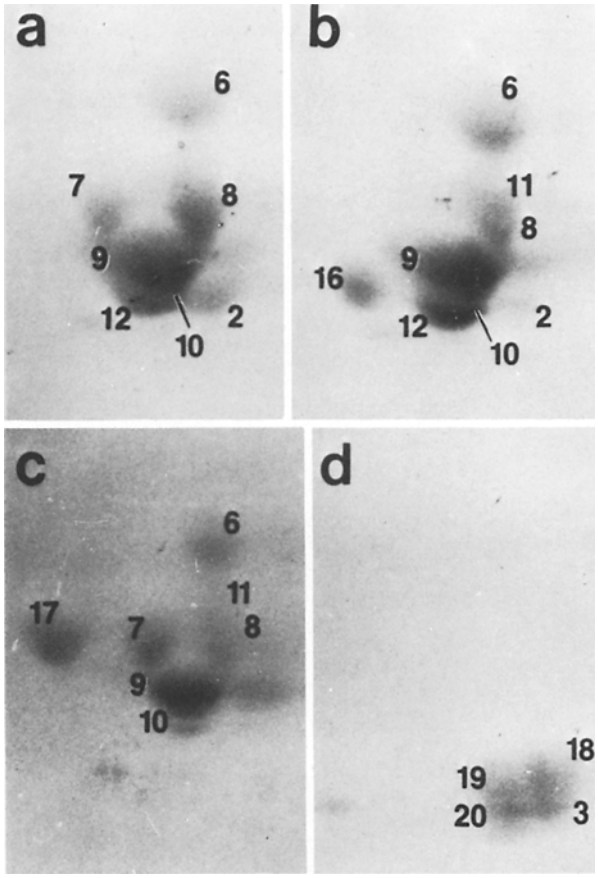

Fig. 4a – d. Enlargements of the two-dimensional patterns of G2/albumin groups from bean cultivars (Fig. 3). **a** 'PI 310878'; **b** 'PI 312041'; **c** 'California Small White 59' and 'Kerman'; **d** 'California Dark Red Kidney'.

Isolation of Active Agglutinating Polypeptides

The two-dimensional electrophoretic patterns of proteins extracted from rabbit erythrocytes agglutinated with protein extracts from 'Tendergreen', 'Protop P-1', and 'Sanilac' and from trypsin-treated cow erythrocytes agglutinated with a protein extract from 'Bunsi' are shown in Figs. 5a–d respectively. In all cases the polypeptides in the patterns obtained from agglutinated trypsin-treated cow erythrocytes were more diffuse than those from agglutinated rabbit erythrocytes, making photographic reproduction more difficult (compare Fig. 5d and Figs. 5a–c). Protein extracts made using

Table 3. G2/albumin patterns and agglutination ratios of 'Triple R-26' segregants and F_1 progeny

Cultivar/cross	Phaseolin type	G2/albumin type	Agglutination Ratio	
			segregant/F_1	parental
'Triple R-26'	T	V_{G2}	16	
'Triple R-26'	T	T_{G2}	1.0	
'Triple R-26'	T	T_{G2}/V_{G2}	8	
F_1 ('Porrillo 70' × 'Protop P-1')	S/T	P_{G2}/Pr_{G2}	0.5	< 0.032; 8
F_1 ('Porrillo 70' × 'Tendergreen')	S/T	P_{G2}/T_{G2}	0.125	< 0.032; 0.5
F_1 ('Porrillo 70' × 'Viva')	S	P_{G2}/V_{G2}	0.25	< 0.032; 16
F_1 ('Tendergreen' × 'Sanilac')	S/T	T_{G2}/S_{G2}	2	0.5; 16
F_1 ('Viva' × 'Bunsi')	S	V_{G2}/B_{G2}	2	16; 1.0
F_1 ('Tendergreen' × 'Bunsi')	S/T	T_{G2}/B_{G2}	0.5	0.5; 1.0
F_1 ('Pinto 111' × 'Protop P-1')	S	Pi_{G2}/Pr_{G2}	16	0; 8
F_1 ('Protop P-1' × 'Pinto 111')	S	Pr_{G2}/Pi_{G2}	16	8; 0

the first extraction procedure (SDS added to the PBS extract) contained a distinguishable amount of phaseolin (compare Fig. 5 a, b and Fig. 5 c, d). The addition of the SDS solution to PBS appears to cause precipitation of salts from solution and thus precipitation of some phaseolin, which requires relatively high salt concentrations for solubility (Sun and Hall 1975) The precipitated phaseolin formed part of the final pellet. In extracts using the second extraction procedure (where protein was directly extracted with 'cleared' SDS/PBS solution) the phaseolin remained in the supernatant and was not evident to any degree in the pellet material (Fig. 5 c, d).

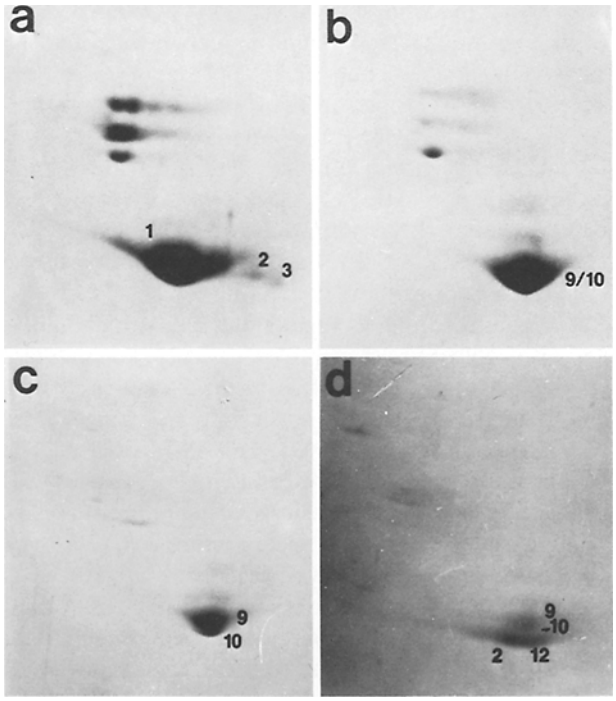

Fig. 5 a – d. Two-dimensional electrophoretic patterns of protein extracted from rabbit and trypsin-treated cow erythrocytes agglutinated with protein extracts of different bean cultivars. **a** Rabbit/'Tendergreen' (T_{G2}); **b** Rabbit/'Protop P-1' (Pr_{G2}); **c** Rabbit/'Sanilac (S_{G2}); **d** Cow/'Bunsi' (S_{G2})

Comparison of the pattern of 'Tendergreen' (rabbit) (Fig. 5 a) with 'Tendergreen' total protein (Fig. 1 a) and to T_{G2} (Fig. 2 a) showed a clear increase in concentration of polypeptide 1 relative to that of phaseolin and polypeptides 2 and 3. Similarly, 'Protop P-1' (rabbit) (Fig. 5 b) showed enhancement of polypeptides 9 and 10 when compared to the total 'Protop P-1' protein (Fig. 1 f). Polypeptides 9 and 10 were also predominant in the pattern of 'Sanilac' (rabbit) (Fig. 5 c) and polypeptides 2, 9, 10, and 12 were extracted from 'Bunsi' (cow) (Fig. 5 d). Due to the similarity in molecular weight of polypeptides 9 (36,500 daltons) and 10 (35,500 daltons) it is often difficult to resolve these polypeptides (viz. Figs. 1–4). Reduced sample loading allowed their resolution, but for the purposes of photographic reproduction it was necessary to load larger amounts of protein.

The pattern of 'Tendergreen' (cow) contained only polypeptides 2 and 3 of the T_{G2} pattern (gel not shown) and those of 'Sanilac' (cow), 'Viva' (rabbit and cow), 'Protop P-1' (cow), 'Bunsi' (rabbit) and 'M-1' (rabbit) contained only polypeptides 9 and 10 (gels not shown) as in 'Sanilac' (rabbit) (Fig. 5 c). The pattern of 'M-1' (cow) was identical to that of 'Bunsi' (cow) (Fig. 5 d) containing polypeptides 2, 9, 10, and 12, and that of 'Porrillo 70' (cow) contained only polypeptides 2 and 12 (gel not shown). These results are summarised in Table 2. 'Porrillo 70' (rabbit) and 'Pinto UI 111' (cow and rabbit) showed no G2/albumin polypeptides as expected, and these extractions together with extracts from non-agglutinated cow and rabbit red blood cells acted as controls.

Discussion

Correlation Between G2/albumin Patterns and Haemagglutinating Activity

Jaffé et al. (1972; 1974) were able to distinguish among a number of cultivars of *P. vulgaris* on the basis of, firstly, their agglutinating activity on blood cells from different animal species and, secondly, their mitogenic activity. The agglutination ratio (cow : rabbit) introduced here provides a measure of the ability of bean protein extracts to agglutinate rabbit and trypsin-treated cow red blood cells, and clearly distinguished the four classes found by Jaffé et al. (1972; 1974) (Table 2).

In our studies we have included as many lines as possible which were the same or similar to those used by Jaffé et al. (1972; 1974). Of sixteen such lines eight were in agreement: 'Saxa' (T_{G2}) – A type; 'Cubagua' (V_{G2}) – B type; four of the five 'Porrillo' lines (P_{G2}) – C types; and two of the four 'Great Northern' lines (Pi_{G2}) – D types. The cultivar 'Juli' reported by Jaffé et al. (1974) as a B type was found here to be a D type and one of the 'Porrillo' lines was found to be an A type (B_{G2}). The accession of 'Hallado' used here, 'Hallados Grandes', had a T_{G2} pattern and was therefore an A type; 'Triguito' and the two 'Kaiser Wilhelm' lines had S_{G2} patterns and two of the four 'Great Northern' accessions had V_{G2} patterns and were all B types. The discrepancies between these two studies are probably due to the use of slightly different accessions, between which variation can occur (viz. 'Great Northern' and 'Porrillo' lines in Table 1) and to the possibility of some variation within seed lots due to mixing and outcrossing in the field.

The results of this study agreed with those of Jaffé et al. (1972) in that the A types (T_{G2}, B_{G2}, M_{G2}) were the most abundant (56 lines), followed by the B types (S_{G2}, V_{G2}, Pr_{G2}) with 34 lines, and the C and D types were far less common with only 4 and 7 lines respectively being identified here.

The agglutination ratios were strongly correlated with specific G2/albumin polypeptide patterns. Thus, the cultivars whose agglutination ratios fell in the classes of 0.25 to 1.0, 8 to 32, <0.032, and no detectable agglutination corresponded to type A, B, C, and D cultivars respectively. They also had T_{G2}, B_{G2}, or M_{G2}; S_{G2}, V_{G2}, or Pr_{G2}; P_{G2}; and Pi_{G2} G2/albumin polypeptide patterns respectively (Tables 1 and 2).

The correlation between agglutination ratio and polypeptide pattern was further exemplified by the intermediate agglutination ratios of the 'Triple R-26' segregants with the T_{G2}/V_{G2} pattern, and the F_1 progeny of crosses between parents with different G2/albumin patterns (Table 3). Thus, seed with a mixture of G2/albumin patterns could still be correlated with agglutination ratio.

Although we have identified eight different G2/albumin electrophoretic patterns into which the majority of the lines studied can be classified, more variation may exist as suggested by the patterns of 'California Dark Red Kidney', 'California Small White 59', 'Kerman', 'PI 310878' and 'PI 312041' (Fig. 4).

Identification of Agglutinating Polypeptides

The active lectin polypeptides contained in the G2/albumin patterns were identified from comparisons of the similarities and differences in both polypeptide composition and haemagglutinating activity of protein extracts from the various cultivars (Figs. 2 and 4; Table 2) and from isolation of the polypeptides from agglutinated erythrocytes. In the first case, it was assumed that if the presence or absence of a particular polypeptide did not affect the agglutination ratio then it did not convey agglutinating activity with regard to rabbit or trypsin-treated cow red blood cells.

Cultivars containing the T_{G2}, B_{G2}, and M_{G2} G2/albumin patterns have high agglutinating activity for both cow and rabbit blood cells giving agglutination ratios of 0.5 or 1.0. Those containing the S_{G2}, V_{G2} and Pr_{G2} patterns did not agglutinate cow blood cells strongly. The B_{G2} pattern contained all of the polypeptides which made up the pattern of V_{G2}, namely polypeptides 6, and 8 to 11, plus polypeptides 2 and 12. Thus, polypeptides 2 and/or 12 appeared to be responsible for high trypsin-treated cow red blood cell agglutinating activity. Similarly, the pattern of 'PI 310878' contained the S_{G2} pattern plus polypeptides 2 and 12, and its agglutination ratio was 0.5 instead of 16 or 32 as expected for an S_{G2} pattern. Cultivars with the P_{G2} pattern agglutinated trypsin-treated cow erythrocytes to the same extent as those containing the T_{G2}, B_{G2}, and M_{G2} patterns, did not agglutinate rabbit blood cells, and also contained polypeptides 2 and 12. Polypeptides 2 and 12 were extracted from trypsin-treated cow erythrocytes agglutinated with protein extracts from cultivars with the B_{G2}, M_{G2}, and P_{G2} patterns. The agglutination reaction of these two polypeptides was apparently specific for trypsin-treated cow erythrocytes as no rabbit erythrocyte agglutination was observed with cultivars of the P_{G2} type. Only polypeptide 2 was common to the T_{G2}, B_{G2}, M_{G2}, and P_{G2} patterns and it therefore appeared to be at least partly responsible for the high trypsin-treated cow red blood cell agglutination activity of the cultivars containing the T_{G2} pattern. Polypeptides 2 and 3 were isolated from trypsin-treated cow erythrocytes agglutinated with protein extracts from cultivars with the T_{G2} pattern. By inference, the rabbit agglutinating activity of these cultivars ought to

be a property of polypeptides 1, 4, or 5 (Fig. 2a) and indeed polypeptide 1 was extracted from agglutinated rabbit erythrocytes in enhanced concentrations (Fig. 5a).

Four of the G2/albumin types contained polypeptide 6: S_{G2}, V_{G2}, Pr_{G2}, and B_{G2}. Fractionation of a protein extract from 'Sanilac' (S_{G2}) into G2 and albumin fractions showed this polypeptide to be present only in the albumin fraction (Brown et al. 1981b). Both fractions agglutinated rabbit erythrocytes (Brown et al. 1981b) and trypsin-treated cow erythrocytes (Osborn and Brown, unpublished data) suggesting that polypeptide 6 had no agglutinating properties with regard to these particular erythrocytes. This was supported by a comparison of the polypeptide compositions of B_{G2} and M_{G2} where the latter lacked polypeptides 6 and 8, and where agglutination activity was unchanged. The polypeptide compositions of S_{G2} and V_{G2} differed in that the former had polypeptide 7 present but 11 absent while the latter had polypeptide 11 present but 7 absent. 'Kerman' and 'California Small White 59' contained the S_{G2} pattern plus polypeptides 11 and 17. The identical agglutination activities of these lines suggested that polypeptides 7, 11, and 17 did not agglutinate rabbit or trypsin-treated cow erythrocytes. Thus, in the cultivars with the S_{G2}, V_{G2}, and Pr_{G2} patterns the active lectin components, with regard to rabbit and trypsin-treated cow erythrocytes, appeared to be polypeptides 9 and/or 10. These two polypeptides were isolated from rabbit and trypsin-treated cow erythrocytes agglutinated with protein extracts from cultivars containing the S_{G2}, V_{G2}, and Pr_{G2} patterns. Polypeptides 9 and 10 were also the only ones isolated from B_{G2}- and M_{G2}-agglutinated rabbit erythrocytes and were extracted, with polypeptides 2 and 12, from B_{G2}- and M_{G2}-agglutinated trypsin-treated cow erythrocytes, further emphasizing the specificity of 2 and 12 for cow erythrocytes and the apparent dual reaction of 9 and 10 with both rabbit and trypsin-treated cow erythrocytes. Thus, these observations based on comparisons of similarities and differences between the G2/albumin types and their respective agglutination ratios were consistent with the identification of the active lectin polypeptides by extraction from agglutinated erythrocytes.

Acknowledgement

The authors would like to acknowledge Mr. K. A. Ausloos for technical assistance, and Mrs. B. A. Brown and Ms. J. K. Pregler for typing the manuscript. This research was supported by the Science and Education Administration of the U.S. Department of Agriculture under Grant Nos. 5901-0410-8-9953-0 and 5901-0410-9-0357-0; Agrigenetics Corporation; NSF Grant No. PCM 78-11803; the Herman Frasch Foundation; and the College of Agricultural and Life Sciences, University of Wisconsin, Madison.

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Received March 12, 1982
Communicated by H. F. Linskens

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