Relation of a 36 000-Dalton Arachin Subunit to Blanchability in Peanuts (*Arachis hypogaea* L.)

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Seed proteins from 22 different peanut cultivars were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF). The comparison of electrophoretic profiles revealed the presence of a 36K dalton, major polypeptide in most of the cultivars and breeding lines with poor blanchability, those whose seed coat adheres to the cotyledon tightly. It is not known whether or not this polypeptide is directly responsible for the poor blanchability of these cultivars and lines. However, this character, namely presence vs. absence of the 36K polypeptide, could be used as a reliable indicator of blanchability in peanut cultivars and breeding lines. It may also serve as an important criterion in the assessment of the quality of peanuts for processing purposes.

INTRODUCTION

Peanuts (*Arachis hypogaea* L.) are an important source of vegetable oil and protein in many parts of the world. Quality assessment in peanuts relies primarily on chemical analysis of total oil content and fatty acid composition. A thorough characterization of peanut protein however has yet to be undertaken in spite of its potential importance as a source of food and feed protein (Lusas, 1979). Recently reports dealing with the amino acid compositions of the peanut protein and genetic variability among cultivars with respect to their essential amino acid profiles have been presented (Heinis, 1972; Amaya et al., 1977). These studies showed that the peanut protein was rather low in three essential amino acids, methionine, lysine, and tryptophan.

Utilization of peanut as a potential source of food and feed protein has begun recently (Martinez, 1979) and thus the characterization of peanut proteins should become an area of active research in the future. The available data on peanut proteins are mainly limited to the two major globulin fractions, arachin and conarachin, which together constitute about 97% of the total meal protein (Cherry and Ory, 1973). A number of reports by Cherry and co-workers (1971, 1973, 1974, and 1977) deal with qualitative and quantitative comparison of peanut proteins as affected by growing area, the potential of peanut as a source of oil and protein, and the variability of its protein during seed development. Basha and co-workers (1976, 1978, and 1979) have published several papers focusing on characterization of peanut protein from various cultivars, changes during germination, and one on two dimensional electrophoretic analysis which showed cultivar specific polypeptides. Basha (1982) also reported on the amino acid composition and characteristics of a basic protein fraction and isolation of two cryoproteins in peanuts. Yamada and his collegues (1979 and 1980) reported studies on the isolation, properties, and accumulation pattern of arachin and its subunits.

One of the important characteristics that directly affect the quality of large-seeded Virginia-type peanuts is blanchability, that is, the ease with which the skin (seed coat) separates from the cotyledon. Milling quality, flavor, blanchability, and maturity in peanuts are factors which could be used as a measurement of quality as reported by the peanut quality standard committee (Sexton et al., 1966). On the other hand, blanchability was shown to be a factor mainly related to genotype, seed size, degree of maturity, as well as the time and temperature of the postharvest storage period (Mozingo, 1979). However, not much is known about the biochemical genetic basis of blanchability. This study was undertaken to search for marker proteins that can be used to distinguish peanut cultivars and breeding lines with good blanchability from those with poor blanchability by electrophoretic procedures.

MATERIALS AND METHODS

Plant Materials. Seed of 22 cultivars and breeding lines used in this study was from the 1983 crop grown at the Tidewater Research Center in Suffolk, VA. Nine varieties out of 22 belonged to the "early" and 13 to "mid-late" maturity groups. For each cultivar 12 kernels were selected randomly, seed coats were then removed, and the cotyledons were cut with a razor blade into two halves. The seed meal was prepared by grinding the thin cotyledon slices cut from the middle of the seed to a fine powder in a mortar with a pestle. The meal was then defatted with ethyl ether thoroughly by using a meal weight to solvent volume ratio of 1:10 (3 times, 24-h each) and stored in the freezer.

Protein Extraction. Cotyledon meals were extracted with a 6 M solution of urea containing 5% mercaptoethanol (2-ME) at a meal weight to solvent volume ratio of 1:10 for a period of 24 h. Extract was recovered by centrifugation at 14 000*g* for 5 min. All operations were performed between 0 and 5 °C unless otherwise stated.

Sample Preparation and Electrophoresis. For isoelectric focusing (IEF) analysis 25 μL of each extract were directly applied on a 5% polyacrylamide gel containing 2% ampholyte (Pharmalyte, pH 4–10.5), and IEF was performed as described by Hu and Esen (1982). Samples for SDS-PAGE analysis were prepared by mixing 3 parts of extract with 1 part of the SDS sample buffer (Laemmli, 1970) and heating them in boiling water for 2 min. SDS-PAGE analysis were performed according to the procedure of Laemmli (1970) with either 10–15% gradient or 12% homogeneous gel slabs.

Blanchability Test. Blanchability tests were performed in duplicate on 250-g samples of peanuts from seven environments for each of 22 lines according to the procedure and device described by Wright and Mozingo (1975).

RESULTS AND DISCUSSION

Both the SDS sample buffer (Laemmli, 1970) and 6 M urea, 5% 2-ME were judged to be suitable for extracting

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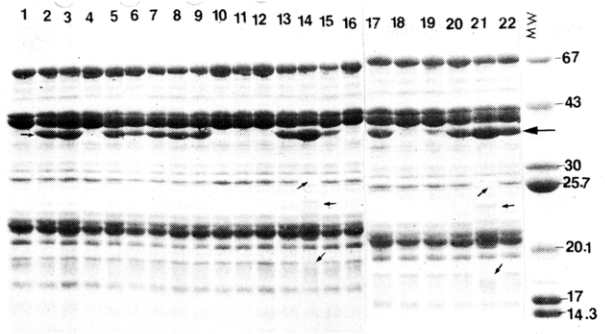

Figure 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of proteins of peanut seed meals from 22 different cultivars or breeding lines. 1, Florigiant; 2, VA bunch 46-2; 3, NC 5; 4, NC 2; 5, NC 6; 6, VA 56 R; 7, NC 4; 8, VA 61R; 9, GA 119-20; 10, NC 8C; 11, GK 3; 12, Avoca 11; 13, VA 72R; 14, NC-Fla 14; 15, NC 9; 16, VA 81B; 17, NC 7; 18, Keel 29; 19, VC 1; 20, Shulamit 6; 21, NC 17; 22, early bunch. The arrows indicate polypeptides whose occurrence varies with the cultivar or genotype. Mobility differences between corresponding bands are due to variability in electrophoretic conditions; the patterns in lanes 17–22 were from a gel which was run 15 min longer than those in lanes 1–16.

the peanut protein in one step with one solvent. Although these two solvents are ideal for electrophoretic analysis of peanut proteins under dissociating conditions as well as for reducing the possibility of proteolysis, they cannot be used when one is interested in isolating proteins in their native or near native state. In this case, the extraction buffer of Basha and Pancholy (1982) and others (e.g., Yamada et al., 1980) may be used.

Figure 1 shows the SDS-PAGE profiles of the 22 peanut cultivars and breeding lines used in this study. These profiles include polypeptides that range in size from approximately 10 000 to 65 000 daltons. Each profile contains 5–6 predominant polypeptides which together appear to constitute 80–90% of the total protein. Based on comparisons of our gel profiles with those of Yamada et al. (1979 and 1980) and using their nomenclature we identified the major polypeptide with the highest M_r, 65 000 daltons, to be conarachin (Figure 1, top arrow). Similarly the 3 major polypeptides with M_r between 36K and 41K and those with M_r between 19K and 21K are identified to be the subunits of arachin. The most striking difference among the 22 cultivars and breeding lines were the absence of the 36K subunit of arachin in 7 of them (Figure 1, lanes 1, 4, 10–12, 16, and 18) and its presence in the remaining 15. Among these 15 cultivars and breeding lines the level of the 36K arachin subunit was lowest in VC 1, NC 9, and VA 56R (Figure 1, lanes 6, 15, and 19) and greatest in NC 17 and NC-Fla 14 (Figure 1, lanes 14, and 21 and arrows) with other being intermediate. Another striking difference was a 25K polypeptide missing in NC 17 and NC-Fla 14 (Figure 1, lanes 14 and 21 and arrows) but present in others. Likewise a 23K polypeptide was present in these two lines; but an 18K polypeptide occurred in trace amounts as opposed to the absence of the former and the presence of the latter in all other lines. Interestingly enough, NC 17 and NC-Fla 14 contained the 36K polypeptide at the highest levels. The relation of the high level of the 36K polypeptide to the absence of the 25K and low level of 18K polypeptide is not known.

Isoelectric focusing profiles of the same 22 cultivars and lines are shown in Figure 2. A comparison of these profiles reveals the presence of a doublet band (Figure 2, bar) in the same 15 lines which had the 36K subunit of arachin (see above). This doublet band was absent in seven lines which lack the 36K subunit of arachin. These results,

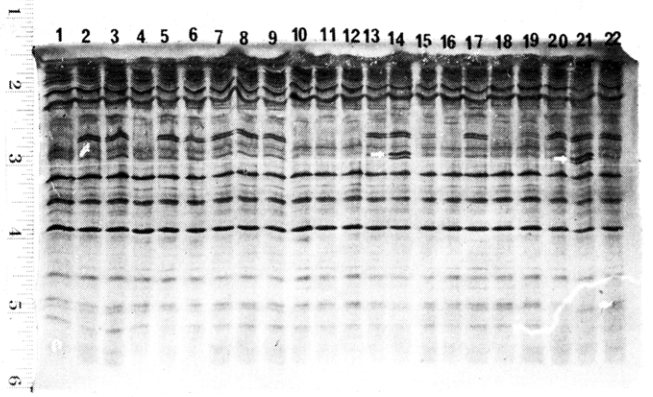

Figure 2. Isoelectric focusing (IEF) patterns of seed proteins from 22 different cultivars and breeding lines of peanuts. The legend is the same as in Figure 1. The arrow indicates the doublet band corresponding to the 36K dalton arachin polypeptide that occurs in some cultivars or breeding lines.

namely the perfect correspondence between a 36K polypeptide in SDS-PAGE and a doublet band in IEF profile of 15 cultivars and breeding lines, strongly indicate that the 36K subunit of arachin is made up of two charge species. Whether these two different charge components represent products of two different alleles of the same gene or one arises from the other by posttranslation modifications is not known. However, the first possibility is a plausible one in view of the fact that peanut is tetraploid and has four copies of each gene. For example, a self-pollinating tetraploid having two different alleles of the same gene in the duplex (say AAaa) configuration would regularly produce this configuration and two different gene products if homologeous chromosomes with identical alleles consistently form bivalents in meiosis.

The fact that the 36K polypeptide is a subunit of arachin was verified as follows. Protein was extracted from two sources, Florigiant (Figures 1 and 2, lane 1) in which the 36K polypeptide is missing and NC 5 (Figures 1 and 2, lane 3) in which it is present, under nondenaturing conditions. Samples were separated by electrophoresis in an alkaline gel system. The gel was briefly stained to visualize the major components. The major band (arachin R_f 0.78) was cut as a strip, placed in 1.5-mL Eppendorf tube, dehydrated with alcohol, and homogenized in the SDS-sample buffer. The hemogenate was left standing for about 24 h, heated for 5 min at 97 °C, and centrifuged and the supernate was applied on a SDS gel and subjected to electrophoresis. The resulting SDS-PAGE profile (not shown) was essentially made up of the polypeptides referred to as the subunits of arachin in Figure 1. As expected, the arachin profile of Florigiant lacked the 36K polypeptide while that of NC 5 contained it.

Blanchability is an important factor in determining the quality of peanuts used as cocktail peanuts. Limited genetic data suggest that poor blanchability is a dominant or semidominant trait. With this in mind, we searched for marker proteins in SDS-PAGE and IEF profiles that might be useful in distinguishing poor blanching peanuts from good blanching ones. This search led to suggesting a strong association between the presence of the 36K subunit of arachin and poor blanchability. Blanchability of 70% or above is considered to be good while that below 70% poor. Table I lists the 22 cultivars and breeding lines, their blanching data, and status with respect to 36K polypeptide. Examining these data shows that all of the seven cultivars and breeding lines that lack this polypeptide have blanchability above 70%. Similarly, the two lines (VC 1 and NC 9) that contain low levels of this polypeptide have

Table I. Blanchability of 22 Peanut Cultivars and Lines and Their Status with Respect to the 36K Polypeptide

cultivar or breeding line	blanchability, %[a]	36K polypeptide: (+) present, (−) absent
NC 8C	87.6	−
Florigiant	84.3	−
VC 1	82.0	trace
NC-Fla 14	80.5	+
NC 9	75.8	trace
Keel 29	74.2	−
GK 3	74.1	−
NC 7	72.4	+
NC 2	72.4	−
Avoca 11	71.8	−
VA 81B	71.0	−
Shulamit	70.5	+
VA 56R	69.2	+
VA 72R	69.0	+
GA 119-20	68.8	+
VA 61R	67.5	+
VA bunch 46-2	62.9	+
early bunch	61.8	+
NC 5	61.2	+
NC 17	57.3	+
NC 4	56.0	+
NC 6	53.8	+

[a] The mean of seven different analyses in duplicate with 250 g each of peanuts white roasted, cooled, and blanched for 3 min as described in Wright and Mozingo (1975).

above 70% blanchability. In contrast, only two of the 13 lines that contain the 36K polypeptide had above 70% blanchability, the rest varying from 54% to 70%. Blanchability of peanut is affected by such factors as seed size, maturity, and moisture content. Small, immature (somewhat shrivelled) seeds blanch only partially or not at all regardless of their genotype. However, the ranking of cultivars and breeding lines remain essentially the same when seed of similar size and maturity levels are compared. This indicates that blanchability is genotype-dependent when other variables remain constant.

It is not known how the presence of 36K polypeptide adversely affects blanchability should it be indeed responsible for this defect. It is possible that the polypeptide in question is a "sticky" glycoprotein which leaks out of the cotyledon or is excreted into the space between the cotyledon and seed coat resulting in tight adherence of the seed coat to the cotyledon. This explanation does not account for good blanchability of two lines which have the 36K polypeptide. However, these two lines may not be excreting this protein. Alternatively, the 36K polypeptide may have no direct bearing on blanchability but the responsible gene coding for this peptide may be closely linked with the gene(s) responsible for poor blanchability. Should this postulated linkage be broken by recombination, genotypes with good blanchability would result even though they have the 36K polypeptide. Further research is therefore needed for a thorough understanding of the biochemical–genetic basis of blanchability. Even if the 36K polypeptide turns out not to have any bearing on blanchability, it may still serve as an important marker in genetic studies and cultivar identification.

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