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CHARACTERIZATION OF ISOPEROXIDASE ACTIVITY IN *ARACHIS HYPOGAEA* CULTIVARS*

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Abstract—Isoperoxidases (both anodic and cathodic) from individual seeds of several peanut cultivars (*Arachis hypogaea*), after ammonium sulfate precipitation of the major storage protein, arachin, from the extracts were characterized by polyacrylamide gel electrophoresis with respect to seed development, maturity and germination and the geographic areas where grown. All cultivars had a major cathodic isozyme near the origin of the gels. Much anodic intra- and inter-varietal isozyme polymorphism was observed in mature seeds collected from different geographic areas. These polymorphic isozymes were consistently present during the earlier stages of seed development (the activities decreased quantitatively and became variable during the later stages of maturation), and most were observed in each peanut upon germination, the latter showing quantitative increases in activity.

INTRODUCTION

THE ABILITY to separate electrophoretically and to detect histochemically the multiple forms of peroxidase from crude extracts of plant materials has proved to be a useful biochemical tool in studies dealing with differentiation and development,[1-4] disease resistance and susceptibility,[5] cellular injury,[6] environmental conditions,[7] protein-hormone interactions,[8] heating and blanching effectiveness,[9] and chemotaxonomy.[10,11]

Proteins and a number of enzymes from crude extracts of immature, mature and germinating peanuts grown in different geographical locations were characterized by gel electrophoretic techniques.[12-14] A number of cultivars from the different peanut types, *Arachis hypogaea* L. subsp. *fastigiata* var. *vulgaris* (Spanish botanical type) and *A. hypogaea* L.

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subsp. *hypogaea* var. *hypogaea* (Virginia botanical and Virginia and runner market types) were included in these studies. These data showed that the gel patterns of proteins and enzymes including leucine aminopeptidase, acid phosphatase and esterase from mature peanuts contained molecular polymorphism not typical of immature or germinating seeds, and catalase, alcohol dehydrogenase and oxidase revealed no variability in these same studies. Furthermore, the observed polymorphism was consistent with the different cultivars examined.

Accordingly, the goal of this investigation was to examine the isoperoxidase patterns of individual immature, mature (dormant) and germinating peanuts from a number of commercial cultivars grown in different geographical locations.

RESULTS AND DISCUSSION

In an earlier examination of Virginia 56R peanuts for isoperoxidase activity, the absence of this enzyme in dormant seeds was reported.[15] Our initial experiments on crude extracts of the total proteins from mature peanuts confirmed this observation. Attempts were made to clarify the electrophoretic patterns by removing part of the storage protein, arachin, which comprises about 30–40% of the total protein in the peanut, by precipitation at 40% ammonium sulfate saturation. This resulted in clear and repeatable isozyme patterns (Fig. 1). Addition of arachin back to partially purified peroxidase fractions again suppressed most of this isozyme activity. This suggested that arachin, or something that precipitates with it, was masking or interfering with the measurement of peroxidase in peanuts.

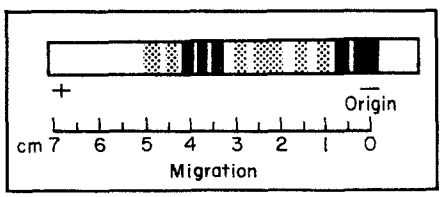

FIG. 1. DRAWING OF TYPICAL POLYACRYLAMIDE GEL ELECTROPHORETIC PATTERNS OF MAJOR AND MINOR ANODIC ISOPEROXIDASE ACTIVITY IN PEANUTS.

Examination of a large number of seeds collected from individual plants of Virginia 56R peanuts grown in Louisiana showed that much anodic intra-varietal peroxidase isozyme polymorphism existed. Zymograms containing one major (dark staining) band at the origin, one in region 0·7 cm and three in region 3·0–4·0 cm, and distinguished by the presence or absence of a minor (light staining) band in region 1·0 cm was typical of most of these patterns (58 of 84 seeds). The presence of one major band in region 3·0–4·0 cm (6), the absence of isozymes at the origin and/or in region 0·7 cm (5) and the presence or absence of minor bands in regions 1·0, 1·5 and 5·0 cm (15) distinguished the other zymograms.

Seeds of other cultivars (Spanish botanical type: Tifspan, Starr, Argentine, Spancross, Comet, Spanhoma; Virginia botanical types: (a) runner market: Early Runner, Florunner, Virginia Bunch 67; (b) Virginia market: Virginia 56R, Virginia 61R, Florigiant, NC 17, NC 5, NC 2) examined from the different geographic areas (Oklahoma, Texas, Georgia, Virginia) showed the same type and frequency of polymorphism as observed with Virginia 56R seeds (216 of 300 seeds), plus some additional patterns (84). The absence of major band

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activity in regions 0·7 and 3·4 cm (2; Virginia-grown Florigiant), the presence of a major band in region 2·9 cm (1: Virginia-grown Virginia 61R) and variation in minor bands in regions 1·0–2·7 cm and 4·0–5·0 cm (81; Virginia-grown Early Runner, Florunner, Virginia Bunch 67, NC 5; Georgia-grown Argentine, Spancross; Oklahoma-grown Spanhoma) distinguished this latter group of zymograms from those typical of Louisiana-grown Virginia 56R.

When the electrical poles were reversed for electrophoresis, only one major isozyme moved toward the cathode and remained near the origin, regardless of the seeds' ontogeny or the cultivar examined.

Most of the major and minor isozyme bands which varied in mature seeds were consistently present during the early stages of peanut development and after 24 hr germination (Georgia-grown Florigiant seeds were used in these studies[14]). For example, minor bands showing polymorphism in regions 1·0–2·7 cm and 4·0–5·0 cm of mature seeds were consistently present during the early and intermediate stages of peanut development and germination. These observations suggest that conditions in the mature peanut may be conducive to the synthesis and storage of isoperoxidases usually formed during earlier stages of development and in some cases may vary between seeds of cultivars grown in different geographical locations. Perhaps, at maturity all seeds in a sample(s) may not have reached the same level of development and subsequent isoperoxidase synthesis. In contrast, similar studies using other selected enzymes showed that their variable patterns from mature peanuts could be distinguished from those of immature seeds.[14]

Accordingly, these data stress the importance of examining a number of different protein and enzyme systems in many individual seeds at various ontogenetic stages for comparative gel electrophoretic studies.

EXPERIMENTAL

The materials and methods used have been presented by Cherry *et al.*,[12] and Cherry and Ory.[14] The benzidine stain used to detect isozyme bands having isoperoxidase activity was described by Scandalios.[2]