An Immunochemical Survey of Proteins In Species of **Arachis**

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ABSTRACT

A comparative immunological survey of the proteins in 36 species accessions of the genus **Arachis** as grouped by Gregory **et al.** (1973) conducted by using immune sera from cultivated peanuts showed both quantitative and qualitative differences in protein contents. Sections I (Axonomorphae) and II (Erectoides) contained higher concentartions of the major peanut globulin, α-arachin than sections III (Caulorhizae), IV (Rhizomatosae), V (Extranervosae), VI (Pseudoaxonomorphae) and VII (Triseminalae). The precipitin patterns of total proteins in sections I, II, and IV appeared very similar to the precipitin pattern of the cultivar Virginia 61R. In general, the total precipitin patterns of sections III, V, VI, and VII showed relatively higher quantities of the albumins and the conarachins than did precipitin patterns of the other sections and their total patterns were less similar to the total pattern of Virginia 61R. Both intraspecific and interspecific polymorphism of proteins existed throughout the seven sections as evidenced by immunochemistry.

At present there exist approximately 50-60 wild species of *Arachis* native to South America (Banks 1972). The potential economic value of these relatives of the cultivated peanut is apparent because they offer an important source of diverse germ plasm for beneficial hybridization. Taxonomic differentiation of these wild species has been the objective of many researchers since the middle of the nineteenth century. Detailed reviews on the genetic resources of peanuts were recently reported by Gregory *et al.* (1973) and Hammons (1973).

For taxonomic purposes, earlier studies on the classification of peanuts were based on plant morphology, geographical areas where the samples were collected, species cross-compatibility, and hybrid fertility among species. In 1970, Seeligmann showed that the distribution of a C-glycoside (presumably vitexin) among different species of *Arachis* could be used as a chemotaxonomic marker. More recently, Cherry, 1974, investigated the electrophoretic properties of proteins and enzymes in 36 species accessions of *Arachis* and used these data to correlate a subordination of these accessions into categories obtained by classical genetic techniques. This chemotaxonomic study supported the classical data from which the accessions of species of *Arachis* were grouped into seven sections by Gregory *et al.* (1973). The present work is a followup survey of the major proteins in some of these accessions using the more species-differentiative immunochemical techniques. All analyses were carried out with immune sera made from cultivars Virginia 56R and Virginia 61R; these originated as individual plant selections from the same bacis genetic stock. Immune sera made against the fractions were available only from those of Virginia 56R.

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Materials and Methods

Seeds — Individual seeds of all species accessions were homogenized with a mortar and pestle in phosphate buffer, pH 7.9, 0.01 ionic strength and the homogenates were centrifuged at 39,000 x g for 30 minutes (Cherry, 1974). Supernatants from each seed were analyzed both separately and as the pools of extracts from five seeds within each species.

Analytical Techniques — Immunoelectrophoretic analysis (I.E.A.) in agar was performed according to Daussant **et al.**, 1969B, using a constant voltage gradient of 4 v/cm for 2 hours at room temperature. Antibody-in-gel crossed electrophoresis was done according to Laurell, 1966, by using anti-α-arachin from Virginia 56R; electrophoresis proceeded for 16 hours at constant voltage of 100 v and 11 ma. All experiments were carried out in 0.025 M veronal buffer, pH 8.2 at room temperature. After the unreacted serum proteins were washed out, the precipitin reactions were stained in 0.1% amido black in 7.0% acetic acid and destained with 7.0% acetic acid. Protein content of each sample was determined according to Lowry **et al.**, 1951.

Immune sera against the total proteins from Virginia 61R and fractions of Virginia 56R isolated on DEAE-cellulose (Groups I and II + III according to Dechary **et al.**, 1961) were prepared by Antibodies Incorporated,* Davis, California 95616.

Results

Classification of Arachis — The classification of wild species accessions by Gregory *et al.* (1973) were used in this study (Table I). All species were identified in each figure by the P.I. numbers (plant introduction numbers) of the U.S. Department of Agriculture.

Qualitative I.E.A. — The immunoelectrophoretic characterization of some isolated peanut proteins in cultivated seed was reported by Daussant *et al.* (1969B). At least 14 antigenic reactions were detected with immune serum made against a total cotyledonary extract. The identification of arachin with anti-arachin showed one major and three minor components. For purposes of identification, the major elongated component was named -arachin and three minor serologically distinct proteins were designated as "α-arachin contaminants." Similar identification of α-conarachin showed two serologically distinct proteins that were named α_1 - and α_2-conarachin.

Figures 1 to 4 show the precipitin patterns of proteins in pooled extracts of five seeds of some of the species accessions listed in Table 1. In each figure, the results presented were obtained in the same experiment; hence, slight variations in electrophoretic migrations of proteins in various sec-

Table 1. Subordination of Species of Arachis L. (n = 10) a/

Section	Series	Species	Collection No.	P. I. No.
I		Axonomorphae		
	1	Annuae (2n)		
		A. duranensis	7988	219823
		A. sp.	10038	263133
	2	Perennes (2n)		
		A. correntina	7830	262137
		A. correntina	7897	262134
		A. correntina	9530	262808
		A. chacoense	10602	276235
		A. villosa	22585	298636
	3	Amphiploides (4n)		
		A. monticola	7264	219824
		A. hypogaea	-	-
II		Erectoides		
	2	Tetrafoliolatae (2n)		
		A. paraguariensis	9646	262842
		A. benthamii	9764	262859
		A. sp.	9835	262308
		A. sp.	9841	262278
		A. sp.	9990	261877
		A. sp.	10002	262140
		A. sp.	10543	276209
		A. sp.	10573	276225
		A. sp.	10576	276228
		A. sp.	10580	276229
		A. paraguariensis	11462	331188
		A. paraguariensis	11488	331187
	3	Procumbensae (2n)		
		A. rigonii	10034	262142
III		Caulorhizae (2n)		
	1	A. pintoi	12787	338447
IV		Rhizomatosae		
	1	Eurhizomatosae (4n)		
		A. sp.	9553	262801
		A. sp.	9570	262817
		A. sp.	9667	262848
		A. sp.	9815	262794
		A. glabrata	9830	262797
		A. sp.	9882	262286
		A. sp.	9935	262301
		A. sp.	10105	276200
		A. sp.	10596	276233
V		Extranervosae (2n)		
	1	A. villosulicarpa	14445	336985
VI		Pseudoaxonomorphae (2n)		
	1	A. sp.	12943	338452
	2	A. sp.	12946	338453
VII		Triseminalae (2n)		
	1	A. pusilla	12922	338449

a/ Excerpted from Gregory et al., 1973

tions are relative to Virginia 61R in any given experiment. The total I.E.A. patterns are presented in A of each figure and the patterns of specific fractions are presented in B and C. For the analyses of specific fractions, immune sera from Virginia 56R were used. Virginia 56R and Virginia 61R originated as individual plant selections from what is presumed to be the same basic genetic stock—they appear identical immunochemically.

In sections I, II, and IV of Table 1, the precipitin patterns of total proteins (A in Figures 1, 2, and 3) appeared very similar to those of cultivated seed. Because of the complexity of the patterns, however, the identity of previously characterized proteins (particularly α_1-conarachin and α-arachin) was difficult in some cases. For the isolated fractiosnns (B and C in Figures 1, 2, and 3), slight differences in electrophoretic migrations and intensities of staining were more clearly observed. In section I, the patterns of the classic Gp I fraction (B) are more similar to the pattern of Virginia 61R than sections II and IV. (It should be pointed out here that this fraction is still under investigation in our laboratory as to the exact number of antigenic components and their relative position within the total pattern). Comparative analyses of the classic Gp (II and III) fraction in C of Figures 1, 2, and 3 clearly showed protein heterogeneity. In Figure 1, e.g., the leading edge of one of the precipitin lines showed an anodic shift for P.I. 262137 that was not observed for P.I. 219824 (arrows 1 and 2). These differences were also well-defined in sections II and IV (Figs. 2 and 3). In some cases, e.g., Figures 2-C and 3-C, certain proteins were not detected in some of the species (arrows 1 and 2). Since these analyses were based on equal quantities of protein in each well the data can be interpreted at least semi-quantitatively. Species accessions in sections III, V, VI, and VII (Figure 4) showed similar patterns but the patterns of total extracts and isolated fraction from sections VI and VII, in particular, showed the greatest similarity to each other. In general, the total patterns were less similar to the total pattern of Virginia 61R and contained high contents of protein in the classic Gp (II and III) fraction. Here again, as in the other sections, differences in electrophoretic migrations were observed.

Semi-quantitative Analysis of α-Arachin — In principle, the technique applied here can be described as follows: The antigen (in the wells) and antibody (dispersed in an agar matrix) interact forming precipitation zones shaped like "rockets." Free antigen within the cone migrates electrophoretically into the area of precipitation and redisolves the precipitate. The final position of the cone apex in relation to the origin is determined by the concentration of the antigen and by the relative electrophoretic mobility of antigen and antibody.

Figure 5 shows the semi-quantitative analysis of α-arachin (the major peanut globulin) in all of the species accessions. These results showed that seeds in Sections I and II generally contained more α-arachin than those in Sections III through VII. In sections III and VII, only traces of α-arachin were visualized. Note that *A monticola*,

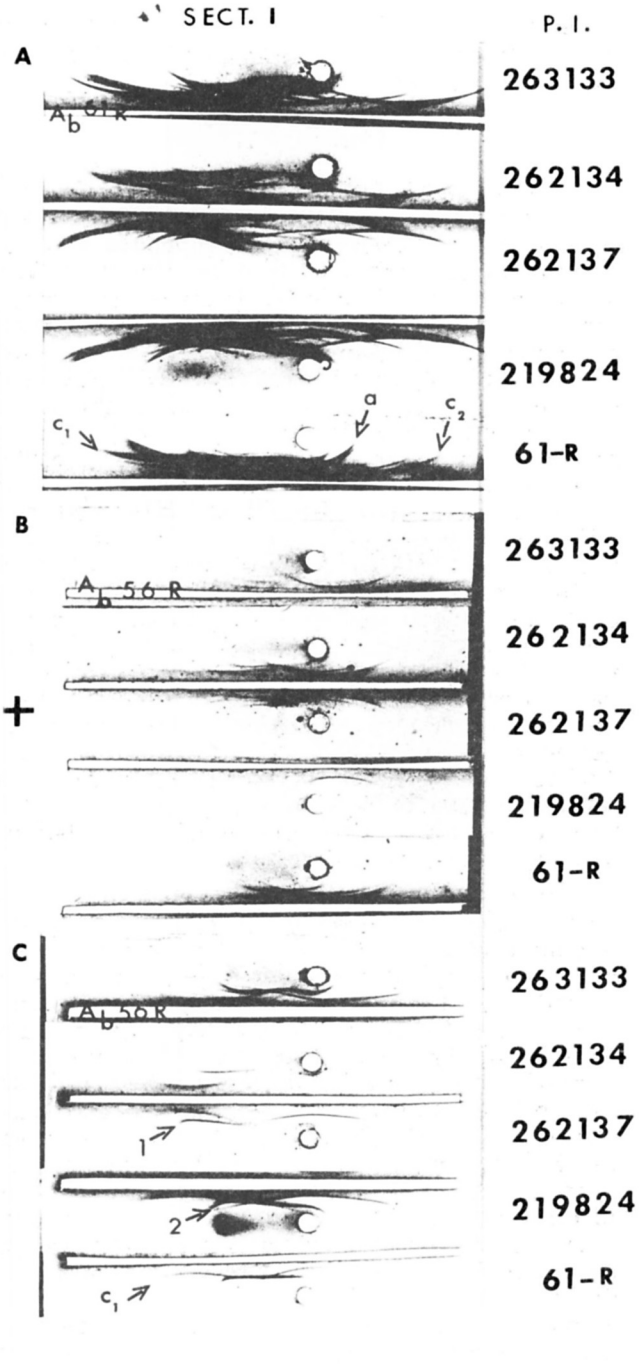

FIGURE 1

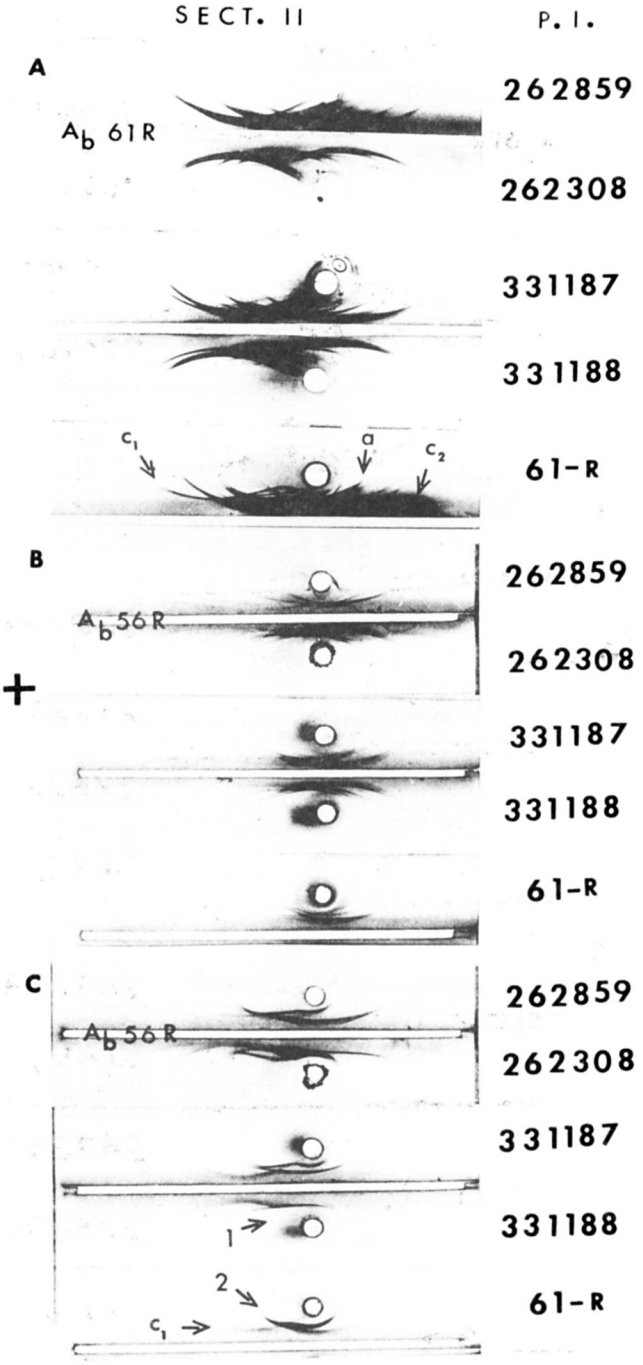

FIGURE 2

Figs. 1 to 4. Qualitative I.E.A. of Arachis sections I-VII. Each well was filled with approximately 0.75 mg protein from pooled samples of five seeds before electrophoresis; thereafter, all troughs were filled four times with immune sera made against:
(A) total proteins of Virginia 61R;
(B) Group I fraction of Virginia 56R (Dechary, et al., 1961) and
(C) Groups (II and III) fraction of Virginia 56R (Dechary et al., 1961). Samples are designated by P.I. numbers in Table I. Conditions for electrophoresis are given in the text.

(P.I. 219824) a reputed close relative of *A. hypogaea,* contained an amount of α-arachin comparable with that of Virginia 56R. On the other hand, one of the species in the Annuae (P.I. 219823), contained only a trace of α-arachin. In Section II, the precipitin intensity ranged from trace (e.g., P.I. 262142 and P.I. 331188) to a high content (e.g., P.I. 262859). It should be mentioned here, however, that although the migrations of the conical peaks in Section II are high in some cases (e.g., P.I. 262859), their intensities are less than those in Section I. These anomalies could be due to either variations in concentration and/or the number of active determinant groups on the antigen. Hence, the implications here point to either different forms and/or quantities of α-arachin. Varied

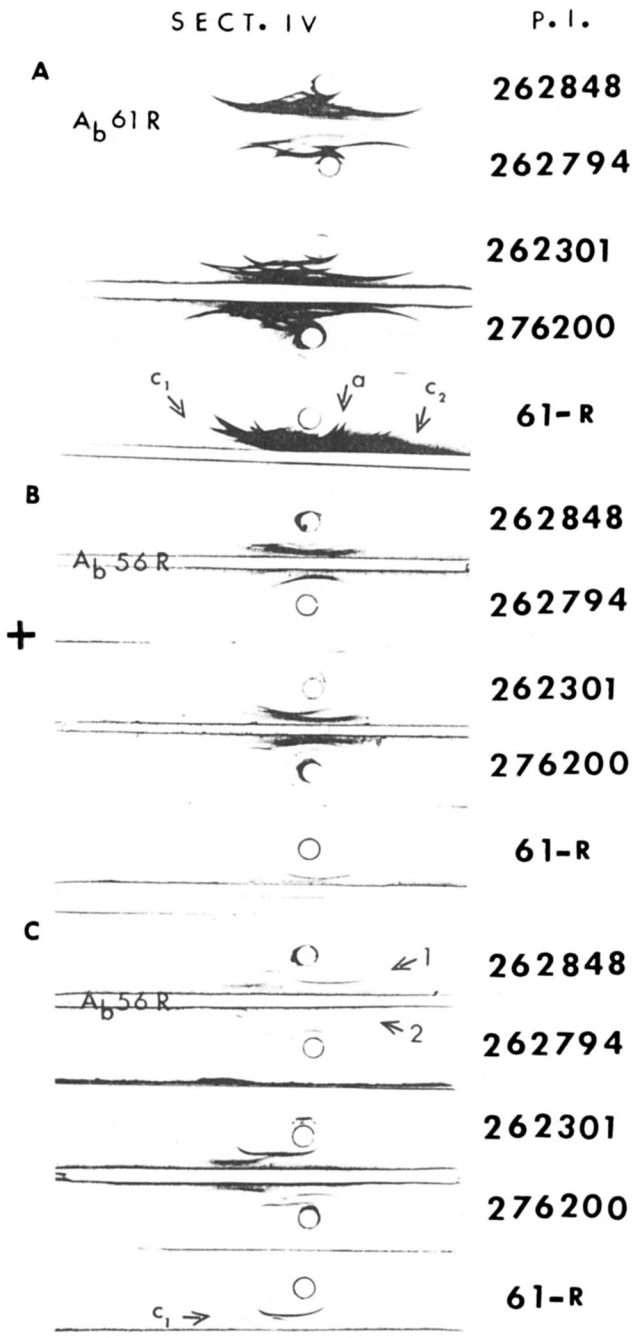

FIGURE 3

forms of arachin in cultivated peanuts have been reported by Tombs and Lowe (1967).

Intraspecific Protein Variations — Comparative analyses from individual seeds showed extensive heterogeneities in protein migrations within most species. Figure 6 represents typical I.E. A. comparisons of individual seeds in species from two sections. Out of five seeds from Section I (part A), P.I. 219823, three of the patterns were very similar but two of the seeds showed what appeared as different concentrations of α-arachin (arrows 1 and 2). Similar differences within species accessions in all of the other sections (e.g., as illustrated for section II in B) suggest the occurrence of heterogeneity in metabolic activity for production of specific proteins among seeds.

Discussion

The data showed evidence of protein synthesis in some wild *Arachis* species accessions comparable to that in cultivated *A. hypogaea* although both qualitative and quantitative differences were observed. As in other plant systems, mechanisms that control fruit development and biochemical syntheses are complex and of considerable interest to plant breeders (Aldana *et al.* 1972). Although certainly not conclusive, our data suggest that modern cultivars have retained some genetic information from their wild progenitors. In particular, the albumins or Gp I fraction and the conarachins contained in Gp (II and III) isolated on DEAE-cellulose (Dechary *et al.*, 1961) were most prominent (or stained more intensely) in Sections II through VII. Conversely, of all the sections, Section I, which includes *A. hypogaea,* and Section II showed the highest concentrations of some form of the major globulin, α-arachin in cultivated peanuts. Since α-arachin is considered to be the major nitrogen source for utilization during germonation and growth (Daussant *et al.*, 1969A) perhaps its presence in larger quantities than other proteins is beneficial to seed germination and development of the plant under selected soil and/or climatic conditions.

Because the primary structures of the peanut proteins are not known, it was difficult to explain unequivocally the differences in electrophoretic migrations and antigenic anomalies that were observed in this study. One significant factor, however, is the size, charge, and type of determinants on the antigens that react with their respective antibodies. These can be divided into so-called "sequential" and "conformational" determinants (Sela *et al.* 1967). Sequential determinants result result from specific amino acid sequences in a randomly coiled protein molecule whereas conformational determinants result from the quaternary structure of the protein. Thus, any antigen can provoke antibodies against many different determinants whereby diverse cross - reactions among related species can occur. Therefore in these wild species accessions of Arachis as in other plant systems (Exposito and Ulrich 1965) undoubtedly some of the proteins common to the cultivars can vary in the number of determinant groups, submit size, overall net charge, and conformation. These factors may be genetically controlled and can determine both the quality of antigenic reactions and the electrophoretic behavior of the antigen.

Heterogeneities in disc polyacrylamide electrophoretic patterns of proteins and enzymes from the genus *Arachis* were aso observed by Cherry, 1974. This study showed similarity index values among species accessions that supported classic techniques used for taxonomic classification of *Aarchis.* For example, cultivated *A. hypogaea* showed protein and enzyme patterns similar to

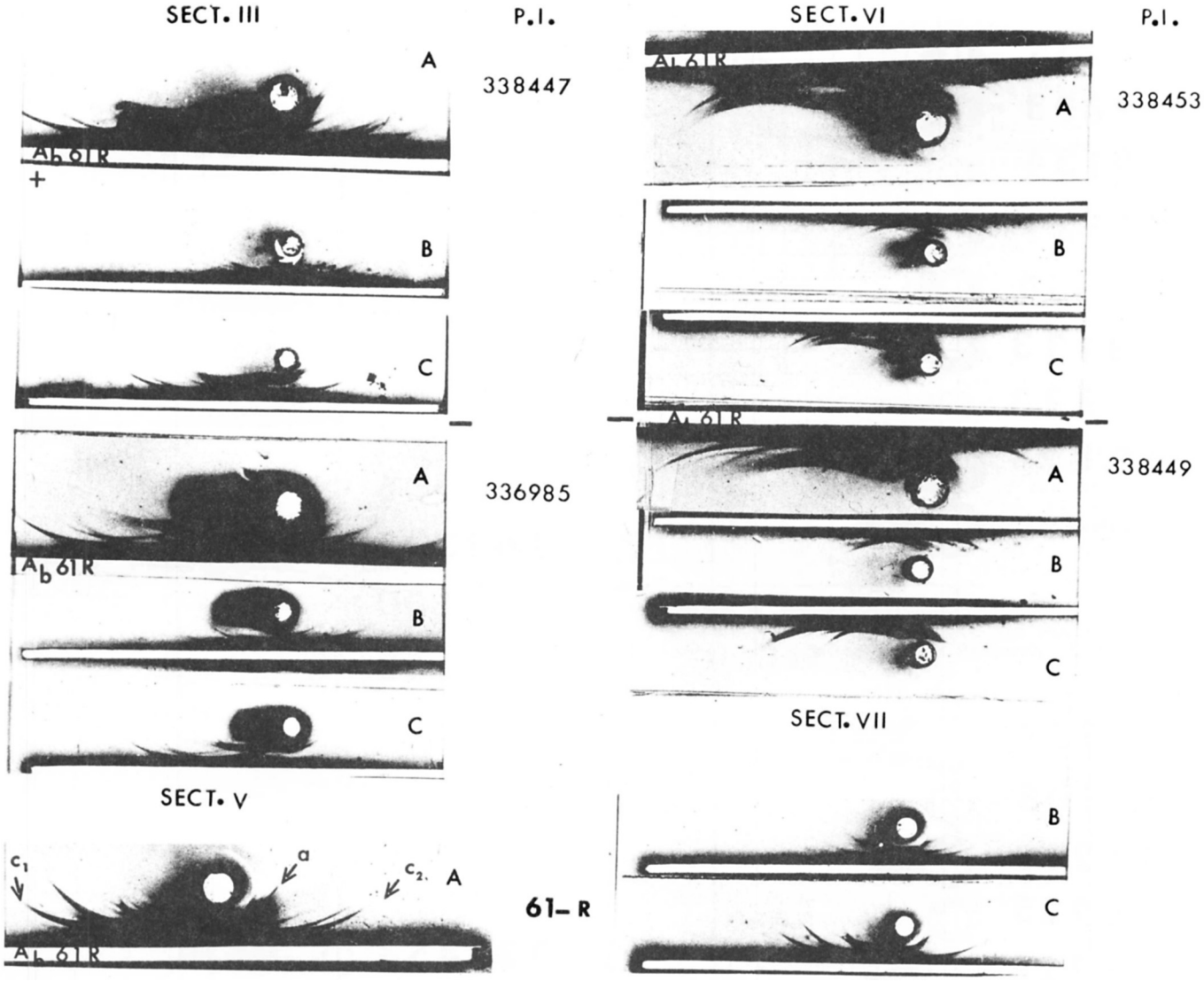

FIGURE 4

A. monticola (P.I. 219824) verifying the classical grouping already established by Gregory *et al.* (1973).

Although the exact chronology of events leading to the evolution of modern cultivars is not known, successful hybrids within and among sections of *Arachis* have been reported. Gibbons and Turley, (1967), reported successful hybrids between species of Section I. Smartt and Gregory (1967) and Gregory (1967) reported the first successful hybrids between species of different sections. Some of these include hybrids between sections II and VI and between Sections II and III. More recently, Hammons (1970) released the first commercial cultivar in the world that was derived from interspecific hybridization of *A. hypogaea* (spancross) and *A. monticola* (P.I. 219824).

Since the sources of heritable variation depend on the gene pool of a species, broadening of our understanding of this variability for practical purposes (e.g., disease resistance or amino acid composition) is increasingly dependent on genetic and biochemical studies. It is well known that induced mutations find practical uses in plant improvement (Shepherd and Mayo 1972). Traditionally, commercial varieties are improved by hybridization with a not-too-distantly related type having certain desirable characteristics. The present survey of proteins in wild species common to cultivated peanuts could help establish favorable species variations for practical purposes and for conservation of the genus *Arachis*.

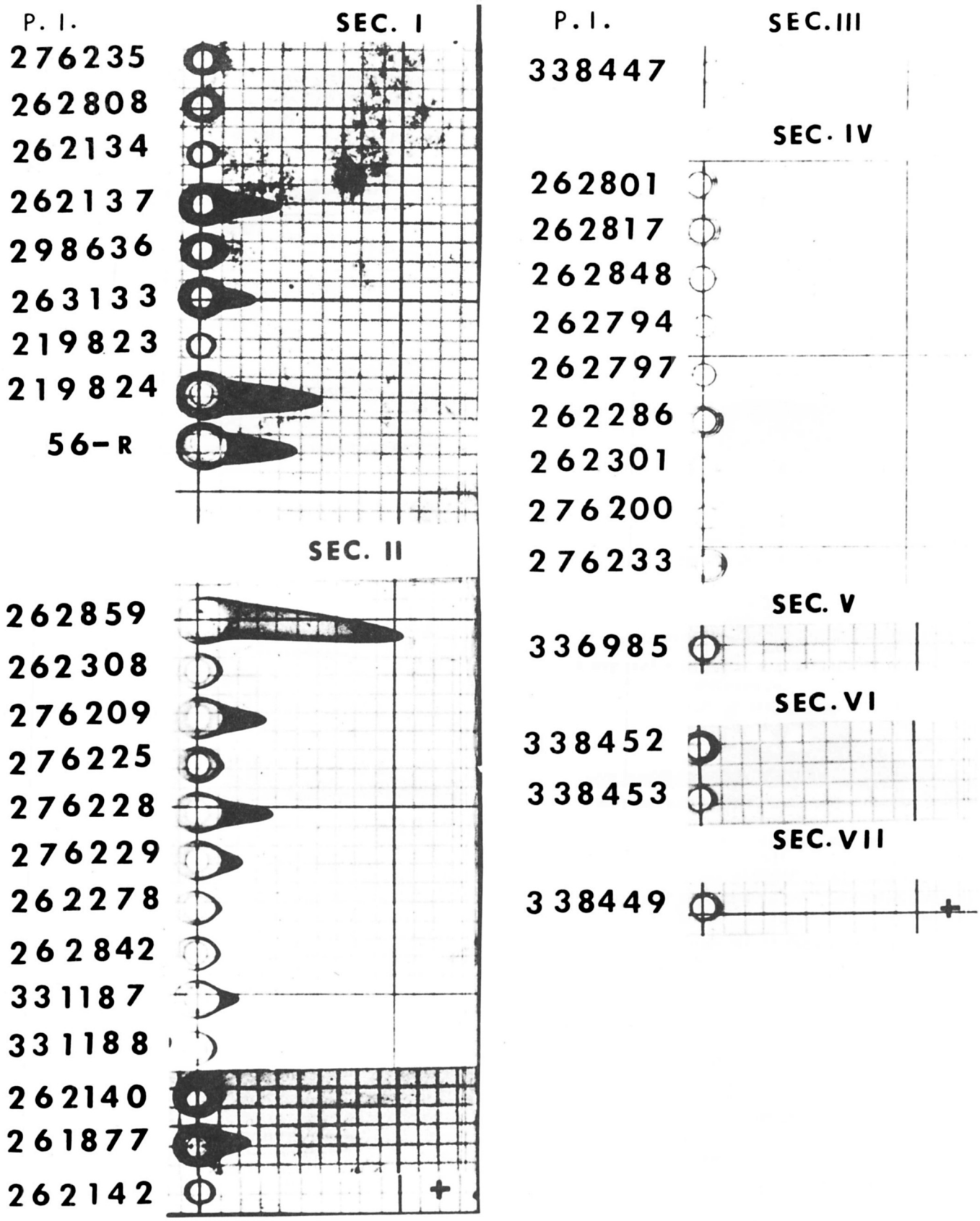

Fig. 5. Semi-quantitative analysis of α-arachin in Arachis sections I through VII by antibody-in-gel electro-phoresis. Each well designated by its P.I. number was filled with 20 μg protein from pooled samples of five seeds before electrophoresis. The agar contained 1.0% immune serum against α-arachin from Virginia 56R. Conditions for electrophoresis are given in the text.

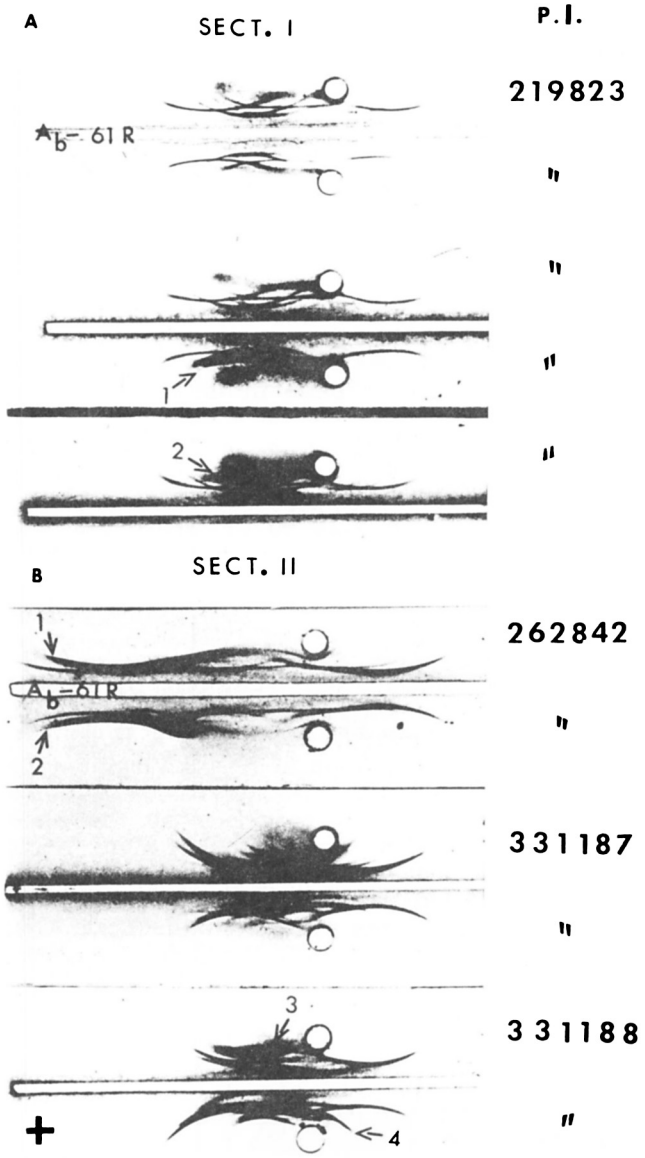

Fig. 6 Variations in protein pattern among individual seeds of four different species accessions in sections I and II. Note the differences in migration of specific proteins (arrows 1 and 2 in part B) relative to the said antigens in other species and quantitative differences (arrows 1 and 2 in part A, 3 and 4 in part B) between seeds of the same species. Experimental conditions were the same as in Figures 1-4.

Acknowledgments

The authors gratefully acknowledge the assistance of Dr. Walton C. Gregory, Dept. Crop Science, and Dr. M. Pfluge Gregory, Dept. of Genetics, North Carolina State University, Raleigh, North Carolina, both for their advice on the subject matter and for supplying the collection of seeds. The authors also wish to thank Jack Bergquist for making the photographs.

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