COMPARISON OF NON-CELLULAR ENDOSPERM STARCH FROM SEVERAL GENOTYPES OF PEANUTS (*ARACHIS HYPOGAEA* L.) USING BIREFRINGENCE END-POINT TEMPERATURES[1]

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Initial and final birefringence end-point temperatures (BEPT) were determined for the starch granules of the non-cellular endosperm from ovules at three stages of development of eight genetically diverse peanut cultivars. The results indicated that cultivars P-326 (P.I. 280688) and P-1286 (narrowleaflet) possess starch granules that are different from those of the other genotypes. The greatest differentiation occurred at the young and intermediate ages (approximately 14 and 21 days after pollination) for both initial and final BEPTs. The differences detected are assumed to be under genetic control.

We have been investigating some of the characteristics of peanut endosperm to elucidate reasons why some interspecific crosses in *Arachis* fail, and to gain further knowledge on the nature and extent of endosperm development in cultivated peanuts. Earlier it was reported that starch granules are conspicuous constituents of peanut endosperm (1). Subsequently, experiments were initiated to determine whether or not genetically diverse peanut cultivars might possess different kinds of starch granules. Some preliminary studies indicated that the starch granules from peanut endosperm are fairly round in shape and range in size from less than 1 μ (becoming smaller than the resolution of our compound microscope) to about 8 or 9 μ.

The physical properties of starch granules have been shown to be under genetic control, as evidenced by x-ray diffraction and birefringence end-point temperature measurements (2, 3, 4). Several methods have been described for ascertaining BEPTs (4-7). All of these, however, rely on the same principle; when a suspension of starch granules is heated, the granules swell, burst, and lose their anisotropy or birefringence (called the gelatinization point by some authors). This phenomenon is easily detected by examining the starch granules microscopically under polarized light as they are heated and by observing the disappearance of the "Nicol's cross" (3).

MATERIALS AND METHODS

Eight genotypes of peanuts, chosen because of their distinctive plant, fruit, and seed phenotypes, were used in this study (Table 1). The plants were grown in single row plots at the Agronomy Research Station, Perkins, Oklahoma during the summer of 1971. Seeds were planted in early June and the resulting plants were cared for by conventional agronomic methods throughout the growing season. Pods, containing the ovules (immature seeds), were harvested during a two-day period in late September. Several pods of varying maturities from two plants for each genotype were collected, taken to the laboratory and washed in tap water. The basal ovules were removed from the pods and grouped by size into three classes, (a) young (0.3 x 0.15 cm), (b) intermediate (0.6 x 0.3 cm), and (c) old (0.8 x 0.4 cm). These sizes correspond fairly closely, according to other studies, to 14-, 21-, and 28-day old ovules (after pollination). Endosperm starch from each ovule was obtained by cutting the fresh ovule in half with a razor blade on a clean microscope slide

[1] Based on cooperative investigations of the Plant Science Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma. Approved as Manuscript No. J-2426, Oklahoma Agricultural Experiment Station. This paper is based on portions of a thesis submitted by the senior author in partial fulfillment of the requirements for the Master of Science degree, Oklahoma State University, Stillwater, Oklahoma, May, 1972.

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and allowing the liquid endosperm to flow onto the surface. Approximate amounts of endosperm used were 2, 6, and 10 μl for the young, intermediate, and old ovules, respectively. The starch granules were not isolated from the endosperm but were used in their natural fluids (except for the salt treatment below). A salt treatment was applied to one group of the old ovules by adding 1 drop (approximately 30 μl) of 1 M $Ca(NO_3)_2$ to the endosperm on the slide. This salt treatment was used because Pfahler *et al* (4) had shown that the starches from some maize genotypes could be further differentiated by its use. Our procedures for ascertaining BEPTs were similar to those described by Schoch and Maywald (7). The endosperm on the slide was ringed with high viscosity mineral oil and a cover glass placed on it; the starch suspension was completely surrounded by the oil without the presence of air bubbles. Except in the case of the salt treatment where observations were delayed for five minutes, the slide was immediately placed on a microscope equipped with a Kofler hot stage and polarizing filters. The rate of temperature increase of the hot stage was about 2 C per minute (transformer set at 23 volts). The observations were made at 312 magnifications and each field contained several hundred starch granules. The temperature was recorded when the first three or four granules lost their birefringence (initial BEPT). The final BEPT was recorded when all but two or three granules showed this character. Two samples, each consisting of one ovule, were examined for two plants of each genotype for each age and treatment (salt *vs.* no salt). Duncan's Multiple Range Test (8) was employed to determine whether or not the differences obtained in the BEPTs were statistically significant.

RESULTS AND DISCUSSION

Statistical analyses of the results are given in Tables 2 and 3. Table 2 shows the initial BEPT of the starches from the various peanut genotypes by age and treatment. At the young age, P-161 had the lowest BEPT and P-1286 had the highest. Note that the BEPT of all genotypes was lower for the intermediate age than for the young age, except for P-161 and P-1286 where the reverse was true, or for P-935 where essentially no change occurred. At the old age, P-112 alone showed an increase in BEPT, whereas the other genotypes either decreased or remained essentially the same. P-936 showed a decrease in BEPT as the endosperm matured, but P-935 showed about the same BEPT at all ages.

The final BEPT data are presented in Table 3 and Figure 1. Genotypes P-326, P-112, P-935, and P-2395 showed a decrease in BEPT from the young to the intermediate age. The other genotypes showed increases in BEPT at these ages. P-326 and P-1286 showed conspicuously higher final BEPTs for all ages (without salt) than

TABLE 1. *Phenotypic descriptions of eight peanut genotypes.*

Genotype[a]	Name and/or P. I. No.	Botanical[b] type	Seed coat color	Other characteristics
P-112	Spanhoma	S	Flesh	Typical Spanish
P-161	Tenn. Red	S (Val)	Red	Typical Valencia
P-326	Guanajuato-2 P.I. 280688	V	Purple	Purple pigmentation in stem, leaf, and flower
P-935	Mani Pintar II P.I. 268837	V	Red and white mottled	Typical Bunch Virginia
P-936	P.I. 262129	S (Val)	Flesh with purple streak	Large leaves, thick stems with glandular hairs on stipules
P-1284	Aureus	S	Flesh	Golden color leaflets
P-1286	Narrowleaflet	S	Flesh	Dwarf plant with very narrow leaflets
P-2395	Nambyquare	V	Purple and white mottled	Large seed, prostrate habit

[a]P-numbers assigned by the Oklahoma Agricultural Experiment Station.
[b]S = Spanish; Val = Valencia; V = Virginia.

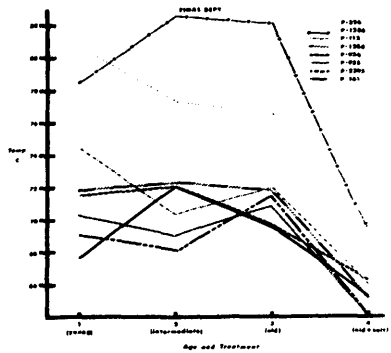

FIGURE 1. Final birefringence end-point temperature (BEPT) of the starch granules from eight peanut genotypes by age and treatment.

did the other genotypes. The latter had final BEPTs below 75 C. Noteworthy is the contrast between the BEPTs of P-161 and P-112 as their endosperms of different ages were tested. The former showed an increasing then decreasing BEPT with age, whereas in the latter the reverse trend was apparent. P-935 and P-2395 reacted similarly to P-112 but at slightly lower temperatures.

As illustrated above, many comparisons are possible with these data. Our major conclusions are as follows:

1. The starch granules of P-326 and P-1286, although not distinguishable (except in the case of the initial BEPT for the intermediate ages), are obviously different from those of several of the other genotypes with respect to both their initial

TABLE 2. *Mean initial birefringence end-point temperatures (BEPT) of peanut endosperm starch from eight genotypes at three ages with salt treatment at one age.*

	Initial BEPT[a]			
	Endosperm age[b] and treatment			
Genotype	Young	Intermediate	Old	Old, salt treated[c]
P-1286	68.5 p	69.2 p	64.1 rst	58.5 xyz
P-326	67.6 pq	64.6 qrs	61.5 rstuvwx	58.4 xyz
P-112	64.8 qr	62.6 rstuv	63.2 rstu	57.9 yz
P-2395	62.0 rstuvw	60.9 tuvwxyz	60.9 tuvwxyz	57.6 z
P-1284	61.4 stuvwx	60.1 uvwxyz	59.8 vwxyz	57.8 z
P-936	61.2 tuvwxy	59.4 vwxyz	58.8 wxyz	59.2 vwxyz
P-935	60.8 tuvwxyz	60.6 uvwxyz	60.1 uvwxyz	57.5 z
P-161	60.4 uvwxyz	62.5 rstuv	59.9 uvwxyz	57.6 z

[a] In centigrade. Means not followed by a common letter (p,q,r,s,t,u,v,w,x,y,z) are significantly different at the 5% level according to the Duncan Multiple Range Test.
[b] Young, intermediate, and old = approximately 14, 21, and 28 days after pollination, respectively.
[c] 1 M $Ca(NO_3)_2$ applied to the endosperm 5 min before microscopic examination.

TABLE 3. *Mean final birefringence end-point temperatures (BEPT) of peanut endosperm starch from eight genotypes at three ages with salt treatment at one age.*

	Final BEPT[a]			
	Endosperm age[b] and treatment			
Genotype	Young	Intermediate	Old	Old, salt treated[c]
P-326	80.8 pq	77.4 pqrs	76.5 pqrst	64.9 vw
P-1286	78.5 pqr	82.6 p	82.1 p	69.5 tuvw
P-112	74.5 qrstu	70.4 stuvw	72.0 rstuv	66.0 vw
P-1284	71.9 rstuv	72.2 rstuv	71.9 rstuv	65.0 vw
P-936	71.5 rstuv	72.0 rstuv	69.6 tuvw	66.6 vw
P-935	70.4 stuvw	69.0 uvw	70.9 stuvw	64.0 w
P-2395	69.1 tuvw	68.1 uvw	71.8 rstuv	64.0 w
P-161	67.6 uvw	72.0 rstuv	69.5 tuvw	65.4 vw

[a] In centigrade. Means not followed by a common letter (p,q,r,s,t,u,v,w,x,y,z) are significantly different at the 5% level according to the Duncan Multiple Range Test.
[b] Young, intermediate, and old = approximately 14, 21, and 28 days after pollination, respectively.
[c] 1 M $Ca(NO_3)_2$ applied to the endosperm 5 min before microscopic examination.

and final BEPTs at the young and intermediate ages. It is interesting that the phenotypes of P-326 and P-1286 are distinctly different from each other and from the other genotypes we studied (Table 1).

2. The greatest differentiation of genotypes by initial and final BEPTs occurred at the young and intermediate ages.

3. The salt treatment depressed both initial and final BEPTs of all genotypes except in the case of P-936, where little effect was noted in the initial BEPT.

We should point out that whereas Brown *et al* (3) noted the greatest differentiation of maize genotypes by starch granule BEPTs at the older ages (24 days after pollination), we found more differences in peanuts at the earlier ages. These variances may be due to differences in the developmental stages and physiological changes that take place in the starch granules of these two diverse taxa. Starch in maize endosperm is accumulated as a food reserve to be used later by the germinating and developing seedling, whereas in peanuts, the endosperm is rapidly depleted during seed maturation and is essentially absent in the mature seed. Hence, a more logical comparison might be made between starch from maize endosperm versus starch from peanut cotyledons. We hope to study starch from peanut cotyledons in the near future.

The significance of starch granule structure as to biological behavior or adaptation in various plant species is unknown. However, it does appear that some peanut genotypes differ in regard to their starch granule structure and these differences are probably under genetic control. A study of hybrids made between some the cultivars we tested might help elucidate the genetics involved. We have already produced some of these hybrids and will study them in the future.

ACKNOWLEDGMENTS

The authors wish to thank Dr. G. R. Waller, Department of Biochemistry, Oklahoma State University, for use of the Kofler microscope hot stage.

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