

Effect of Planting Date on Maize Starch Thermal Properties¹

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ABSTRACT

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Starches isolated from kernels of two maize (*Zea mays* L.) inbreds and their F1 progeny, grown after four planting dates, were evaluated for differences in thermal properties. Differential scanning calorimetry (DSC) was used to compare onset (T_o) and peak (T_p) temperature, range (R_n), and total enthalpy (ΔH) values of gelatinization. Amylose content (%AM) of samples was determined colorimetrically, and image analysis was used to determine average diameters of granules. Significant ($P \leq 0.05$) increases for T_p and ΔH were observed with later planting dates.

Significant genotypic differences also were seen for T_o , T_p , and ΔH . Later planting dates had no effect on %AM or starch granule size. Genotypes ranked similarly for %AM across each environment, and no differences were observed for average granule diameter. The presence of environmental effects on thermal properties of the starch suggests that if small differences (1–2°C or <0.2–0.3 cal/g) are to be identified among nonmutant genotypes, growing conditions may need to be controlled.

Qualitative genetic variations in starch characteristics have long been observed among maize endosperm mutants. Wide variations in thermal properties among these mutants also have been well characterized by differential scanning calorimetry (DSC) (Brockett et al 1988, Sanders et al 1990, Wang et al 1992). Use of DSC has been suggested for detecting quantitative variations in thermal properties among normal (nonmutant) maize starch. Genetic variability measured by DSC may be useful in breeding programs aimed at screening maize germ plasm for desired starch properties on the basis of these values. Several studies have indicated genetic variability among nonmutant sources of maize by DSC. For example, Krueger et al (1987b) reported significant variations in DSC values, especially total enthalpy of gelatinization (ΔH)

and peak height index for maize starches of different varieties. White et al (1990) observed significant variations for onset temperature (T_o), range (R_n), and ΔH values within and among several genetically variable, open-pollinated populations of maize. Similarly, Li et al (1991) observed variability within several exotic populations, suggesting that selection might be possible within populations to obtain genotypes with specific starch properties.

Environmental factors also influence the quality of starch. Studies in several plant species have indicated that temperature during grain filling, soil fertility, planting date, year, and location may influence starch amylose content (%AM), as well as temperature of gelatinization (T_p) (Dunn et al 1953, Juliano et al 1969, Kongseree and Juliano 1972, Lempiannen and Henriksnas 1979). The importance of environmental factors affecting starch quality was demonstrated by Beachell and Stansel (1963), when they found that a lower temperature during the ripening of rice grains resulted in a higher %AM and lower T_p . A recent study by White et al (1991) revealed differences in the shapes of DSC thermograms from genotypes grown in temperate versus tropical locations. Further information regarding nongenetic factors affecting DSC values will be necessary if this technique is to be used in screening for genetic variability.

The objectives of this study were to determine the effects of planting date on DSC parameters of starch from three normal (nonmutant) endosperm genotypes of maize, and to identify differ-

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ences among the maize genotypes. Amylose and granule size also were examined to determine whether these measurements explained the observed differences in DSC values.

MATERIALS AND METHODS

Plant Materials

The inbred lines Oh43 and A632 and the F1 progeny Oh43 × A632 were planted at four dates (Table I) at Ames, IA, in 1991, in a factorial randomized complete-block design with two replicates. Plots consisted of 12-foot rows thinned to 25 plants. Plants were self-pollinated; flowering date was recorded as the number of days to pollination following an arbitrarily selected date (June 30). The initial planting date was May 15, and subsequent plantings were made at approximately one-week intervals (Table I). Later planting dates resulted in a progressive delay in flowering for each of the three genotypes (Table I). Fluctuations in daily high temperatures throughout the 1991 growing season in relation to the four planting dates are shown in Figure 1. Ears were harvested on a single date (October 15), when all entries had reached physiological maturity (presence of black layer). They were dried for 48 hr at 38°C. Two ears per replicate were selected for analysis, from which 100-kernel weights (HKW) were recorded. Only kernels selected from the center one-third portion of the ear were included in the analysis.

Starch Isolation

Starch was isolated from five kernels per ear. Kernels were steeped (48 hr in 0.45% sodium meta-bisulfite at 50°C), degermed by hand, homogenized in a laboratory microblender, filtered to pass a 30- μ m sieve, washed with distilled H₂O, and decanted (White et al 1990). Starches were further purified by toluene emulsification, as described by Krueger et al (1987a).

Differential Scanning Calorimetry

A calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) equipped with a thermal analysis data station was used. Approximately 3.5 mg (dwb) of starch was weighed into an aluminum pan and 8 mg of distilled water was added. The pan was sealed, allowed to equilibrate for approximately 1 hr, and heated from 30 to 102°C at a rate of 10°C/min (White et al 1990). T_o , T_p , and ΔH were recorded directly from the computer software. The R_n of gelatinization was calculated as $2(T_p - T_o)$, according to Krueger et al (1987a). Values for each replicate represented the mean of four DSC runs, averaged from two five-kernel samples per ear and two ears per replicate.

Apparent Amylose

A rapid method was used to determine %AM from starches as described by Knutson (1986). This procedure is as accurate and sensitive as conventional colorimetric methods (Knutson 1986). Approximately 5.0 mg of starch was dissolved in 10 ml of 90% dimethyl sulfoxide containing $6 \times 10^{-3} M$ iodine. Dissolved sample (1 mg) was diluted to 9 ml with H₂O, and the absorbance was measured at 600 nm on a spectrophotometer (Hitachi U-2000, Tokyo, Japan). Purified amylose was prepared from maize starch as described by Schoch (1942), and used to construct a standard curve.

Starch Granule Size

Isolated starch granules were dispersed in ethanol and mounted on slides. The preparations were placed on a Laborlux light microscope fitted with a video camera. The microscope system was attached to a digitizing Colorado video unit (Boulder, CO) linked to a Kevex Delta IV instrument with an image analysis software program (Kevex, San Carlos, CA). Fields of starch grains from different samples were viewed with a 40 \times objective and then digitized and processed for image analysis. Data collected were expressed in Waddell diameter (diameter calculated from a circle with an equivalent area to a granule).

Statistical Analysis

A factorial design was used to determine significance of genotypic and planting date effects on DSC parameters, %AM, and HKW. Analysis of variance (ANOVA) and correlation analyses for the data were computed (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Thermal Properties

Significant effects on DSC values as a result of planting date and genotype are shown in Table II. The date of planting had a highly significant effect on values for T_p ($P \leq 0.01$) and a significant effect on ΔH ($P \leq 0.05$) (Fig. 2), which increased with later planting dates. Although not significant, a trend for increased R_n also was observed for each genotype (Fig. 2). The extent to which planting date influenced certain DSC parameters, such as T_o , may have been dependent on genotype. This observation was further demonstrated by the significant interaction between genotype and planting date on T_p (Table II). Previously, White et al (1991) also observed environmental effects on DSC values in which starch of several maize populations had more narrow endotherms when grown in a tropical environment than they had in a temperate environment. These data further support the idea that DSC parameters of starches can be influenced by environmental factors. For example, differences in planting date may alter granule characteristics such as %AM and granule diameter, which may result in the observed changes in DSC values. The ranges for DSC parameters in this study were generally small

TABLE I
Planting and Average Flowering Dates
for Two Inbred Lines and an F1 Hybrid

Genotype	Flowering Days ^a According to Planting Dates			
	May 15	May 23	May 30	June 6
Oh43	15	20	27	34
A632	21	25	34	39
Oh43×A632	13	22	28	34

^a Recorded as the average number of days until self-pollination after June 30.

TABLE II
Mean Squares of Differential Scanning Calorimetry (DSC) Parameters,
Amylose Percent (%AM), and 100-Kernel Weight (HKW)
for Three Maize Genotypes

Source	DF	DSC Parameters ^a					
		T_o	T_p	R_n	ΔH	%AM	HKW
Replicate	1	0.59	0.09	4.61	0.03	0.59	0.92
Planting date (PD)	3	0.16	1.27** ^b	5.25	0.04**	1.56	23.6*
Genotype (G)	2	4.40**	3.70**	0.93	0.005**	3.61*	33.3*
G × PD	6	1.04	0.71**	0.40	0.20	0.07	16.7
Error	11	0.59	0.05	1.80	0.005	0.59	6.6

^a Onset of gelatinization (T_o); peak of gelatinization (T_p); range of gelatinization (R_n); enthalpy of gelatinization (ΔH).

^b Significant at $P \leq 0.05$ (*) and $P \leq 0.01$ (**).

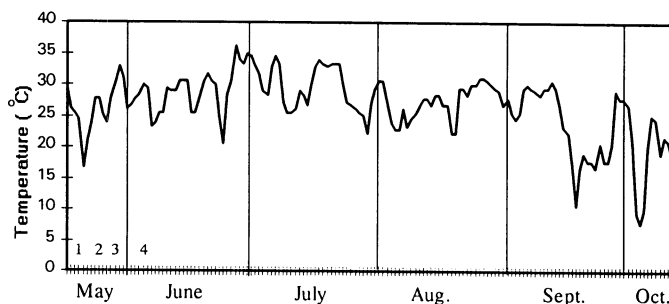


Fig. 1. Daily high temperatures at Ames, IA, for the 1991 growing season in which maize genotypes were planted on May 15, May 23, May 30, and June 6 (1–4, respectively).

compared to the larger ranges among starches from endosperm mutants such as *ae*, *du*, *su*, and *wx*, as demonstrated in previous studies (Brockett et al 1988, Sanders et al 1990, Wang et al 1992).

Many significant genotypic effects were seen among the DSC parameters, including T_o , T_p , and ΔH (Table II). Oh43 consistently showed greater values for these parameters than did A632 across each planting date (Fig. 2). Generally, the hybrid Oh43×A632 remained intermediate for these measures relative to the parent inbreds for most planting dates. The heterogeneous nature of segregating kernels collected from the hybrid Oh43×A632 might result in increased variability, thus masking trends due to planting date for T_o and T_p . Similar data suggesting genetic variation among nonmutant maize genotypes for DSC parameters have been detected. For example, Krueger et al (1987b) found differences among DSC endotherms of two inbred lines, and suggested that DSC endotherms could be used as a method for identifying inbreds. Other studies have shown wide variations for several DSC parameters within and among several genetically variable maize populations (Li et al 1991, White et al 1991). Although evidence suggests an abundance of genetic variability in thermal properties among exotic sources of maize, clearly there is a need to further investigate variability within commercially available U.S. germ plasm.

Amylose Content

No significant effect of planting date was seen on %AM for the three genotypes (Table II). These results are in agreement with Williams et al (1958), who found no relationship between %AM and date of planting among U.S. rice varieties at four dates of planting. However, Helm et al (1968) found that later planting dates were associated with a higher %AM among high-amylose maize genotypes. In the present work, fluctuations in the %AM were parallel among the genotypes. Possibly, environmental factors may have influenced %AM of each genotype in a similar manner during the growing season (Fig. 3). This observation may have been the effect of transient temperature changes during the growing season, especially during grain filling.

A significant effect of genotype on %AM was found (Table II). Genotypes ranked consistently in %AM across each planting date (Fig. 3). The influence of genotype among nonmutant sources on %AM also has been reported. Previous studies have shown differences in %AM among collections of normal (nonmutant) accessions of maize (Deatherage et al 1955). Environmental effects,

however, may have resulted in the differences because the collections were not necessarily grown in similar environments.

100-Kernel Weight

HKW was significantly affected by both date of planting and genotype (Table II). Later planting dates were associated with decreased HKW for genotypes Oh43 and Oh43×A632, whereas A632 was less affected (Fig. 3). The low HKW for A632 may be due to a later flowering date than that of the other genotypes (Table I). The HKW of Oh43×A632 generally exceeded those of the inbred parents, which is likely a result of the increased vigor of the hybrid genotype.

Starch Granule Size

Average starch granule diameters are shown in Table III for starches of each genotype collected from the first and fourth planting dates. Average granule diameters among the genotypes from these two planting dates ranged from 5.4 to 6.3 μm . No significant differences in granule diameter were observed among starches from the different planting dates. In contrast to the results seen in this study, previous studies with rice have indicated that environment may affect starch granule size (Kongseree and Juliano 1972).

There is some indication that differences among endotherms of nonmutant maize starches may be explained by differences in average granule size. For example, Knutson et al (1982) found a slight broadening and flattening of endotherms for smaller granules when fractionated by sedimentation as compared to those of larger granules. In our study, there was no evidence to support a relationship between starch granule size and shape of the endotherm.

Correlation Analysis

Correlation analyses were conducted among DSC values, %AM, HKW, and flowering date (Table IV). Correlations among several DSC parameters such as T_o and T_p were highly significant ($P \leq 0.01$), indicating that a certain degree of redundancy may exist among these measures. A highly significant reduction in HKW occurred for the later flowering date, as indicated by the negative correlation. A highly significant negative correlation also was seen between flowering date and R_n (Table II). Differences in days to flowering among genotypes within planting dates may have confounded detection of this effect in the ANOVA. Although

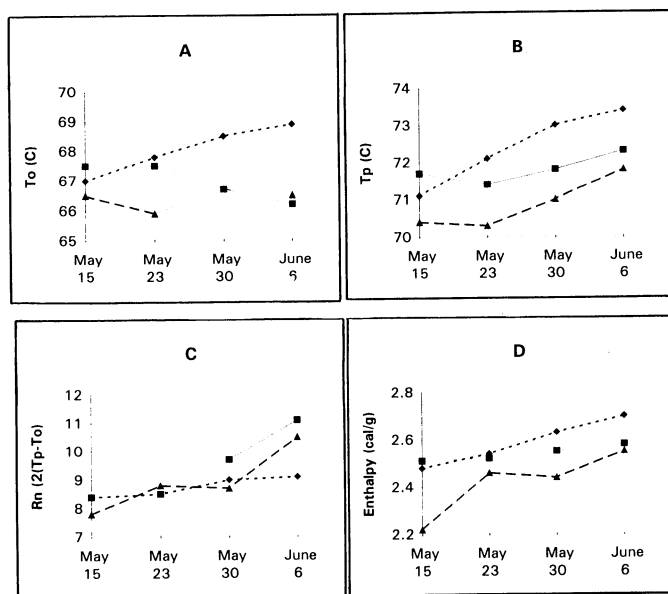


Fig. 2. Genotypes Oh43 (◆), A632 (▲), and Oh43×A632 (■) planted on May 15, May 23, May 30, and June 6. A, Mean onset temperature (T_o). B, Gelatinization peak temperature (T_p). C, Range of gelatinization (R_n). D, Total enthalpy of gelatinization (ΔH).

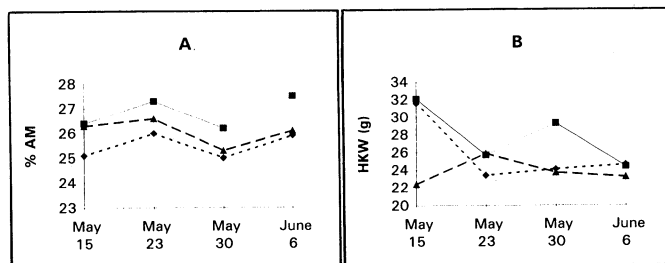


Fig. 3. Genotypes Oh43 (◆), A632 (▲), and Oh43×A632 (■) planted on May 15, May 23, May 30, and June 6. A, Mean amylose content (%AM). B, 100-kernel weight (HKW).

TABLE III
Mean Starch Granule Diameter and Standard Deviation (SD)
for Starch of Oh43, A632, and Oh43×A632

Genotype	Number of granules	Planting Date 1 (May 15)		Planting Date 4 (June 6)		
		Mean (μm)	SD	Number of Granules	Mean (μm)	SD
Oh43	243	5.8	1.6	203	5.4	1.6
Oh43×A632	215	5.4	1.7	218	5.6	1.8
A632	251	5.9	2.0	208	6.3	1.9

TABLE IV
Correlation Analysis of Differential Scanning Calorimetry (DSC)
Parameters,^a 100-Kernel Weight (HKW), Flowering Date (FD),
and Percent Amylose (%AM) from Genotypes Planted at Four Dates

	T_p	R_n	ΔH	HKW	FD	%AM
T_o	0.74*** ^b	-0.53**	0.46*	-0.02	-0.17	-0.38
T_p	...	0.17	0.70**	-0.02	0.22	-0.17
R_n	0.20	0.01	0.52**	0.34
ΔH	0.02	0.27	-0.10
HKW	-0.53**	-0.15
FD	0.08

^aOnset of gelatinization (T_o); peak gelatinization (T_p); range of gelatinization (R_n); enthalpy of gelatinization (ΔH).

^bSignificant at $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.0001$ (***)

the %AM did not greatly influence any DSC parameters, a negative correlation with T_o did approach significance ($P = 0.07$).

CONCLUSIONS

Planting date and genotype affected DSC parameters in this study. Differences in DSC parameters were not large, but the effects on several parameters were highly significant, thus demonstrating the extreme sensitivity of this method for determining differences among starch thermal properties. Increases in T_p , ΔH , and R_n for starches of all genotypes generally were observed with later planting dates. The DSC values were strongly influenced by genotype; starch from Oh43 had greater T_o , T_p , and ΔH values than did starch from A632. The R_n also was significantly correlated with the number of days to flowering. There was no indication that planting date or genotypic differences in starch granule size accounted for differences in thermal properties among the starches. Differences among planting dates are likely because of environmental fluctuations, such as variations in daily high temperatures (Fig. 1), as well as day length during vegetative growth and the grain-filling period.

The present work suggests that the DSC is a good tool to use for screening starches with unusual properties. Further studies are required to determine the extent to which the differences in DSC values reflect differences in functional properties of starches. Future use of DSC in programs designed to screen and select germ plasm may require a more thorough examination of the potential influence of environmental effects and the interaction of these effects with genotypic differences.

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