Thermal Properties of Starch from 62 Exotic Maize (*Zea mays* L.) Lines Grown in Two Locations[1]

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ABSTRACT

The variability in thermal properties among 62 S_3 lines derived from a high-yielding exotic corn (*Zea mays*) population, Antigua 1 (PI 484990), was evaluated by differential scanning calorimetry (DSC). The S_3 lines were grown in Puerto Rico (1990–1991) and Georgia (1994). Separate single-kernel starch extractions for five kernels (five replicates) from each line grown in each location were performed, and the starch was analyzed. The DSC values reported included gelatinization onset (T_{oG}), range (R_G), enthalpy (ΔH_G), and peak height index (PHI) and retrogradation onset (T_{oR}), range (R_R), enthalpy (ΔH_R), and percent retrogradation (%R) (an indication of the stability of gelatinized starch after storing at 4°C for 7 days). Significant differences ($P < 0.05$) were found among the 62 lines of Antigua 1 for T_{oG}, R_G, and PHI and highly significant differences ($P < 0.01$) were found for ΔH_G. The starches from plants grown in Georgia (1994) had significantly ($P < 0.05$) greater T_{oG}, ΔH_G, and PHI but a significantly lower R_G than those from Puerto Rico (1990–1991). These data suggest that the starch from plants grown in Georgia (1994) might have a greater degree of crystallinity than that from Puerto Rico (1990–1991). None of the retrogradation values were significantly different among starches of the 62 lines of Antigua 1 and the starches from plants grown in the two locations.

The importance of starch from completely "natural" sources has grown dramatically in the food industry during recent years. The introduction of starches containing single-, double-, and triple-mutant combinations with properties similar to chemically modified starches has resulted in a number of patents (Wurzburg and Fergason 1984; Friedman et al 1988a–g; Friedman et al 1989a, b; Mauro et al 1991; Furscik and Mauro 1991a, b; Furscik and Deboer 1991a, b; Zallie et al 1984; Pearlstein and Ulrich 1994; White et al 1994; Hauber et al 1996).

Screening germplasm is important because the corn hybrids grown in the United States use only about 5% of the total germplasm available worldwide (Goodman 1985). This limited use of germplasm can translate into increased genetic vulnerability to insects, diseases, and stress and eventually affects agronomic performance. Tracy (1990) stressed the importance of exotic maize germplasm as an excellent source of improved quality and, possibly, improved agronomic performance in corn because "much of the maize grown outside of the United States is consumed directly by humans and has undergone centuries of selection for flavors, aromas and textures."

Differential scanning calorimetry (DSC) has been used to evaluate thermal properties of starches since Stevens and Elton (1971) first reported the DSC properties of cereal starches. A differential scanning calorimeter is easy to operate and requires only a small sample size. In addition, it is relatively rapid compared with more traditional methods of studying starch gelatinization, making it useful for screening maize germplasm (Campbell et al 1995). Several studies have used DSC to screen corn germplasm. White et al (1990) reported differences in thermal properties revealed by DSC among and within five open-pollinated corn populations. The greatest differences were observed for gelatinization onset (T_o), range (R_G), and total enthalpy (ΔH). Campbell et al (1995) reported significant differences in DSC properties among a set of exotic and domestic inbred corn lines. Starches from exotic lines generally had lower T_o, peak temperature (T_p), and ΔH. Li et al (1994) reported large variations in thermal properties among and within 35 tropical and subtropical maize populations. The means of T_o, T_p, peak conclusion (T_c), and ΔH were 66.3°C (64.3–69.6°C range), 71.0°C (70.1–73.9°C range), and 77.8°C (76.8–79.6°C range), and 2.5 cal/g (2.0 to 2.9 cal/g range), respectively.

Properties of endosperm starches are affected by environmental factors during the development of plants. Fergason and Zuber (1962) reported a negative relationship between amylose synthesis and temperature in maize. Asaoka et al (1984) examined the effect of environmental temperature on properties of rice starch. A higher environmental temperature seemed to increase the amount of long B chains and decrease that of short chains of amylopectin in rice starch. Shi et al (1994) studied the effect of temperature during grain filling on starches from several wheat cultivars in growth chambers. Elevated temperatures caused low test weight and shriveled kernels, reduced starch accumulation and starch granule size, and deformed starch granules. In addition, the starch lipid levels increased markedly, amylose content increased slightly, and gelatinization temperatures of starches were greater. White et al (1991) found that starches from corn grown under tropical conditions rather than temperate conditions gave an elevated and narrow gelatinization temperature range; after retrogradation, there was an elevated onset temperature.

Our objectives were to study the thermal properties of 62 S_3 lines of an exotic corn population and to examine the effect of growing location on the thermal properties of the same 62 lines.

MATERIALS AND METHODS

Plant Material

Antigua 1 (PI 484990) is a native corn (*Zea mays* L.) accession with yellow semident grain that originated from the Caribbean Island of Antigua. Development of inbred lines proceeded as follows. The first self-pollination was done during the winter near Homestead, FL, in 1989–1990, and the second was done during the summer near Isabela, Puerto Rico, in 1990. Sixty-two lines (S_3 generation) of Antigua 1 were developed during the winter near Isabela, Puerto Rico, in 1990–1991 (planting date December 17,

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1990) and near Tifton, GA, during the summer of 1994 (planting date April 5, 1994), using the same S_2 plants grown in Puerto Rico in 1990–1991. The average monthly temperatures and precipitation of the growing seasons for S_3 development in Puerto Rico and Georgia are shown in Table I. Grain filling occurred between January and February in Puerto Rico and between June and July in Georgia. The field experiments were planted in a completely randomized design. One random ear in each row was selected for DSC analysis. Ears were dried at 38°C for 48 hr to a moisture content of ≈13%. All samples were stored at 4°C in 40% rh until analyzed.

Starch Extraction

Single-kernel starch isolations were conducted on five random kernels from each ear of each line according to the method of White et al (1990), with slight modifications (Krieger et al 1997). A single kernel was degermed by hand, and the pericarp was removed. The endosperm was put in a 50-ml centrifuge tube, and 5 ml of distilled water was added. A Tekmar tissue homogenizer (600 W, Ultra-Turrax T25, Cincinnati, OH) at 2,052 × *g* (17,000 rpm) was used to blend the endosperm and distilled water for 25 sec in the centrifuge tube. The filtering and drying processes were conducted as described by White et al (1990). After extraction, samples were dried at room temperature (22–24°C) with a fan circulating air over the starch and then stored at room temperature in air-tight vials until evaluation by DSC.

TABLE I
Average Monthly Temperatures (°C) and Total Precipitation Near Isabela, Puerto Rico (1990–1991) and Tifton, GA (1994)

Location Month	Temperature: Average Maximum	Average Minimum	Average	Precipitation (cm)
Puerto Rico[a]				
November	30.9	21.7	26.3	10.3
December	28.9	20.2	24.6	15.2
January	28.9	19.7	24.3	6.6
February	28.8	19.9	24.4	15.8
March	29.1	20.0	24.6	6.5
Georgia[b]				
April	27.2	12.3	19.8	6.7
May	28.7	15.0	21.9	4.0
June	31.6	20.4	26.0	23.5
July	31.6	20.9	26.3	19.2
August	31.4	20.1	25.8	17.4

[a] National Climatic Data Center (1990, 1991). Grain-filling occurred during January and February.
[b] National Climatic Data Center (1994). Grain-filling occurred during June and July.

Differential Scanning Calorimetry

For DSC analysis, a Perkin-Elmer DSC-7 (Norwalk, CT) equipped with a thermal analysis data station was used. Approximately 4 mg of starch dwb was weighed in an aluminum pan, and 8 µL of distilled water was added. Samples were heated from 30 to 110°C at a rate of 10°C/min. The starch gelatinization peaks and the peak caused by melting the amylose-lipid complex were completed at 110°C. Further heating provided no additional information about the starches. For all the DSC analyses, an empty pan with 8 mg of distilled water was used as a reference. The DSC parameters recorded included onset (T_{oG}), range (R_G), enthalpy (ΔH_G), and peak height index (PHI). Onset (T_{oG}), peak (T_{pG}), and enthalpy (ΔH_G) were computed directly by the DSC software. At the water level used, the DSC endotherms were essentially symmetrical, which allowed the total gelatinization range to be computed as $2(T_{pG} - T_{oG})$ according to the method of Krueger et al (1987). The PHI, which is the ratio $\Delta H_G/(T_{pG} - T_{oG})$, was calculated to allow quantitative evaluation of variations in peak shape (Krueger et al 1987). To determine retrogradation characteristics, starch samples used for gelatinization were stored for 7 days at 4°C and rescanned on the DSC from 30 to 90°C at the same heating rate as was used for the gelatinization run. By this method, we determined onset temperature (T_{oR}), range (R_R), enthalpy (ΔH_R), and starch gel retrogradation (%R), as described by White et al (1989). Further heating above 90°C provided no additional changes on the DSC thermograms. The %R is a measure of the tendency of the gelatinized starch to retrograde after storage. The lower the %R, the less tendency there is to retrograde, and the more stable is the gelatinized starch paste. All DSC values reported are the average of five scans, one from each single-kernel starch extraction. Enthalpies were calculated on a starch dwb.

Statistical Analysis

A completely randomized design was used to investigate the DSC properties of the starch among 62 lines of the population Antigua 1 and between the two locations. Analysis of variance (ANOVA) and least significant difference (LSD) analyses (α = 0.05) were computed by the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Variations Among Lines

There were significant differences ($P < 0.05$) for T_{oG}, R_G, and PHI and highly significant differences ($P < 0.01$) for ΔH_G among the 62 Antigua 1 lines. Individual values are not shown (Ng

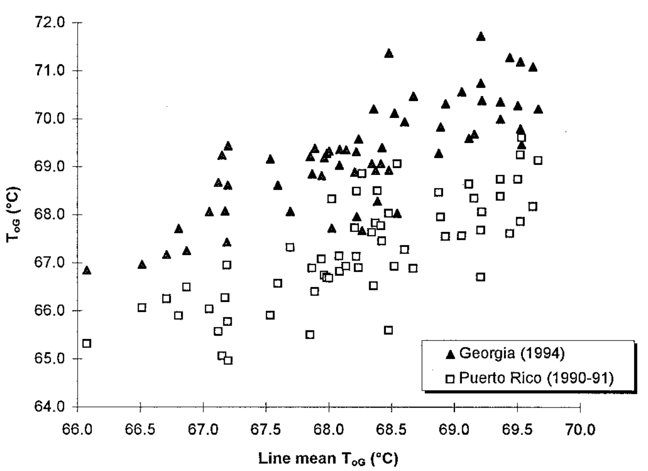

Fig. 1. Effects of growing location on gelatinization onset temperature (T_{oG}) of 62 S_3 corn lines of Antigua 1.

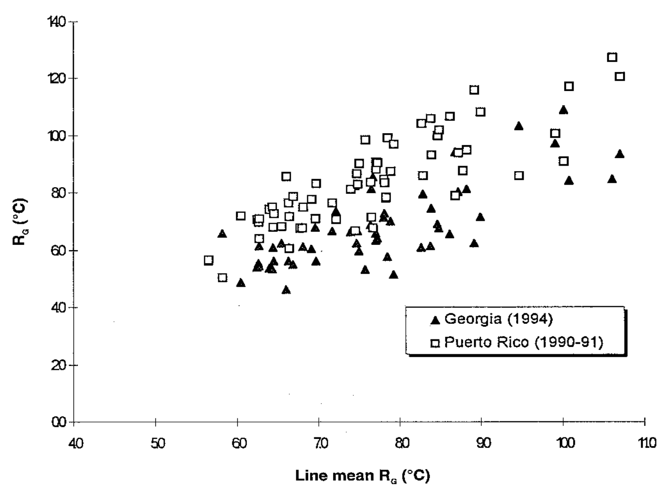

Fig. 2. Effects of growing location on gelatinization range (R_G) of 62 S_3 corn lines of Antigua 1.

1997). However, scatter plots of the values for the 62 lines of these DSC parameters are shown in Figs. 1–4. None of the retrogradation characteristics, however, were significantly different among the lines. In each generation of self-pollination, the percentage of heterozygotes decreases by 50%. These corn kernels were S_3 lines, so 87.5% of genes within a line would be expected to be fixed. The fact that the lines are fixed for different genes likely accounts for the differences in thermal properties observed in this research. Antigua 1-20 had the lowest T_{oG}, 66.1°C, whereas Antigua 1-56 had the highest T_{oG}, 69.7°C when averaged from both locations (data not shown). For R_G, Antigua 1-56 had the narrowest R_G, 5.7°C, whereas Antigua 1-20 had the widest R_G, 10.7°C. The ΔH_G ranged from 2.63 cal/g for Antigua 1-20 and 3.35 cal/g for Antigua 1-57. For PHI, Antigua 1-20 had the lowest value, 0.51, whereas Antigua 1-57 had the highest value, 1.17 (Antigua 1-56 had a similar high PHI of 1.15). The respective values of T_{oR}, R_R, ΔH_R, and $\%R$ ranged from 39.4°C (Antigua 1-27) to 43.4°C (Antigua 1-55), 19.0°C (Antigua 1-51) to 24.2°C (Antigua 1-80), 1.32 cal/g (Antigua 1-55) to 1.67 cal/g (Antigua 1-25), and 41.4% (Antigua 1-55) to 55.8% (Antigua 1-67).

Li et al (1994) evaluated the intrapopulation variability of thermal properties within 35 tropical maize populations. Significant variations were found within all populations for T_{oG}, T_p, and T_c, except for T_c in two populations (Tuson Bai III and Tuson × (Mo44[2] × Tuson Cub) 75% F_3). Thirty of thirty-five populations showed significant variation for R_G, and only six populations showed significant variation for ΔH_G. The maize population Yungueño showed the greatest variation for T_{oG} (64.1–68.2°C), R_G (11.0–16.8°C), and ΔH_G (2.1–3.8 cal/g). White et al (1990) also evaluated the variability of thermal properties within the same population with 20 plants from a Corn Belt population, Weekly. The T_{oG}, R_G, and ΔH_G ranged from 59.6 to 64.7°C, 10.5 to 19.5°C, and 1.83 to 2.83 cal/g, respectively. A comparison of these values to those found in the current study on Antigua 1 lines (66.1–69.7°C for T_{oG}, 5.7–10.7°C for R_G, and 2.63–3.45 cal/g for ΔH_G, respectively) (data not shown) showed greater variation in populations Yungueño and Weekly than in Antigua 1. In the current study, the fact that only the gelatinization parameters and none of the retrogradation parameters were different among the Antigua 1 lines suggests that the native states of the starches, such as the degree of crystallinity, were different. Krueger et al (1987) reported that T_o and ΔH increased, but the range decreased in dent corn lines that had undergone an annealing treatment (heating starch samples in water at a subgelatinization temperature). They

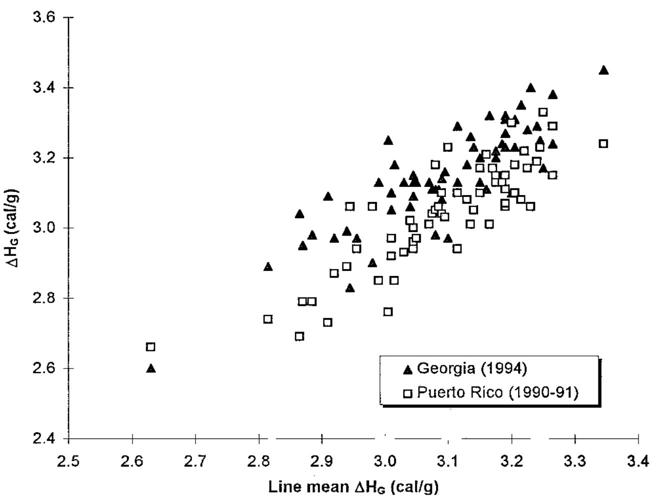

Fig. 3. Effects of growing location on gelatinization enthalpy (ΔH_G) of 62 S_3 corn lines of Antigua 1.

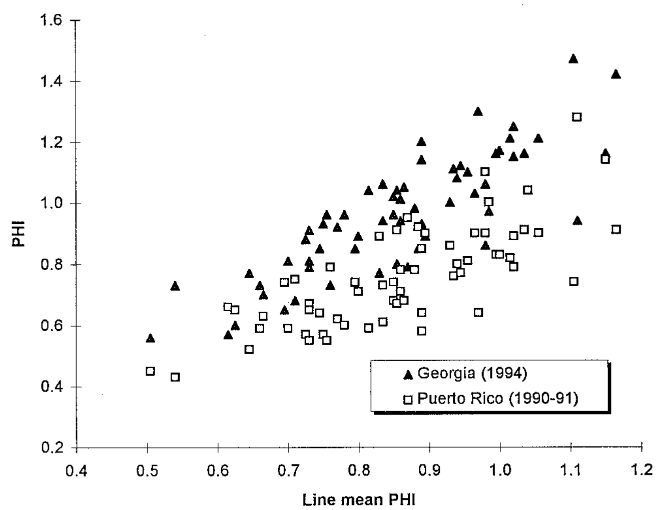

Fig. 4. Effects of growing location on peak height index (PHI) of 62 S_3 corn lines of Antigua 1.

TABLE II
Means and Ranges of Gelatinization and Retrogradation Values for 62 S_3 lines of Antigua 1 Grown at Two Locations Determined by Differential Scanning Calorimetry (DSC)

	Gelatinization				Retrogradation			
Location	T_{oG}[a]	R_G[b]	ΔH_G[c]	PHI[d]	T_{oR}[e]	R_R[f]	ΔH_R[g]	$\%R$[h]
Puerto Rico (1990–1991)								
Mean	67.2	8.4	3.04	0.76	41.1	21.3	1.49	49.1
Min	65.0	5.0	2.66	0.43	37.6	18.6	1.14	40.1
Max	69.6	12.7	3.33	1.28	44.3	26.8	1.69	56.8
Georgia (1994)								
Mean	69.2	6.9	3.15	0.96	41.4	21.4	1.52	48.5
Min	66.8	4.6	2.60	0.56	38.2	18.6	1.26	38.5
Max	71.7	10.9	3.45	1.47	44.8	24.5	1.80	58.5
$LSD_{0.05}$[i]	10.4	10.5	0.04	0.05	10.4	10.5	0.04	11.3

[a] Gelatinization onset temperature (°C).
[b] Gelatinization range (°C).
[c] Enthalpy of gelatinization (cal/g).
[d] Peak height index = $\Delta H_G/(T_{pG} - T_{oG})$, as described by Krueger et al (1987).
[e] Retrogradation onset temperature (°C).
[f] Retrogradation range (°C).
[g] Enthalpy of retrogradation (cal/g).
[h] $\%R = (\Delta H_G/\Delta H_R) \times 100$.
[i] Least significant difference ($\alpha = 0.05$).

proposed that the shifts were attributed to an increase in crystallinity of the starch molecules that occurred during annealing. Marchant and Blanshard (1978) proposed that annealing allows partial melting of some crystallites and a general realignment of starch chains in the amorphous phase, leading to an increase in hydrogen bonding. White et al (1990) attributed significant differences among five open-pollinated corn cultivars for T_o, range, and ΔH to the differences in the native alignment and hydrogen bonding of the starch molecules. Perhaps, some variations in the gelatinization characteristics among the lines in the current study were a result of different size distributions of starch granules. In a study by Knutson et al (1982), gelatinization temperature and ΔH of amylomaize increased as granule size decreased; however, these trends were not observed in normal dent corn. Knutson et al (1982) also noted that larger starch granules gelatinized over a smaller range. Possibly, the range and PHI values in the current study might be useful in predicting starch granule size.

Starches with narrow gelatinization ranges would make production more efficient by making gelatinzation more rapid (Hegenbart 1996). Antigua 1-56 and Antigua 1-5 had narrow gelatinization ranges of 5.7 and 5.8°C (data not shown), respectively. Li et al (1994) reported gelatinization ranges of 35 tropical and subtropical maize populations that varied between 9.8 and 13.0°C. The gelatinization ranges of starches from five open-pollinated corn populations studied by White et al (1990) varied between 8.7 and 16.4°C. The Antigua 1 lines may be worth further investigation for this and other functional properties that could be useful in possible industrial applications.

Variations Between Locations

The average values and ranges of the DSC parameters (T_{oG}, R_G, ΔH_G, PHI, T_{oR}, R_R, ΔH_R, and %R) of starch from corn grown at two locations are presented in Table II. The LSD ($\alpha = 0.05$) for each parameter also is listed. The differences for T_{oG}, R_G, ΔH_G, and PHI between locations were highly significant ($P < 0.01$). The effects of growing location on T_{oG}, R_G, ΔH_G, and PHI for all 62 lines are illustrated in Figs. 1–4. The T_{oG}, ΔH_G, and PHI in starches from corn grown in Puerto Rico (1990–1991) were significantly lower ($P < 0.05$) than for corn grown in Georgia (1994). The R_G, on the other hand, was significantly greater for starches from corn grown in Puerto Rico than from corn grown in Georgia. As noted for differences among the 62 Antigua 1 lines, there were differences in gelatinization properties, but not in retrogradation properties, between the starches grown in the two locations. Again, the data suggest differences in the native state of starches from corn grown in the two locations. Starches from plants grown in Georgia, which had higher T_{oG}, ΔH_G, and PHI and narrower R_G (results similar to the effect of annealing), may have a higher degree of molecular order than those from Puerto Rico, as suggested by Krueger et al (1987). Perhaps part of the variation in the gelatinization properties of starches may have been a result of partial annealing due to the higher temperatures and precipitation in Georgia than in Puerto Rico during the grain-filling period (June and July in Georgia and January and February in Puerto Rico, respectively [Table I]).

Increased temperatures during grain-filling cause a higher gelatinization temperature of starch from barley, wheat, and maize (Tester et al 1991, Shi et al 1994, Lu et al 1996). The increase in T_{oG} of starches from corn grown in Georgia in the current study, where higher temperatures occurred during the grain-filling period, agreed with those studies. Tester et al (1991) attributed the increase in gelatinization temperature of starch to differences in the perfection of amylopectin crystallinity, which increased with the temperature at which barley was grown. Lu et al (1996) reported that the increase in T_{oG} of starches from corn grown at higher temperatures correlated with an increase in chain length of the medium branch-chain fractions of amylopectin. They did not report information on the retrogradation properties of the starches.

Variations in starch thermal properties of corn between the two locations studied in this research might have been a combination of many factors, including location effects, year differences, and genotype by environment interactions. Possibly, the longer storage period of the Puerto Rico corn than of the Georgia corn affected these properties, although the seed was kept in controlled environments. Further experiments might be necessary to isolate these factors, if possible, to determine their effects on starch thermal properties. The cause of the geographic differences and the relations between thermal starch properties, structural variations in the starch, and production characteristics of the corn may be of future interest.

CONCLUSIONS

Significant differences were found among the 62 S_3 lines of one corn population, Antigua 1, when averaged over two locations for the gelatinization properties (T_{oG}, R_G, ΔH_G, and PHI) but not for retrogradation characteristics. Highly significant differences ($P < 0.01$) were observed for the gelatinization properties of the lines grown in two locations, but not for the retrogradation properties. These differences in starch thermal properties suggest that there may be differences in native alignment and hydrogen bonding within the starch molecules.

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