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Changes in nucleic acids, protein and ribonuclease activity during maturation of peanut seeds [1]

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Changes in weight, protein, RNA, DNA and ribonuclease activity during maturation of the peanut seed have been studied. The investigation period began with fruits harvested four weeks after the pegs turned at right angles in the soil and continued through the fourteenth week. Fresh weights of both cotyledons and embryonic axes increased rapidly during early maturation (8 weeks) and continued in the cotyledons through 12 weeks. The water content at maturity was still adequate for enzymatic processes at 45% and 39%, respectively, for embryonic axes and cotyledons. The levels of DNA and RNA also increased rapidly during early maturation of both cotyledons and embryonic axes. DNA content decreased during the remainder of maturation while RNA decreased from 8 to 11 weeks in the cotyledons and then increased during the after-ripening period to a value near its previous maximum at 8 weeks. RNA levels in the embryonic axes underwent a gradual increase throughout maturation. The large decrease in cotyledonary RNA during weeks 8 through 11 could be related to increased RNase activity during the period.

Peanut seeds have been subjected to innumerable studies during germination. However, there are only a few reports dealing with the maturative events which prepare peanuts for this heterotrophic period of growth. Morphological changes and embryonic development in the maturing peanut seed have been described by Smith (*1–3*), and Brennan (*4*), but there are only a few studies of the biochemical basis for these changes (*5–9*).

The biochemical aspects of seed maturation have been investigated previously in several plant species including grasses (*10–13*), peas (*14*), beans (*15–18*), and oil plants (*19–21*). The study of peanut seed maturation offers a contrast to some aerially borne seeds (*22*) since the former develop within subterranean fruits and dehydration does not become critical until after the mature fruits are harvested. We report here on some of the changes which occur during peanut seed development.

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Materials and methods

Peanuts (*Arachis hypogaea* L. var NC-2) were grown in field plots and maturative stages of the fruits determined by a sampling method based on the order of reproductive and vegetative branching of the plant (*23*). With this technique, fruits were picked weekly from the second internode of the first lateral reproductive branch. The sampling period was from the 4th week after the pegs had turned at right angles in the soil through the 14th week. Although peanut seeds are considered mature at about the 12th week (*9*), later samples were taken to ascertain any initial after-ripening effects.

Harvested fruits were transported to the laboratory in ice buckets before dissecting embryonic axes from the cotyledons. Three to five replications were conducted on lots of 25 axes and 25 cotyledon pairs at each sampling period. Cotyledons from weeks 4 and 5 and all axes were ground in a chilled mortar and pestle; cotyledons from later stages were homogenized for 3–5 min at full speed in an ice-jacketed Sorvall Omnimixer[3].

Fresh weights were determined immediately after separation of the embryonic axes from the cotyledons while dry weights were obtained after desiccating the plant material at 137°C for 24 hr. The difference, water content, was expressed as the percentage of fresh weight.

Nucleic acid content was determined by the method of Smilie and Krotkov (*24*) as modified by Nitsan and Lang (*25*). Homogenization of the cotyledon pairs and embryonic axes was carried out in 0.5 M NaCl containing 0.1 M EDTA, pH 8.0; the homogenates were filtered through cheesecloth and glass wool. After extraction, ribonucleic acid (RNA) was estimated from supernatants as phosphorus (*26*) and deoxyribonucleic acid (DNA) was determined by the diphenylamine reaction (*27*).

For the determination of ribonuclease activities (*28*), 25 axes or cotyledon pairs were homogenized in 0.01 M Tris containing 0.06 M KCl and 0.01 M $MgCl_2$, buffered at pH 6.7. After 30 min in ice, the homogenates were centrifuged at $12{,}000 \times g$ for 15 min in the cold before decanting the resulting supernatants through cheesecloth and glass wool. These filtrates served as crude enzyme preparations.

For protein determinations, tissues were homogenized in 0.01 M Tris buffered at pH 6.7. After filtering the resulting brei through cheesecloth lined with glass wool, the homogenates were centrifuged at $3{,}000 \times g$ for 10 min. Lipids, which largely collected as a semi-solid layer at the top of the centrifuge tubes, were skimmed off with a spatula. In order to remove further lipid material, supernatants were refiltered through cheesecloth and glass wool. Aliquots of 1.0 ml were removed and precipitated with equal volumes of 10% trichloroacetic acid (TCA). After collection by centrifugation, the pellets were washed twice with 10% TCA followed by resuspension in 0.1 N NaOH. Duplicate aliquotes were then assayed for protein (*29*).

The protein synthesizing capacities of the seed parts were estimated by following the incorporation of ^{14}C-leucine. Axes and cotyledon pairs in lots of 25 were surface-sterilized with Chlorox (1 : 4), washed several times with distilled water

[3] Mention of specific trade names is made for identification only and does not imply endorsement by the U. S. Government over similar products.

and incubated in 1.0 mM citric acid, pH 3.5 containing 10 μCi of uniformly labeled ^{14}C-L-leucine (Volk-180 mCi/mmole) for 3 hr at room temperature. Cotyledons, at and after 7 weeks of age, were sliced into 1–3 mm thick pieces before surface sterilization in order to facilitate isotope penetration. After incubation, TCA extracts were obtained according to procedure described by Marcus et al. (*30*).

Results and discussion

The chemical events described in this report began with seeds harvested 4 weeks after the pegs turned at right angles in the soil. At this time the endocarp was still fleshy, the liquid endosperm had largely been absorbed, and the seed consisted mainly of small succulent cotyledons.

Fresh weights of both cotyledons (Fig. 1) and embryonic axes (Fig. 2) increased rapidly during early development; in the cotyledons, this increase was sustained until the 12th week and was due in large part to corresponding increments in dry weight (Fig. 1). In the axes, the major increase occurred by week 6 (Fig. 2).

Water content (Fig. 3) dropped from 87 to 45% and from 80 to 39% in the axes and cotyledons, respectively. Even at maturity, the cotyledons and axes were hardly desiccated, and indeed enzymatic processes continue during curing of harvested peanuts until the moisture percentage becomes critical (*31*). Dehydration apparently does not become significant until after curing is initiated. In this respect,

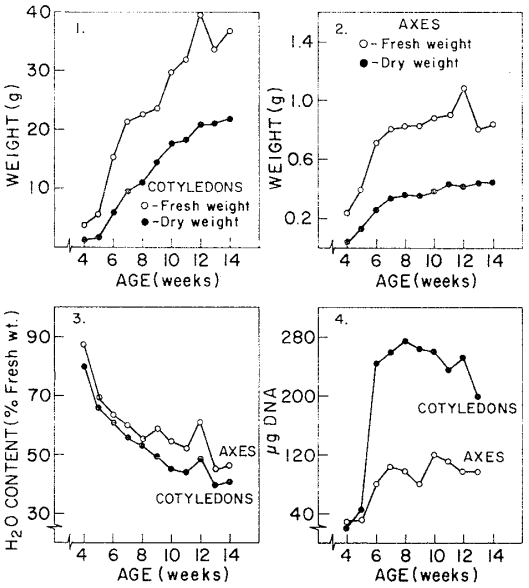

Fig. 1. *Changes in fresh and dry weight of 25 cotyledon pairs.* (○-○) fresh weight; (●-●) dry weight.

Fig. 2. *Changes in fresh and dry weights of 25 embryonic axes.* (○-○) fresh weight; (●-●) dry weight.

Fig. 3. *Changes in the water content (percent fresh weight) of axes* (○-○) *and cotyledons* (●-●).

Fig. 4. *DNA content changes per cotyledon pair* (○-○) *and per axis* (●-●).

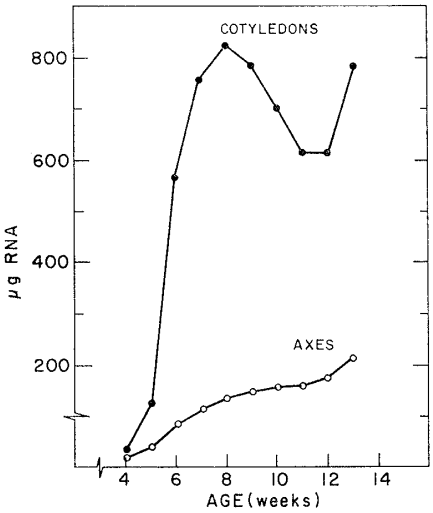

Fig. 5. *Changes in RNA content per cotyledon pair* (●-●) *and per axis* (○-○).

peanuts differ from maize (*13*), sunflower, dill, poppy (*22*), and castor bean (*19*) which become quite desiccated during the final stages of maturation.

Fig. 4 shows the amount of DNA in maturing peanut cotyledons and axes. Since the amount of DNA per cell within a given tissue is nearly constant (*32*), increases in DNA content can serve as a approximation of cell number. During weeks 4 through 7, copious amounts of DNA were synthesized in the cotyledons, with DNA levels remaining relatively constant thereafter. Thus most cells in the cotyledonary tissue are apparently formed by the end of the sixth week and the subsequent growth (Fig. 1) is primarily through cell enlargement.

Changes in DNA content in the axes (Fig. 4) were similar to those in the cotyledons, achieving a maximum by 7 weeks. Since increases in fresh weight had ceased by this time (Fig. 2), it appears that the embryonic axes grow mainly by cell division with cell expansion remaining nominal until germination. Graham et al. (*10*)

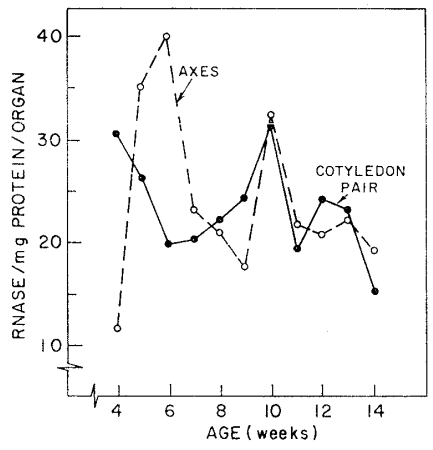

Fig. 6. *Changes in ribonuclease activity in the cotyledons* (●-●) *and embryonic axes* (○-○).

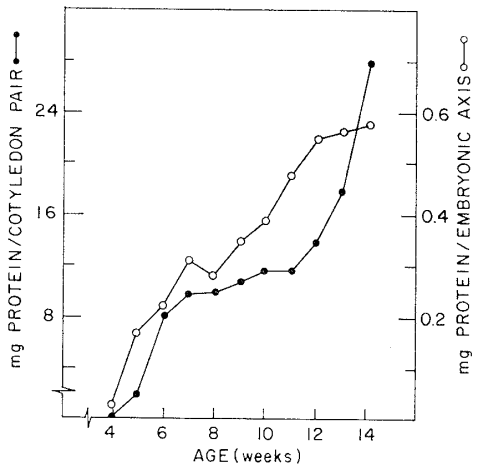

Fig. 7. *Changes in protein content of cotyledons* (●-●) *and embryonic axes* (○-○).

working with wheat embryos, Ingle et al. (*13*) with corn, and Wheeler and Boulter (*17*) using *Vicia faba* all reported basically the same findings for DNA content.

The RNA content of cotyledonary tissues increased rapidly from weeks 4 through 8 (Fig. 5), the period of highest cell proliferation (Fig. 1 and 4). During weeks 9 through 11, RNA levels in the cotyledons declined by 32%; however, during the terminal days of maturation, RNA content again increased and became nearly equivalent to the maximum observed at 8 weeks. Similar RNA profile changes have been obtained for peanut seeds maturing during a subsequent growing season (*33*).

The large decrease in cotyledonary RNA content during weeks 9 through 11 seems to be correlated with ribonuclease (RNase) activity (Fig. 6) in that the RNase activity profile increased during the period of diminishing RNA content. However, RNase in the axes (Fig. 6) followed a pattern similar to cotyledonary RNase changes even though RNA content continued to increase (Fig. 5). Thus, the apparent relationship between RNA levels and RNase activities in the cotyledons may simply be fortuitous. This period of cotyledon development is distinctive in that total volatiles (including methanol, ethanol, acetaldehyde, pentane and hexanal) as well as alcohol dehydrogenase and lipoxidase activities (*9*) display profile changes similar to those shown here for RNA. However, the significance of these observations remains unclear.

Data summarizing the weekly changes that occurred in protein content of maturing peanut embryos are shown in Fig. 7. Both cotyledonary and axial tissue demonstrated an increasing amount of protein over the entire period of maturation.

The protein synthesizing capacity of maturing seed was estimated by following the course of ^{14}C-leucine incorporation into TCA soluble and insoluble fractions. As indicated in Fig. 8, efficiency of incorporation (*30*) in the cotyledons was elevated initially but rapidly declined despite the accumulation of large amounts of protein (Fig. 7). In contrast, efficiency in the axes, initially equivalent to the cotyledons, increased until the 12th week before declining during weeks 13 and 14 (Fig. 8).

The failure of the cotyledons to exhibit amino acid incorporation might have

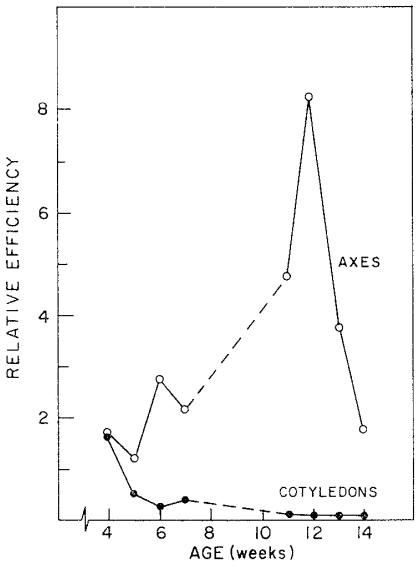

Fig. 8. *Relative efficiencies of amino acid incorporation into acid insoluble precipitates in cotyledons* (●-●) *and embryonic axes* (○-○).

been due to a lack of isotope penetration into the cotyledonary protein bodies demonstrated by Dieckert et al. (*34*). If so, the "cytoplasmic" system in the cotyledons apparently synthesized only nominal amounts of protein during late maturation or else the protein formed was much lower in leucine content than that in the axes. A third possibility exists. The observations of high relative efficiency of amino acid incorporation in the axes when protein was not rapidly accumulating in the tissue suggest that some of the biosynthetic activity in the axes during late maturation may possibly be directed toward the production of protein for transport to, and storage in, the cotyledons.

References

(*1*) Smith, B. W.: *Arachis hypogaea.* Aerial flower and subterranean fruit. *Amer. J. Bot.* 37: 808–815 (1950).

(*2*) Smith, B. W.: *Arachis hypogaea.* Normal sporogenesis and syngamy with occasional single fertilization. *Amer. J. Bot.* 43: 81–89 (1956).

(*3*) Smith, B. W.: *Arachis hypogaea.* Embryogeny and the effect of peg elongation upon embryo and endosperm growth. *Amer. J. Bot.* 43: 233–240 (1956).

(*4*) Brennan, J. R.: The peanut gynophore. *Biologist* 51: 71–82 (1969).

(*5*) Pickett, T. A.: Composition of developing peanut seed. *Plant Physiol.* 25: 210–224 (1950).

(*6*) Schenk, R. U.: Development of the peanut fruit. *Georgia Agricultural Experiment Station Technical Bulletin N. S.* 22, 53 pp. (1960).

(*7*) Schenk, R. U.: Respiration of the developing peanut fruit. *Crop Sci.* 1: 103–106 (1961).

(*8*) Pattee, H. E., A. E. Purcell, and E. B. Johns: Changes in carotenoid and oil content during maturation of peanut seeds. *J. Am. Oil Chem. Soc.* 46: 629–631 (1969).

(*9*) Pattee, H. E., J. A. Singleton, E. B. Johns and B. C. Mullin: Changes in the volatile profile of peanuts and their relationship to enzyme activity levels during maturation. *J. Agr. Food. Chem.* 18: 353–356 (1970).

(*10*) Graham, J., A. C. Jennings, R. K. Morton, B. A. Polk, and J. K. Raison: Protein bodies and protein synthesis in developing wheat endosperm. *Nature* 196: 967–969 (1962).
(*11*) Jennings, A. C. and R. K. Morton: Changes in carbohydrates, protein and non-protein nitrogenous compounds of developing wheat grain. *Aust. J. Biol. Sci.* 16: 318–331 (1963).
(*12*) Jennings, A. C., and R. K. Morton: Changes in nucleic acids and other phosphorus-containing compounds of developing wheat grain. *ibid.* 16: 332–341 (1963).
(*13*) Ingle, J., D. Beitz, and R. H. Hageman: Changes in composition during development and maturation of maize seeds. *Plant Physiol.* 40: 835–839 (1965).
(*14*) Flinn, A. M. and J. S. Pate: Biochemical and physiological changes during maturation of the fruit of the field pea (*Pisum arvense* L.). *Ann. Bot.* 32: 479–495 (1968).
(*15*) Gresiuk, S., T. Mierzwinska, and E. Sojka: On the physiology and biochemistry of seed development in broad bean. *Fiziol. Rast.* 9: 544–552 (1962).
(*16*) Kloz, J., V. Turkova, and E. Kozova: Proteins formed during maturation and germination of seeds of *Phaseolus vielgari*. *Biol. Plant.* 8: 164–173 (1966).
(*17*) Wheeler, C. T. and D. Boulter: Nucleic acids of developing seeds of *Vicia faba* (L.). *J. Exp. Bot.* 18: 229–240 (1967).
(*18*) Briarty, L. G., D. A. Coult, and D. Boulter: Protein bodies of developing seeds of *Vicia faba*. *ibid.* 20: 358–372 (1969).
(*19*) Marre, E.: Ribosome and enzyme changes during maturation and germination of castor bean seed, pp. 76–104. *In* A. A. Moscona, and A. Monroy (ed.), *Current Topics in Developmental Biology*, Vol. 2. Academic Press, New York (1967).
(*20*) Zeman, Ivo and Vladimir Ksatochvil: Changes in the composition of winter rape oil during seed maturation. *Biol. Plant.* 9: 11–14 (1967).
(*21*) Galitz, D. S. and R. W. Howell: Changes in individual nucleotides of developing soybean seeds. *Physiol. Plant.* 21: 1296–1300 (1968).
(*22*) Prokof'ev, A. A. and V. P. Kholodova: Features of the changes in water content in ripening seeds. *Fiziol. Rast.* 15: 859–867 (1968).
(*23*) Gupton, C. U., D. A. Emery, and J. A. Benson: Reproduction efficiency of Virginia-type peanuts. III. Relating the time of peg placement to the branching patterns of the plant. *Oleagineux.* 23: 247–250 (1968).
(*24*) Smilie, R. M., and G. Krotkov: The estimation of nucleic acids in some algae and higher plants. *Can. J. Bot.* 38: 31–49 (1960).
(*25*) Nitsan, J. and A. Lang: DNA synthesis in the elongating non-dividing cells of the lentil epicotyl and its promotion by gibberellin. *Plant Physiol.* 41: 965–970 (1966).
(*26*) King, E. J.: The colorimetric determination of phosphorus. *Biochem. J.* 26: 292–297 (1932).
(*27*) Burton, K.: A study of conditions and mechanisms of diphenylamine reaction for colorimetric estimation of DNA. *ibid.* 62: 315–323 (1956).
(*28*) Wilson, C. M.: Chromatographic separation of ribonucleases in corn. *Biochim. Biophys. Acta* 68: 117–184 (1963).
(*29*) Lowry, O. H., N. Rosebrough, A. L. Farr, and R. J. Randall: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275 (1951).
(*30*) Marcus, A., J. Feeley, and T. Volcani: Protein synthesis in imbibed seeds. III. Kinetics of amino acid incorporation, ribosome activation and polysome formation. *Plant Physiol.* 41: 1167–1172 (1966).
(*31*) Mohapatra, S. C.: Effect of curing time and temperature on the metabolism of lipids and ethanol soluble compounds in peanuts. Ph. D. thesis, North Carolina State University (1970).
(*32*) Mazia, D.: Physiology of the cell nucleus, pp. 77–122. *In* E. G. Barron (ed.), *Modern Trends in Physiology and Biochemistry*. Academic Press, New York (1952).
(*33*) Rudd, T. P. and R. C. Fites: Association of thymidine and uridine kinase activities with changes in nucleic acid levels during peanut fruit ontogeny. *Phytochem.* 11: 1631–1636 (1972).
(*34*) Dieckert, J. W., J. E. Snowden, Jr., A. T. Moore, D. C. Heinzelman, and A. M. Altschul: Composition of some subcellular fractions from seeds of *Arachis hypogaea*. *J. Food Sci.* 27: 321–325 (1962).