PEANUT (ARACHIS HYPOGAEA L.) SEED PROTEIN CHARACTERIZATION AND GENOTYPE SAMPLE CLASSIFICATION USING POLYACRYLAMIDE GEL ELECTROPHORESIS

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Summary: Peanut seed protein has been characterized using polyacrylamide gel electrophoresis. The same general electropherogram pattern as regards numbers and kinds of protein components resolved was observed for all forty-five samples tested. Therefore, a universal standard electropherogram protein pattern appears to exist for all peanut seed samples and is presented. However, quantitative differences of individual proteins may occur. Depending on the genotype examined, four to six components contain most of the protein. Based on amounts of these major components present, all the samples can be separated into one of four groups. Classification by this electropherogram grouping method offers another criterion in determining genetic relatedness.

INTRODUCTION

Polyacrylamide gel electrophoresis (PAGE) has become a widely used method in protein biochemistry, and in the last few years, many researchers of peanut protein have performed various analyses using this technique. It has been employed to monitor and characterize protein development and localization within the seed (1-3), arachin/conarachin fractionation experiments and content (4-11), enzymes and reactions (12-16), and environmental and varietal (11, 15-17) as well as heat and roasting (18,19) effects on the individual proteins.

Data presented in this literature reveal that the total number of electrophoretically resolved proteins was often used as an important control parameter in experimentation, and several reports (15-17) specifically concern this point. In these, seed protein of different peanut cultivars was examined in an attempt to develop and characterize electrophoretic patterns to serve as possible "standards" for various test comparisons. Overall, they report that it was difficult to clearly distinguish between cultivars simply on the basis of the protein patterns, and defined standard patterns were not established. It was

noted though that some cultivars grown in one geographic location could be partially distinguished from the same type grown in another by minor qualitative and quantitative differences in the patterns.

The similarity of electropherogram protein patterns of the different cultivars (15-17) indicates that a universal standard pattern might exist for all cultivars instead of a different one for each. An anodic non-detergent gel system was utilized, however, in these experiments. Data recently obtained in this laboratory reveal that this type PAGE system does not separate and resolve peanut protein components as well as the sodium lauryl sulfate (SLS) detergent type system (20). In light of this information and because more knowledge of the numbers and kinds of protein molecules present in the mature peanut seed would be of great value to the breeder and laboratory investigator, data were accumulated using the SLS PAGE system, and some major findings are reported.

Materials and Methods: Oil was removed from all seed samples by treatment (20 mg ground seed in 100 ml solvent) for 30 min in cold (4°C) 90% acetone containing ammonium hydroxide (50 µl/100 ml). This process was repeated, and deoiled (stripped) sample was collected by sedimentation (20,000g for 30 min at 4°C), quick-frozen, lyophilized to dryness and stored at -20°C until analyzed.

PAGE. Detergent (SLS) gel electrophoresis was performed in 10% gels utilizing a combination of the basic systems of Ornstein and Davis (21,22) and Shapiro, Vinuela and Maizel (23) as refined by Weber and Osborn (24) and Grula and Savoy (25). The capability of this procedure to separate and resolve plant proteins has recently been reported and the methodology given in detail (20). Molecular weight (MW) determination of resolved components was accomplished as previously described (23,24). The proteins of known MW used were: bovine serum albumin, DNase, concanavalin A, lysozyme and cytochrome c. Calibration curves were constructed using protein concentrations varying from 2.5 to 10 µg/gel.

Results and Discussion: Several studies of peanut seed protein have been completed in this laboratory wherein SLS PAGE testing was performed to monitor experimentation. One or more different market types were examined in each study, and the genotypes analyzed consisted of the following: GA 98-3-5, GA 157-1, GA C37, Florunner, Jenkins Jumbo, Senegal 41-95 P.I. 196623, P.I. 268771B, Bassee P.I. 229553, PS 1539 C-1-29-1 C-3, PS 1555 C-2-33-1D, NC 4144 and NC 5 (26).

Comparison of the electropherogram protein patterns obtained from these twelve genotypes revealed that all are alike as regards numbers and kinds of components present except for quantitative differences of some individual pro-

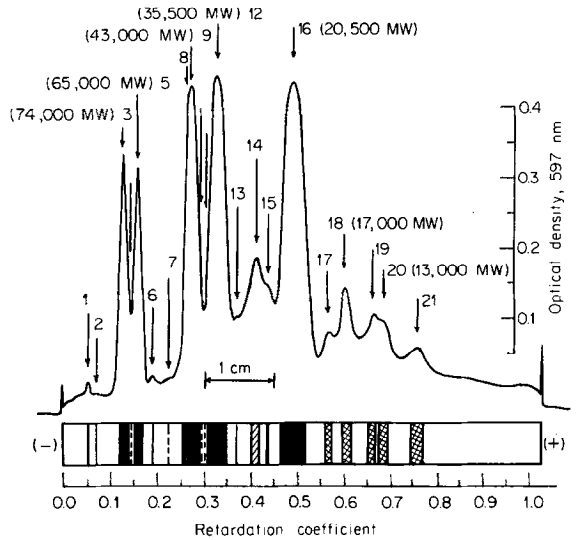

Figure 1. Electropherogram component pattern of stripped peanut protein meal (Jenkins Jumbo) after PAGE in the SLS system. Detection was accomplished using CBB G250, and 60 µg meal (contains 35 to 50 µg protein) were analyzed. Malachite Green in solvent served as the marker dye, and retardation coefficients were calculated by setting the migration distance (leading edge) of the dye to one.

teins. Fig. 1 shows the pattern of Jenkins Jumbo, and it can be used to describe the major pattern characteristics of the others. Each was found to contain at least twenty-one protein components, with the majority of proteins having an approximate MW distribution of 13,000 to 74,000 daltons. These components were determined to be the same in each genotype by comparing retardation coefficients and electrophoretically analyzing all samples simultaneously, with a few on the same gel column. The number reported could be minimal since the quantity of some protein molecules may be insufficient for detection.

The MW values are considered approximate because of the expected deviation of up to 10% (24) and the structural properties of the resolved components in the SLS solvent. This detergent converts most proteins into rod-like particles whose lengths vary uniquely with MW (27). The logarithm of mobility of this type molecule during SLS PAGE is also linear with respect to MW; whereas the mobility logarithm of a globular molecule is linear with the square of its ra-

TABLE I

CLASSIFICATION OF PEANUT MARKET TYPES AND GENOTYPES BASED UPON THE PAGE ELECTROPHEROGRAM COMPONENT PATTERNS

Market Type and Genotype*	Growth Area	Group Classification A	B	C	D
Spanish					
Set No. 1					
Spancross	Headland, AL		X		
GA 116	" "		X		
Starr	" "		X		
GK-19	" "		X		
Comet	" "		X		
GA 123	" "		X		
Spanhoma	" "		X		
Tifspan	" "		X		
Set No. 2					
Spancross	Tifton, GA		X		
Starr	Holland, VA			X	
Runner					
Set No. 1					
GA 194R	Headland, AL				X
Set No. 2					
Florunner	Marianna, FL				X
" "	Headland, AL		X		
" "	Stephenville, TX				X
" "	" "				X
" "	Tifton, GA				X
" "	" "			X	
" "	Yoakum, TX			X	
F439-16	Headland, AL			X	
" "	Yoakum, TX		X		
" "	Marianna, FL		X		
Early Runner	Headland, AL		X		
" "	Marianna, FL		X		
Virginia					
Jenkins Jumbo	Gainesville, FL	X			
Set No. 1					
NC Fla 14	Headland, AL		X		
VA 72R	" "		X		
AU-2	" "				X
Florigiant	" "				X
Set No. 2					
Florigiant	Tifton, GA				X
" "	Yoakum, TX				X
" "	Stephenville, TX				X
P.I. 319178-S5	Marianna, FL		X		
F393-6	Headland, AL		X		
Uncertain					
Set No. 1					
Goldin-I	Headland, AL		X		

*Set number one and Goldin-I genotype were grown in 1974, number two in 1971 and Jenkins Jumbo in 1973.

dius (24,28). Thus, mobility is directly affected by extent of molecular branching and size. At present it is not known whether peanut proteins possess rod-like or globular mobility characteristics in the presence of SLS; most probably become rod-like. However, preliminary evidence indicates that one or more may be a glycoprotein and therefore may demonstrate globular characteristics.

Overall, the similarity in electropherogram patterns of the twelve genotypes strongly supports the idea that a universal standard pattern exists for all peanut genotypes. To test this possibility further, thirty-three additional samples (listed in Table I, and grown in the area shown by AES or ARS cooperators) and a peanut flour (test sample #8160-16) obtained at the 1975 meeting of the American Peanut Research and Education Association (APREA) were analyzed. Regardless of the market type, genotype, growth area or growing season, the pattern described previously for the twelve genotypes was also obtained for each of these samples. These data allow the conclusion that a universal standard pattern does exist for peanut seed proteins. Quantitative differences of individual proteins may occur though.

In comparison of the twelve peanut genotype patterns, it was also noted that only four to six components (numbers 3, 5, 8-9, 12 and 16) contain most of the seed protein, and the number of these major components is seemingly characteristic of the genotype (Fig. 1). Moreover, the twelve genotypes could be separated into three groups based on the intensities of these components, especially using numbers 5 and 12. Subsequent testing of other genotypes has led to a fourth group by subdividing one. Although reproducibility of minor components is poor in a photograph, representative gel columns showing the resolved proteins of these four groups may be seen in Fig. 2. Descriptions of the group classifications follow. Group A. Genotypes of this group contain numbers 3, 5, 8-9, 12 and 16 as major components. Group B. Numbers 3, 8-9, 12 and 16 are the only major components. Number 5 is present, but in trace amounts. Group C. Genotypes of this group contain numbers 3, 8-9 and 16 as major components. Numbers 5 and 12 are present only in trace amounts. Group D. This

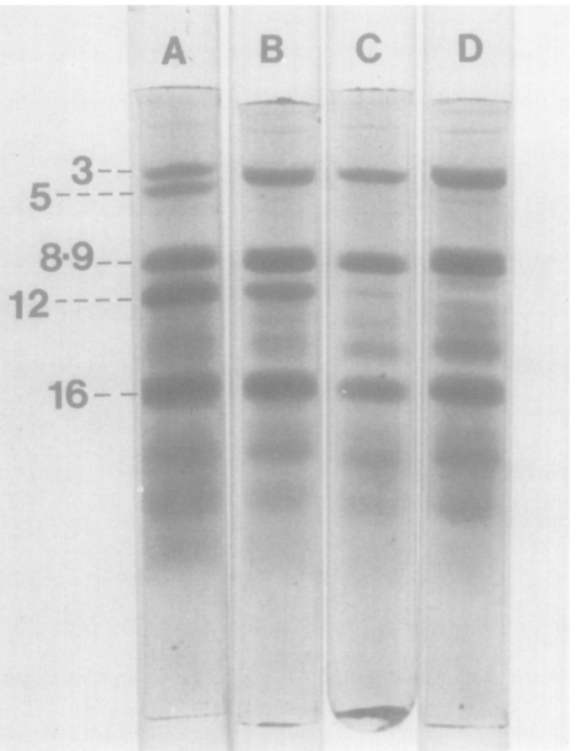

Figure 2. Photographic representation of SLS PAGE columns containing resolved peanut protein components; CBB G250 was used for detection. The four groups of electropherogram characteristics are exemplified by the patterns of the following cultivars: (A) Jenkins Jumbo, (B) GA 98-3-5 (Spanish market type), (C) Florunner and (D) AU-2 (Virginia market type).

group is like Group C except component number 12 appears to be missing or is not present in sufficient quantity for detection.

To determine if other groups might exist, the samples listed in Table I were electrophoretically analyzed. It was found that all the samples can be categorized into one of the four electropherogram groups previously described. Separation of the samples into the market type descriptions provided by the breeder reveals that only those listed as Spanish demonstrate electropherogram grouping similarity. Except for one, all these possess the pattern characteristics of Group B. It cannot be stated though that all the genotypes which possess the Group B characteristics are of this market type since the Runner and Virginia market type genotypes were found to be evenly distributed among

the B, C and D Groups. Thus, classification by electropherogram grouping does not at first appear useful in distinguishing market types; however, the following information needs consideration before such a conclusion is reached.

During the group discussion on peanut genetics and breeding at the 1975 APREA meeting, it was mentioned (29) that peanut germplasm is not as distinguishable as it used to be. Several reasons may be accountable for this. In the last few decades, hybridization breeding methods have been emphasized for developing new cultivars. Thus, nomenclature for subsequent hybrid lines would not be eqivalent to the Spanish, Runner or Virginia terms of the past. Classification is further complicated by the fact that confusion is apparent within the peanut literature between the designations botanical varieties, market types and cultivars. Moreover, the terms Spanish, Runner, Virginia and Valencia have often been used as both type and cultivar designation. As a result, incorrect nomenclature labeling could and probably has occurred.

Therefore, differences in PAGE group classification could be a result but not a necessary event when samples supposedly of the same genotype are analyzed. This information would explain why samples of one genotype grown in different geographic locations all possess the same electropherogram group characteristics (examine those of the Spancross, Early Runner or Florigiant shown in Table I), and others do not (Starr, Florunner and F439-16). Because Groups C and D pattern characteristics are quite similar and those of Group B are significantly different to these, it is possible that one sample in each of the Starr, Florunner and F439-16 should not be so classified.

Overall, these data reveal that classification by electropherogram pattern grouping offers another criterion to distinguish between peanut genotypes. The extent of importance in classifying market types and genotypes remains unknown, however, until evaluation has been made on strict botanical varieties of A. hypogaea.

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