Molecular Plant Pathology

Detection and Partial Characterization of New Polypeptides in Peanut Cotyledons Associated with Early Stages of Infection by *Aspergillus* spp.

J. B. Szerszen and R. E. Pettit

Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, Texas A&M University, College Station 77843.

Research supported in part by Peanut Collaborative Research Support Program, USAID grant DAN-4048-G-SS-2065-00.
We thank our technicians Daniel Gully, Debbie Byars, Ted Oplinger, and Michael Hart for valuable assistance.
Accepted for publication 8 May 1990.

ABSTRACT

Szerszen, J. B., and Pettit, R. E. 1990. Detection and partial characterization of new polypeptides in peanut cotyledons associated with early stages of infection by *Aspergillus* spp. Phytopathology 80:1432-1438.

Polypeptide profiles of peanut cotyledonary tissue from viable kernels of 14 cultivars grown under normal irrigation and five genotypes grown under drought stress were determined before and after invasion by *Aspergillus flavus* and *A. parasiticus*. Inoculated kernels and isolated cotyledons were removed from moist chambers every 6 hr within 48 hr after inoculation. Polypeptide patterns were determined by microprocessor-controlled sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional electrophoresis and visualized after staining with silver. Uninoculated cotyledonary tissue contained 35 comigrating groups of SDS-dissociated proteins (13.5–218.7 kDa), and mapping showed the presence of 257 components within pI range 3.00–8.70. Four new polypeptides (16.4, 18.1, 23.0, and 30.6 kDa; pI 7.95, 8.00, 7.90, and 7.55, respectively) were present in viable intact kernels and live, isolated cotyledons 18–24 hr following inoculation. Two additional polypeptides (19.9 and 22.0 kDa; pI 8.15 and 8.00, respectively) were detected after 24–30 hr of incubation in cotyledons from plants grown under normal irrigation. Drought stress inhibited the synthesis of these polypeptides except in kernels of cultivar TX 798736, which contained five of them, including one specific for this cultivar (37.2 kDa; pI 6.50). Mapping of polypeptides showed their enhanced synthesis with time and variations in amounts among cultivars tested.

Additional keywords: *Arachis hypogaea*, molecular markers of pathogenesis-related genes, pathogenesis-related proteins, peanut proteins, polypeptide mapping.

Peanut (*Arachis hypogaea* L.) cotyledonary tissue contains 25–30% proteins (3), which have been classified as globulins (arachin, conarachin) and albumins (22). Arachin and conarachin together account for approximately 87% of peanut seed proteins (21). Cultivar differences in peanut seed polypeptide composition are known (6,7,18,28), and electrophoretic patterns of peanut proteins are very complex (13). Kernel-invading microorganisms can change these patterns, because protein molecules are altered by microbial enzymatic action, and new proteins of microbial origin are synthesized. Such a situation occurs when the aflatoxin-producing or nontoxigenic strains of *Aspergillus* spp. colonize shells and kernels (8,14–16,30). These changes can be detected electrophoretically as early as 2 or 3 days after inoculation of kernels with *Aspergillus* spp. (14–16).

Aspergilli colonizing viable peanut kernels can be considered as weakly pathogenic (14). There is, nonetheless, preliminary

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evidence that *Aspergillus parasiticus* and *A. flavus* can act as pathogens that invade live aerial peanut pegs and establish themselves within peg tissue (4). Invaded plant tissue can mount an active biochemical defense response (17), including the formation of newly synthesized pathogenesis-related proteins named b-proteins, PR proteins, or PRs (10,19,35). Pathogenesis-related proteins (PRs) are of host plant origin, not of pathogen origin. They have been detected in 16 plant species (35), but not in peanut plants. PRs can be induced in plants by viruses (10,27,35), viroids (10), bacteria (1), fungi (10,19), and culture filtrates from plant pathogenic fungi (26) or bacteria (24). In addition, some chemicals can induce them (34,35). Pathogenesis-related proteins typically have low molecular weights, are extractable at extreme pH, are mostly protease-resistant, and accumulate in the intercellular spaces (35). Their amino acid sequences revealed that they could not be categorized into any known functional class of proteins. Plant tissue, in which PRs have been induced, is more resistant to many pathogens compared to tissue in which the induction did not occur (35). This finding can be linked with a possible role of PRs in limiting multiplication and/or spread of a pathogen within invaded plant tissue (35). Thus, it is thought that induced PRs may be responsible for a systemic acquired resistance.

The objectives of this study were to investigate changes in SDS-dissociated protein patterns in viable peanut kernels infected by *A. flavus* or *A. parasiticus* at specified intervals, search for molecular markers useful to study the expression of pathogensis-related genes in peanut cotyledons infected by pathogenic aspergilli, and provide molecular evidence to serve as a basis for the future search for disease resistance in peanuts.

MATERIALS AND METHODS

Mature peanut kernels from 14 cultivars were examined. These cultivars, ranked here according to their increasing susceptibility to aspergilli, were: TX 798736, SN 55-437, J-11, SN 73-30, PI 337409, SN 73-33, Toalson, Tamnut, PI 341885, PI 343419, Pronto, TX Ag 3, Florunner, and Starr. Kernels were surface-sterilized by soaking for 1.5 min in 1.3% NaOCl, then washed for 10 min in running distilled water and soaked for an additional 45 min in sterile deionized water at room temperature. This hydrated them to 25% moisture. Intact viable kernels, isolated live cotyledons (testae and embryonic axes removed), and homogenized cotyledonary material were used in the experiments. The latter were obtained by grinding isolated cotyledons 5 min with mortar and pestle in the presence of liquid nitrogen. After thawing, the resultant peanut paste was shaped into the form of cotyledons. Plant tissues were sprayed with conidia of *A. flavus* (Fl 102) or *A. parasiticus* (NRRL 2999) (7×10^6 spores per milliliter of sterile distilled water containing 0.03% Tween 20). Controls were sprayed with sterile distilled water containing Tween 20. Treated materials were incubated in the dark, at 32 C, and 95% RH and sampled every 6 hr during the first 48 hr of incubation. After incubation, testae and embryonic axes were removed from the kernels. Additionally, all visible fungal growth was collected from surfaces of seed.

Protein content in water-checked and aspergillus-infected kernels, sampled every 6 hr of incubation, was estimated as described (9). Each sample had five replicates, each replicate comprised five kernels. Extraction of proteins included grinding plant materials for 10 min, at 4 C, in the presence of 50 mM Tris-HCl, pH 8.0, followed by centrifugation at 10,000 *g*, 4 C, for 30 min.

In another experiment, five peanut cultivars were grown in field microplots arranged in a completely randomized design and watered with deionized water. The microplot area was covered with a plastic greenhouse roof. The cultivars grown were: J-11, SN 55-437, Toalson, Starr, and TX 798736 (selection of the cross Toalson/UF 73-4022). There were two treatments: normal irrigation and irrigation following wilting (drought stress). Each treatment had five replicates, each replicate comprised 10 plants. Unstressed plants were watered twice a week with 3 cm of water each time. Drought stress began 100 days after planting and was interrupted only by watering each wilted plant with 1 cm of water to prevent their possible death and was repeated as needed. The wilting was assessed visually at 9 a.m. each day. After digging, the plants were cured in the greenhouse (28–34 C, forced air flow) for 2 wk. Sound mature pods were shelled and processed. Plant materials were surface-disinfested, inoculated, incubated, and sampled as described above.

For the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), samples were ground at 4 C with pestle and mortar, and defatted with a repeated series of cold diethyl ether. Defatted material was reground in the presence of white quartz sand and buffer (1 mM EDTA, 10 mM Tris-HCl, pH 8.0) in ratio 1:1:48 (w/w/v), respectively, followed by filtration and centrifugation at 10,000 *g*, 4 C, for 30 min. SDS (2.5% w/v) and 2-mercaptoethanol (5%, v/v) were added, the samples were heated for 5 min at 100 C and cooled on ice. Bromophenol blue was added as a tracking dye, and the samples

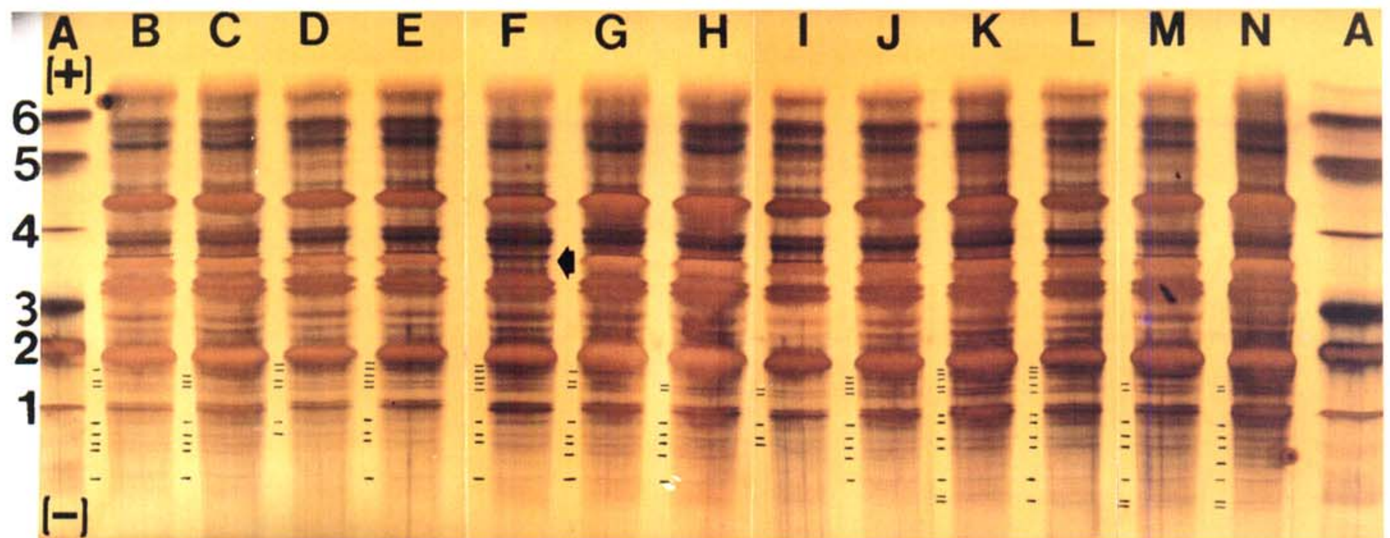

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis banding patterns of proteins extracted from noninfected peanut cotyledonary tissue. **A,** Molecular weight marker (1 = 94.0 kDa, 2 = 67.0 kDa, 3 = 43.0 kDa, 4 = 30.0 kDa, 5 = 20.1 kDa, 6 = 14.4 kDa); **B,** Starr; **C,** PI 341885; **D,** PI 337409; **E,** Tamnut; **F,** Florunner; **G,** TX Ag 3; **H,** Toalson; **I,** SN 55-437; **J,** TX 798736; **K,** SN 73-33; **L,** SN 73-30; **M,** Pronto; and **N,** PI 343419. The arrow indicates the position of a 37.1 kDa major protein component that is not present in cotyledons of Florunner cultivar. Small bars on left side of each lane mark positions of the weakest bands.

were centrifuged for an additional 15 min at 10,000 g, 4 C. The samples were then subjected to horizontal discontinuous SDS-PAGE (PhastSystem, Pharmacia LKB Biotechnology, Piscataway, NJ) with gradient gels (the resolving gradient zone was 8–25% with 2% cross-linking). The volume of samples applied to gels was 0.5 or 0.3 μl (2.8 or 1.9 μg of protein, respectively). Gels measured 43 × 50 × 0.45 mm, with a separation length of 32 mm. Gel buffer in both zones (stacking and resolving) contained 0.112 M acetate and 0.112 M Tris, pH 6.4. The electrophoresis buffer system consisted of 0.20 M tricine, 0.55% SDS, and 0.20 M Tris, pH 7.5. Each run consisted of 250 V, 10 mA, 3 W, for 73 Vhr. Molecular weight markers were as follows: α-lactalbumin (14.4 kDa), trypsin inhibitor (20.1 kDa), carbonic anhydrase (30.0 kDa), ovalbumin (43.0 kDa), albumin (67.0 kDa), phosphorylase b (94.0 kDa), β-galactosidase (130.0 kDa), and ferritin half unit (220.0 kDa).

For isoelectric focusing-polyacrylamide gel electrophoresis (IEF-PAGE), defatted cotyledonary meal was ground at 4 C in the presence of white quartz sand and a buffer (0.1 M Tris, 0.05 M EDTA, 0.1 M KCl, 0.04 M 2-mercaptoethanol, and 0.1 M sucrose), followed by filtration and centrifugation at 10,000 g and 4 C, for 45 min. Proteins were separated in a gradient gel from pH 3.0 to 9.0 containing 5% T (percentage total monomers), 3% C (percentage cross-linker as a percentage of the total monomer), and 22 μM Pharmalyte (ampholytes carrier)/pH unit. Gels measured 43 × 50 × 0.35 mm with a separation length of 37 mm. One half microliter of each sample was applied at the anode and 0.5 μl at the cathode of the gels. A prefocusing step consisted of 2,000 V, 2.5 mA, 3.5 W for 75 Vhr; a loading run consisted of 200 V, 2.5 mA, 3.5 W for 15 Vhr; and a focusing step consisted of 2,000 V, 2.5 mA, 3.5 W for 410 Vhr. Separation bed temperature was maintained at 15 C. Isoelectric point (pI) markers were: amyloglucosidase (pI = 3.50), soybean trypsin inhibitor (pI = 4.55), β-lactoglobulin A (pI = 5.20), bovine carbonic anhydrase B (pI = 5.85), human carbonic anhydrase B (pI = 6.55), horse myoglobin-acidic band (pI = 6.85), horse myoglobin-basic band (pI = 7.35), lentil lectin-acidic band (pI = 8.15), lentil lectin-middle band (pI = 8.45), and lentil lectin-basic band (pI = 8.65).

IEF gels for two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) were cut into strips and equilibrated for 5 min in solution containing 2.5% SDS, 1% dithiothreitol, 0.112 M acetic acid, 0.112 M Tris, 0.260 M iodo-acetamide, and 0.03% bromophenol blue. The second dimension was run with molecular weight markers on polyacrylamide gradient gels (8–25%) for 73 Vhr. Gels were stained as described (20).

Generally, one SDS-PAGE and one 2D-PAGE were performed per one sample; however, additional runs were performed for each sample showing presence of novel polypeptides to ensure repeatability.

RESULTS

Sodium dodecyl sulfate-dissociated cotyledonary proteins from kernels of the 14 cultivars consisted of 35 bands, 16 major, and 19 minor (Fig. 1 and Table 1). The range of their molecular weights was 13.5–218.7 kDa. The majority of the protein components were contained within molecular weight ranges of 13.5–23.6 and 30.7–94.0 kDa (Table 1). There was a visual similarity in banding patterns within all cultivars tested (Fig. 1); however, both qualitative and quantitative differences were evident (Table 1). Florunner lacked the major 37.1 kDa component and variations in other cultivars included bands of high, low, or very low intensity.

A total of 257 components was found in all cultivars by two-dimensional electrophoresis (Fig. 2). Major components were grouped within quadrants 9, 10, 11, 15, 21, and 22. The range of pIs of polypeptides mapped was 3.00–8.70, with the majority of spots within pI = 4.5–7.7. Many components, which were recorded on SDS gels as a single band, were resolved into several distinct spots on two-dimensional gels, e.g., the 51.5 kDa component was resolved into 19 spots (Fig. 2, quadrants 20–23). Most polypeptide spots shown on the two-dimensional map do not overlap each other; however, some had similar molecular weights and pIs, and they appear on the map as superimposed spots.

Inoculation with *A. flavus* resulted in a rapid increase of soluble proteins (Fig. 3). *A. parasiticus* caused cotyledonary proteins that were less soluble in comparison with the water check. SDS or IEF gels did not record changes of protein patterns of water-checked or inoculated plant materials within 42 hr of inoculation (not shown). The only changes of one-dimensional protein patterns were due to the presence of very small quantities of proteins of fungal origin. A zymogram of these SDS-dissociated proteins (mycelia and conidia) collected from surfaces of cotyledons is shown in Figure 4. Separations made in the second dimension within this period also showed the presence of new components that were excluded because they were of fungal origin. These changes included the appearance of four very faint spots in a basic and low molecular weight region of two-dimensional gels (Fig. 5). The presence of these spots of approximate molecular weights of 16.4, 18.1, 23.0, and 30.6 kDa, and pIs 7.95, 8.00, 7.90, and 7.55, respectively, was not detected in aspergillus-inoculated ground cotyledonary material, in water-checked

TABLE 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of peanut cotyledon proteins in 14 cultivars[a]

Molecular weight of protein subunits (Da)	Cultivars[a] lacking the band of SDS-dissociated protein	Quantity[b] of protein subunits
13,500	...	high
15,800	...	high
17,300	...	high
18,000	...	high
19,300	...	high
21,900	...	high
23,600	...	very high
26,900	...	low
28,800	...	low
30,700	...	high
31,900	...	high
33,100	D,E,I,L	high
34,700	...	high
37,100	F	very high
40,200	...	very high
41,900	...	very high
51,500	...	moderate
56,200	...	moderate
62,500	...	moderate
69,500	...	very high
72,200	B,C,G,H,I,J,M,N	low
73,600	C,H,I,M,N	low
76,700	B,D,G,H,I,N	low
81,200	...	low
83,600	...	low
90,100	...	high
94,000	...	high
97,300	B,C,D,E,F,G,H,I,J,M,N	very low
109,600	...	very low
118,100	...	very low
123,200	D	very low
126,300	D,E,F,I,K,L,M	very low
142,900	D,I	very low
215,200	B,C,D,E,F,G,H,I,J	very low
218,700	B,C,D,E,F,G,H,I,J,L	very low

[a]Cultivars investigated: Starr (B), PI 341885 (C), PI 337409 (D), Tamnut (E), Florunner (F), TX Ag 3 (G), Toalson (H), SN 55-437 (I), TX 798736 (J), SN 73-33 (K), SN 73-30 (L), Pronto (M), PI 343419 (N), and J-11 (O).

[b]Quantity estimated according to intensity of protein subunit bands, according to scale: very high, high, moderate, low, very low. For examples of bands classified to certain intensity and scale, see Figure 1, line B, from anode to cathode: very high, seventh band; high, first band; moderate, seventeenth band; low, eighth band; very low, weak bands in the cathodal region.

Fig. 2. Map of protein components detected in 14 peanut cultivars and location of polypeptides (marked a, b, c, d, e, f, and g) induced by *Aspergillus flavus* and *A. parasiticus* in live cotyledonary tissue.

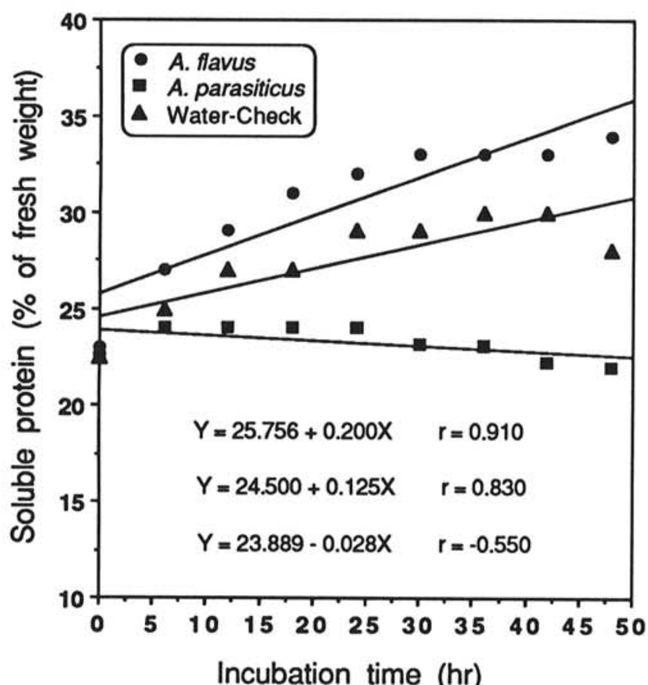

Fig. 3. Changes of soluble protein content in cotyledons from kernels of peanut cultivar TX 798736 inoculated with *Aspergillus flavus* and *A. parasiticus*. Each data point is a mean of five replicates.

samples or in fungal growth collected from surfaces of seed. Their locations relative to all components mapped from all cultivars tested are shown in Figure 2 (spots marked a, b, c, and d). Among all 14 cultivars tested, only two (Florunner and PI 341885) did not produce the new polypeptide spots. Mapping showed gradual increase in concentration of the four polypeptides detected after 24 and 30 hr in isolated cotyledons and intact kernels, respectively, from J-11, Toalson, TX 798736, and SN 55-437 (Fig. 5) inoculated with *A. flavus*, but not with *A. parasiticus*. Also, mapping showed the presence of two additional polypeptides 19.9 and 22.0 kDa; pIs 8.15 and 8.00, respectively) in *A. flavus*- and *A. parasiticus*-inoculated cotyledons and kernels, 24 and 30 hr after inoculation, respectively (Fig. 5). The presence of these polypeptides was detected in all cultivars tested except Florunner and PI 341885. Their location among other protein components is shown in Figure 2 (spots marked e and f). Enhanced concentration of these two polypeptides was detected after 30 and 36 hr of incubation in isolated cotyledons and kernels, respectively, in J-11, SN 55-437, Toalson, and TX 798736 inoculated with *A. flavus*. They were not detected in inoculated ground cotyledonary material or in the uninoculated water check.

Cotyledons from drought-stressed J-11, SN 55-437, Toalson, and Starr did not produce any of the four new polypeptides upon infection with *A. flavus* or *A. parasiticus*. Drought-stressed and *Aspergillus* spp.-infected cultivar TX 798736 exactly followed the above described pattern of a, b, c, and d formation, but failed to produce spots e and f. However, drought-stressed TX 798736 showed the presence of a new polypeptide spot (37.2 kDa, pI

6.50) detected 30 and 36 hr in isolated cotyledons and kernels, respectively, after inoculation with either fungus. Its location within the two-dimensional composite map is shown in Figure 2, spot marked g. This particular spot was found only in drought-stressed and infected cultivar TX 798736 and exhibited enhanced concentration within the 36–42 hr period (Fig. 6). The image of the new spots of the resolved samples taken 42–48 hr after inoculation was diffuse on gels.

DISCUSSION

The most significant finding from this research was the detection of novel polypeptides in viable peanut cotyledons during early stages of infection by *Aspergillus* spp. with cultivar variation and drought stress responses. To achieve this, we first had to establish "standard" polypeptide patterns of unchallenged cotyledons and the fungi themselves. Our experiments resolved 35 SDS-dissociated protein components from peanut cotyledons, which is the most separated to date. Previously, 12–21 components have been separated electrophoretically (6,8,28). The high resolution obtained is attributed to the electrophoretic system, which employed a high-technology microprocessor controlling each separation and staining step. Electrophoretic patterns of proteins from surface-sterilized kernels were identical to the patterns obtained from nonsterilized and nonsoaked kernels, regardless of the electrophoretic techniques we employed. However, soaking may result in a slight increase of soluble proteins (16). Moreover, hydration may increase the susceptibility of kernels to *Aspergillus* spp.

One- and two-dimensional electrophoretic patterns of peanut proteins from kernels inoculated with aspergilli and incubated for 0–3 days were reported as either basically unchanged (8) or changed (14–16,29,31,32). Most often the recorded changes were a decreased staining ability of high molecular weight bands and the appearance of new low molecular weight components. During

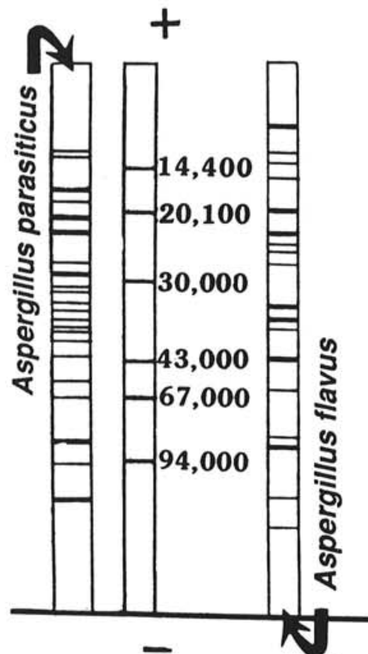

Fig. 4. Zymograms of sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of protein components detected in mycelia and conidia of *Aspergillus flavus* and *A. parasiticus* grown on peanut kernels and testa-free cotyledons.

Fig. 5. Low molecular and basic region of two-dimensional polyacrylamide electrophoretic gels showing formation of novel polypeptides (circled areas) in cotyledons of peanut cultivar SN 55-437 infected with *Aspergillus flavus*. **A,** 12 hr after inoculation, no polypeptide spots present in circled areas; **B,** 18 hr after inoculation, four faint spots present in circled areas, polypeptides marked a, b, c, and d; **C,** 30 hr after inoculation, polypeptides marked a, b, c, and d show enhanced concentration, new polypeptides marked e and f in small circled areas.

advanced colonization of kernels by the aspergilli, intensity of these changes, caused by proteolytic action of the fungi, increased (8,14–16,30). We made an extensive analysis of peanut cotyledonary protein patterns as changed by *Aspergillus* spp. proteolytic enzymes, covering the period 42 hr–12 days after inoculation (30; Szerszen and Pettit, *unpublished*). We detected the formation of newly synthesized polypeptides before the changes due to fungal protease activity were recorded. Scanning 2-D gels of protein separations from noninfected plant materials, infected ground (nonviable) cotyledons, and fungal components revealed a lack of polypeptides previously detected in live infected kernels and cotyledons.

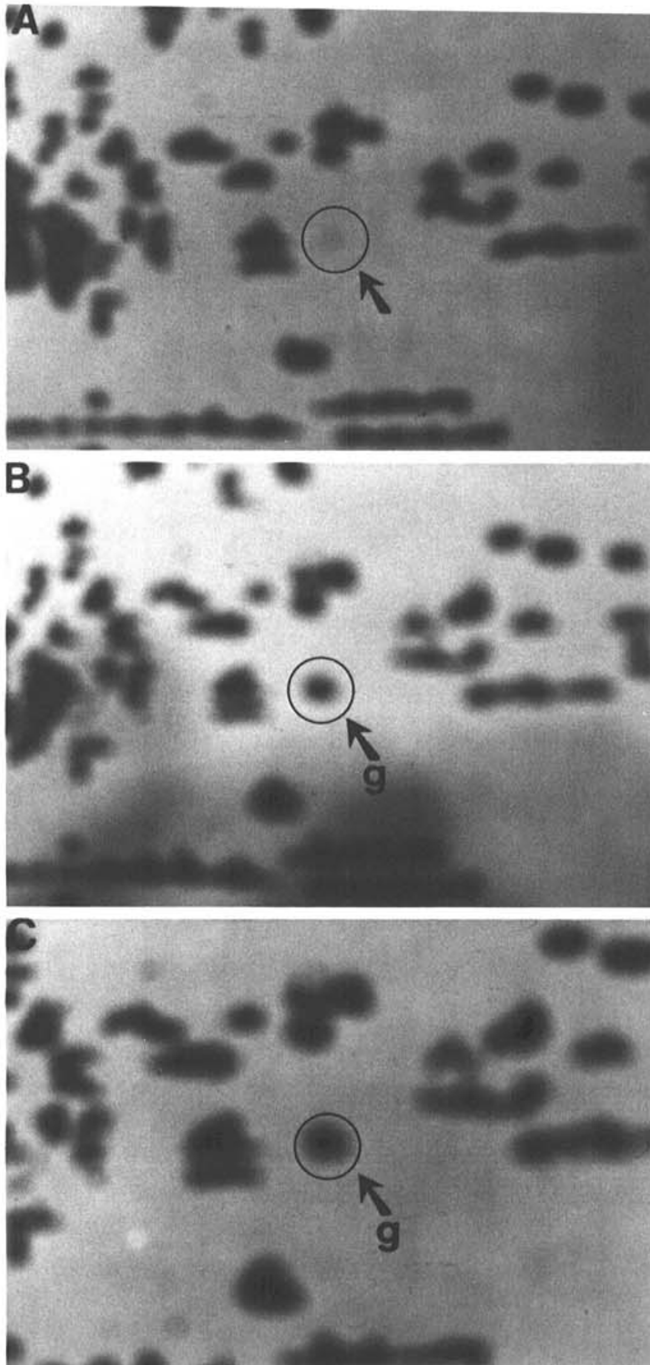

Fig. 6. Novel polypeptide (circled areas) of 37.2 kDa and pI 6.50, exctracted from cotyledons of peanut cultivar TX 798736 grown under drought stress and inoculated with *Aspergillus parasiticus*. **A,** 24 hr after inoculation, circled area covers region of two-dimensional gel in which 6 hr later was found polypeptide marked g (extremely faint spot indicates beginning of synthesis of the polypeptide); **B,** 30 hr after inoculation, presence of polypeptide marked g; **C,** 36 hr after inoculation, enhanced concentration of polypeptide marked g.

Since the presence of the new spots was detected only in *Aspergillus* spp.-infected live plant material, their origin could only have been peanut cotyledonary tissue, not fungal tissue. The enhanced concentration of postinfectional polypeptides (Figs. 5 and 6) indicate a role for them in the host response reaction. These polypeptides could have been subunits of SDS-dissociated pathogenesis-related proteins induced by the aspergilli and present in the peanut tissue in a very low level. However, we do not have evidence that these polypeptides constitute part of a weak resistance mechanism induced in the cotyledons before extensive pathogen development occurs.

In previous experiments (8,11,12,14–16) with similar systems, the researchers sampled aspergillus-infected peanut kernels every 1–4 days, which could have prevented them from detecting protein changes that may occur in viable cotyledons within hours. They used naphthol blue black or Coomassie blue stains to visualize bands. These stains are at least 60 times less sensitive than ions of silver (2,20) and the novel polypeptides, which we detected, could not have been visualized on their gels. Also, most of the research on protein changes in peanut cotyledons challenged by aspergilli was conducted using one-dimensional electrophoretic techniques (11,12,14–16). Two-dimensional separations (8) did not show presence of the novel polypeptides probably because of the reasons mentioned above. It is worth noting that contrary to these researchers we used highly hydrated cotyledons (25% moisture) for our tests.

We used both intact kernels and isolated cotyledons to avoid any possible cut-injury response of incubated live tissue, which sometimes may change protein patterns (33). No detectable change in protein patterns was noted. De novo synthesized polypeptides were not detected in our one-dimensional separations, because the compounds were present in very low quantities, and either their molecular weights or pIs overlapped with other cotyledonary protein components on one-dimensional gels.

Differences in synthesis patterns of novel polypeptides among tested cultivars (i.e., presence or absence of these components, their number, and increased concentration) may provide valuable information about the presence and expression of pathogenesis-related genes, since each PR is a product of a separate gene (35). Our experiments showed that cultivars Florunner and PI 341885 grown under normal irrigation either did not possess these genes, or their de novo expression was not induced by these aspergilli. Similar, but not always identical, formation patterns of novel polypeptides induced by *A. flavus* or *A. parasiticus* possibly could be explained by the close taxonomic relatedness of these species. Four of the five drought-stressed peanut cultivars produced kernels whose cotyledonary tissue did not produce any novel polypeptides upon infection. In full sunlight, plant tissues can experience heat-shock temperatures, especially under drought-stress conditions. Besides temporary production of heat-shock proteins, the synthesis of several major proteins could have been inhibited (23). Either drought stress, or possibly heat shock, inhibited potential de novo expression of the polypeptides. Mechanisms associated with this phenomenon are unknown. Heat shock can turn off transcription of some genes (25); thus normal accumulation of PR-mRNA in tissue frequently heat-shocked could not occur. In the case of cultivar TX 798736, the transcription and translation appeared to function, since drought-stressed kernels of this cultivar produced five novel polypeptides upon inoculation with either fungus. Drought stress- or heat shock-activated transcription of DNA coding for PR-mRNA, or even translation of already accumulated PR-mRNA, could allow for expression of an additional polypeptide found in cultivar TX 798736. Kernels from drought-stressed plants are generally more readily colonized by aspergilli compared to those from plants grown under normal irrigation (36). The exception is TX 798736, whose kernels from drought-stressed plants were colonized at significantly lower frequencies than those from normal irrigation (5; Pettit et al, *unpublished*). It is unknown to date whether the novel components in kernels from drought-stressed cultivar TX 798736 could limit multiplication of aspergilli and result in higher resistance.

Further research should involve: 1) utilization of Western blotting, 2) construction of a cDNA library generated from RNA isolated from aspergilli-infected viable cotyledons of peanut genotypes, which encode the major forms of novel polypeptides, and 3) identification of the pathogenesis-related genes with labeled probes developed from mRNAs that are isolated from infected and water-checked cotyledons.

Known peanut genotypes fail to exhibit satisfactory resistance to aspergilli and to many other pathogens. However, there exists a potential role for using patterns of novel polypeptides to establish models for triggering the host response in peanuts using the interaction of a pathogen with host molecular receptors encoded by pathogenesis-related genes.

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