Variation for Thermal Properties of Starch in Tropical Maize Germ Plasm[1]

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ABSTRACT

The thermal properties of starches during gelatinization are important to the food industry, primarily because of the rapid market growth of convenience foods. Few studies have been conducted on the amount of thermal variation of maize starch in normal maize genotypes. Inter- and intrapopulation variability for thermal properties of laboratory-isolated starch from 35 tropical and semitropical maize populations, representing a range of sources and maturity groups, was evaluated by using differential scanning calorimetry. Starch was isolated from a bulk of 10 kernels from each of 16 plants in each population; two subsamples from each plant were analyzed independently. Endotherms were analyzed to determine enthalpy (ΔH), peak onset (T_o), peak maximum (T_p), and peak conclusion (T_c) temperature. The temperature range of gelatinization was calculated as ($T_c - T_o$). Among 35 populations, highly significant differences ($P \leq 0.01$) for all measured thermal properties were observed. Means of T_o, T_p, and T_c were 63.0, 71.0, and 77.8°C, respectively; ranges were 64.3–69.6°C, 70.1–73.9°C, and 76.8–79.6°C, respectively. Temperature ranges of gelatinization varied between 9.8 and 13.0°C. Average enthalpy (ΔH) was 10.5 joules per gram with a range of 8.2–12.3 J/g. Within each population, highly significant differences were noted in the T_o values of endotherms in all populations; highly significant differences were also noted in the T_p, T_c, and temperature range in most populations. Within each population, there were few significant differences in the ΔH. There was little relationship between starch thermal properties and population origin, kernel color, kernel weight, kernel starch content, or plant maturity group. The results demonstrate that genetic variability for thermal properties of maize starches exists in tropical and semitropical maize germ plasm. Compared to the range of starch thermal properties found in maize endosperm mutants, the range of variability in this germ plasm was limited.

The thermal properties of starches during gelatinization are important to the food industry, primarily because of the rapid market growth of convenience foods. Differential scanning calorimetry (DSC) can be used to evaluate the thermal properties of starch during gelatinization. Since Stevens and Elton (1971) first reported DSC endotherms of cereal starches, DSC has been widely used to study thermal properties of starches.

Most studies involving the thermal properties of maize starches have concentrated on the effect of mutant endosperm genes such as waxy (*wx*), amylose-extender (*ae*), dull (*du*), and sugary-2 (su_2). Stevens and Elton (1971) reported that *wx* starch had a broader peak than did normal starch. The DSC properties of starch from several genotypes (normal, *ae, wx, du,* and su_2) in a dent inbred line were evaluated by Inouchi et al (1984) at different developmental stages. The declining order for enthalpy (ΔH) values was: *wx*, normal, *du*, and su_2 starches. Starches from *ae* and *ae wx* genotypes from a sweet corn, inbred line had higher T_p than did normal starch. The declining order for enthalpy (ΔH) values was: *ae wx, wx, ae,* normal and *du* (Brockett et al 1988). For *ae* starch, there was no clear peak. The ΔH was not completed until about 115°C (Stevens and Elton 1971, Brockett et al 1988). Inouchi et al (1991) indicated that *wx* starches possessed a higher temperature conclusion (T_c) and a larger ΔH than that of normal starch from a dent inbred line, while T_p and T_c of *ae* starches were higher than those of other genes. They also found the temperature onset (T_o), T_p, and T_c were lower for *su2* than other types. Sanders et al (1990) showed genetic backgrounds (three sweet corn and one dent inbred line) affected the thermal properties of mutant genotypes, with highly significant interactions between backgrounds and genotypes with respect to T_p and ΔH.

Few studies have been conducted on the relationship of normal maize genotypes to the thermal properties of maize starch. Krueger et al (1987) noted starches from two different inbred lines exhibited significant variation in thermal properties. More recently, White et al (1990) investigated the thermal properties of starches from five open-pollinated maize populations: four white or yellow dent types from the United States, and one yellow floury type from the Southern Cone, and showed there was significant genetic variation among and within populations. They reported a mean onset temperature of 64.0°C (range 61.6–67.2°C), a mean gelatinization range $2(T_p - T_o)$ of 12.4 (range 8.7–16.4°C), and a mean enthalpy of 11.1 J/g (range 9.8–13.1 J/g). Because normal maize is a major starch source for food and nonfood industries, further study of genetic effects on the thermal properties of starch in normal maize is essential.

The germ plasm base of the yellow dent maize commonly grown in the United States is very narrow, and it is desirable to increase its genetic variability (Darrah and Zuber 1985). Many maize breeders have attempted to incorporate tropical germ plasm into current breeding programs, with little success (Goodman 1985). One reason for this is the lack of enough information about the characteristics of exotic germ plasm. Successful utilization of exotic germ plasm depends on investigation into the agronomic, physiological, pathological, and other specific characteristics of exotic germ plasm. There are several recent reports of variation for isozymes (Doebley et al 1985), yield potential (Gutierrez-Gaitan et al 1986, Holley and Goodman 1988), and agronomic traits (Castillo-Gonzalez and Goodman 1989) in exotic maize germ plasm. There is little information, however, on variation for starch thermal properties in tropical and semitropical maize germ plasm.

The objective of this study was to determine the genetic variation for starch thermal properties during gelatinization among and within a representative sample of 35 Latin American maize populations, and the genetic variation for kernel properties among the same populations.

MATERIALS AND METHODS

Thirty-five tropical and semitropical maize populations (Table II) were selected to represent various regions (Caribbean, Central America, northern South America, Southern Cone, and others), endosperm colors (white, yellow, and mixed white and yellow), and maturities (very early to late). Twenty seven of the populations were evaluated for agronomic characteristics as described by Castillo-Gonzalez and Goodman (1989). Five populations were synthesized at North Carolina by crossing the racial collection with Mo44, a photoperiod-insensitive inbred, chain cross-sibbing two generations, backcrossing to the racial collection, and sibbing for two more generations to obtain photoperiod-insensitive tropical maize germ plasm (D. Uhr and M. M. Goodman, *personal communication*). Short Tuxpeno Planta Baja, SLWD, and SLWD-2 are composites of tropical maize germ plasm from the maize-breeding program at the Centro International de Mejoramiento Maize Y Trigo (CIMMYT) near Mexico City, Mexico. The Cateto races are generally considered to be temperate. All

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populations were grown under short-day conditions in a winter nursery near Puerto Vallarta, Mexico in 1989–90. Random crosses (approximately 64) were made among plants within each population; a plant was used as either a male or a female only once. Ears were individually harvested at maturity and stored in a cold room. Sixteen ears (representing 16 replicates) from each population were randomly chosen and 10 kernels from each ear were randomly selected and bulked for starch extraction and thermal analysis. The 10 kernels were weighed to determine average kernel weight.

A method described by Schoch (1957) and Boyer et al (1976) was employed with modification to extract kernel starches. Ten maize kernels were weighed and placed in a test tube containing 0.45% $Na_2S_2O_5$ in a water bath at 50°C for 48 hr. After the first 24 hr, the germ and pericarp were removed, and fresh $Na_2S_2O_5$ was added. The endosperm was macerated with a mortar and pestle and then homogenized with a Waring blender for two one-minute periods with a one-minute interval. The resulting slurry was filtered through 75-μm and 45-μm sieves, respectively. Any residue remaining on the 75-μm screen was reground and re-

TABLE I
Analysis of Variance of Means of Starch Thermal Properties for 35 Tropical and Semitropical Maize Populations

Source	DF	Thermal Properties[a] T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$[b] (°C)	ΔH (J/g)	DF	Starch per Kernel (mg)	Kernel Weight (mg)	Starch Extract (%/kernel)
Population	34	33.74**[c]	19.74**	14.47**	12.96**	21.81**	34	2.339**	0.067**	0.060**
Plant population	525	1.22**	0.71**	1.03**	0.98**	1.52**				
Error	560	0.08	0.06	0.17	0.20	0.93	510	0.118	0.003	0.003
Coefficient of variation		0.42	0.35	0.53	3.91	9.18				

[a] T_o = peak onset, T_p = peak maximum, T_c = peak conclusion, ΔH = enthalpy.
[b] Gelatinization range.
[c] ** = Significant at the 0.01 level of probability.

TABLE II
Population, Kernel Color, Short-Day Maturity, Origin, and Means of Starch Thermal Properties (T_o, T_p, T_c, T_c-T_o, and ΔH), Starch per Kernel, Kernel Weight, and Percent of Starch per Kernel for 35 Tropical and Semitropical Maize Populations

Population[a]	Origin[b]	Short-day Maturity[c]	Kernel Color[d]	Thermal properties[e] T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$[f] (°C)	ΔH (J/g)	Starch per Kernel (mg)	Kernel Weight (mg)	Starch Extract (%)
Amarillo Salvadoreño Composite	CA	E	Y	66.3	71.3	77.9	11.6	10.6	179	333	54.0
Canilla (P.G.)/Mo44/2/Canilla	CA	I	Y,W	66.4	70.8	76.8	10.4	11.2	196	386	50.8
Cariaco Mag 408	SA	I	Y	68.4	72.8	79.6	11.2	12.3	243	415	58.7
Cariaco Cor 338	SA	I	Y	65.8	70.4	77.8	12.0	11.5	145	235	62.8
Cariaco Ven 408	SA	L	W	67.6	72.2	78.4	10.8	10.9	166	316	52.4
Cateto Assis Brasil RGS XIV	SC	E	Y	65.6	71.4	78.0	12.3	9.8	172	316	54.7
Cateto Sulino Escuro Urg V	SC	E	Y	65.0	70.3	76.9	11.9	9.7	59	117	50.4
Cateto Sulino Urg IV	SC	VE	Y	66.2	71.2	77.5	11.3	10.0	130	243	53.5
Chandelle Ven 460	SA	I	W	67.6	72.4	78.6	11.0	11.0	196	406	48.3
Costeño Atl 314	SA	E	Y	66.6	71.5	77.7	11.1	10.1	225	393	57.2
Costeño Atl 328	SA	E	W	65.6	70.6	77.1	11.4	11.3	226	391	58.1
Costeño Cor 320	SA	L	Y	67.3	72.2	78.4	11.1	9.8	226	389	58.3
Costeño/Mo44/2/Costeño ATL 75% F3	O		Y,W	65.4	70.4	77.1	11.7	10.6	231	375	62.4
Cuban Flint Cub 63	CR	I	Y	66.7	71.5	77.3	10.7	9.4	132	266	49.2
Cubano Amarillo Duro Ecu 904	SA	I	Y,W	64.3	70.1	77.3	13.0	10.9	201	365	54.9
Cubano Dentado Bov 585	SA	E	Y	68.0	72.6	78.6	10.6	8.4	143	326	44.0
Cubano Tusón Ecu 542	SA	I	Y	65.5	70.8	77.2	11.7	10.2	203	349	58.7
Dente Branco Paulista SP V	SC	I	Y,W	66.9	71.9	78.6	11.7	11.1	187	360	52.2
Dente Branco R.G. RGS X #	SC	E	W	65.7	70.7	77.8	12.1	11.4	236	350	67.7
Early Caribbean Mar 9	CR	E	Y	65.2	70.6	77.0	11.7	10.4	180	342	53.1
NAL-TEL AM.T.B. GUA220	CA	VE	Y	69.6	73.9	79.4	9.8	8.2	135	300	45.3
Negrito/Mo44/2/Negrito Mag 75%	O	I	Y,W	66.3	71.0	77.2	10.9	11.0	203	373	54.5
Olotón Gua 383	CA	L	Y,W	66.8	71.5	78.0	11.2	10.9	131	348	37.2
Perla Lim 13	S	I	Y	66.0	71.0	77.9	11.8	10.8	187	409	45.7
Puya/Mo44/2/Puya Mag 322 75% F3	O		W	66.6	71.3	77.5	10.8	10.1	214	412	52.1
Semi-Dentado RGS XV	SC	I	Y	65.7	71.0	77.8	12.1	11.1	171	302	56.7
Short Tuxpeño Planta Baja	O		W	66.3	71.0	77.2	10.9	10.4	186	414	44.9
SLWD 1236	O		W	65.7	71.0	77.2	11.5	9.6	204	421	48.6
SLWD-2 1235	O		W	65.9	71.0	77.1	11.3	10.1	206	456	45.3
Tepecintle Gua 597	CA	I	Y,W	65.5	70.7	77.6	12.1	10.4	197	338	58.5
Tusón Bai III	SC	E	Y	66.2	71.3	77.6	11.4	10.5	183	356	51.3
Tusón Cub 62	CR	I	Y	65.6	70.9	77.8	12.2	10.8	190	350	54.7
Tusón/Mo44/2/Tusón Cub 75% F3	O	I	Y	65.7	71.0	77.6	11.9	10.7	137	269	51.0
Tuxpeño Ven 767	SA	I	W	66.5	71.2	77.9	11.4	11.0	193	349	55.6
Yungueño Bov 362	SA	I	Y,W	66.4	71.6	78.6	12.3	11.3	198	344	57.6
Mean				66.3	71.3	77.8	11.5	10.5	183	346	53.2
Walter-Duncan least significant difference ($P \leq 0.05$)				0.7	0.5	0.6	0.6	0.8	30	50	5.1

[a] N = 16 for each population.
[b] Source region: CR = Caribbean, CA = Central America/Mexico, SA = northern South America, SC = Southern Cone, O = other.
[c] Short-day maturity groups classified by Castillo-Gonzales and Goodman (1989); Short-day maturity equivalent to materials grown in the northern (VE), Central (E), southern (I), and far-southern (L) United States.
[d] Yellow (Y), white (W), or mixed (Y,W) kernels.
[e] T_o = peak onset, T_p = peak maximum, T_c = peak conclusion, ΔH = enthalpy.
[f] Gelatinization range.

screened four times. Extracted starch granules were purified a minimum of 12 times with 0.05M NaCl and one-fifth volume toluene. Purified starch granules were washed twice with double distilled water, once with acetone, and dried at 40°C.

Thermal analysis was conducted with a Mettler 30 DSC and a TC11 TA evaluation and control center (Mettler, Hightstown, NJ). Starch samples were placed in an oven at 40°C for 10–12 hr before thermal analysis to ensure that no moisture remained in the sample. A 3.5-mg starch sample was weighed into a preweighed aluminum hermetic pan, and 8.0 μl of double distilled water was added. The pan was sealed immediately to prevent moisture loss, and the starch was allowed at least one, but not more than 2 hr to absorb water before the thermal analysis. An aluminum pan with 8.0 μl of double distilled water was used as a reference. The rate of temperature increase was 10°C min^{-1}, from 25 to 125°C. Temperature and enthalpy were calibrated using indium. Two starch subsamples were run in duplicate from each ear sample to obtain an estimate of intrapopulation variability. Endotherms were analyzed to determine ΔH, T_o, T_p, and T_c using TA72 Graphware software (Mettler). The temperature range of gelatinization was then calculated as ($T_c - T_o$). An analysis of variance was computed for thermal characters, kernel weight, and starch content with the Statistical Analysis System program (SAS 1989). An F-test was calculated for each trait using the appropriate mean squares to detect differences among and within populations (Snedecor and Cochran 1967). When an F-test was significant at the 0.05 probability level, the means were separated on the basis of the Duncan's multiple range test (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The F-tests from analyses of variance among and within populations detected highly significant ($P \leq 0.01$) differences among and within the 35 populations for the starch thermal properties measured (Table I). This indicates that a range of genetic variability exists for these traits in tropical maize germ plasm. A wide range of values was observed for each trait (Table II), but there was little relationship between maturity, kernel color, origin, or starch thermal properties in this germ plasm (Table II). Mean T_o was 66.3°C, with a range of 64.3°C in Cubano Amarillo Duro, an intermediate maturity, mixed-color population from the Caribbean, to 69.6°C in NAL-TEL AM.T.B., a very early, yellow population from Central America.

Mean T_p was 71.3°C, with a range of 70.1°C in Cubano Amarillo Duro to 73.9°C in NAL-TEL AM.T.B. Mean T_c was 77.8°C, with a range of 76.8°C in Canilla (P.G.) × (Mo44[2] × Canilla) to 79.6°C in Cariaco (Mag 408), an intermediate, yellow population from the Caribbean. Mean gelatinization range was 11.5°C, with a range of 9.8°C in NAL-TEL AM.T.B. to 13.0 in Cubano Amarillo Duro. Mean enthalpy was 10.5 J/g, with a range of 8.2 J/g in NAL-TEL AM.T.B. to 12.3 J/g in Cariaco (Mag 408).

Kernel properties analyzed included starch per kernel, kernel weight, and percent of starch extracted from each kernel (an estimate of the starch to germ ratio in the seed). The F-tests from analyses of variance among populations detected highly significant ($P \leq 0.01$) differences among the 35 populations for the kernel properties measured (Table II). This indicates that a range of

TABLE III
Significance Levels from F-Tests for Intrapopulation Variability of Starch Thermal Properties for 35 Tropical and Sub Tropical Maize Populations[a]

Population[b]	T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$[c] (°C)	ΔH (J/g)
Amarillo Salvadoreño Composite	**[d]	**	**	ns	ns
Canilla (P.G.)/Mo44/2/Canilla	**	**	**	**	ns
Cariaco Mag 408	**	**	**	**	ns
Cariaco Cor 338	**	**	**	**	ns
Cariaco Ven 408	**	**	**	**	ns
Cateto Assis Brasil RGS XIV	**	**	**	ns	ns
Cateto Sulino Escuro Urg V	**	**	**	**	ns
Cateto Sulino Urg IV	**	**	**	**	**
Chandelle Ven 460	**	**	**	ns	ns
Costeño Atl 314	**	**	**	**	ns
Costeño Atl 328	**	**	**	**	ns
Costeño Cor 320	**	**	**	*	ns
Costeño/Mo44/2/Costeño ATL 75% F3	**	**	**	**	ns
Cuban Flint Cub 63	**	**	**	*	ns
Cubano Amarillo Duro Ecu 904	**	**	**	*	*
Cubano Dentado Bov 585	**	**	**	**	ns
Cubano Tusón Ecu 542	**	**	**	**	ns
Dente Branco Paulista SP V	**	**	**	**	ns
Dente Branco R.G. RGS X #	**	**	*	*	ns
Early Caribbean Mar 9	**	**	**	*	ns
NAL-TEL AM.T.B. GUA220	**	**	**	**	**
Negrito/Mo44/2/Negrito Mag 75%	**	**	**	*	ns
Olotón Gua 383	**	**	**	**	ns
Perla Lim 13	**	**	*	*	ns
Puya/Mo44/2/Puya Mag 322 75% F3	**	**	**	**	ns
Semi-Dentado RGS XV	**	**	**	ns	ns
Short Tuxpeño Planta Baja	**	**	**	*	ns
SLWD 1236	**	**	**	**	*
SLWD-2 1235	**	**	*	**	ns
Tepecintle Gua 597	**	**	**	**	ns
Tusón Bai III	**	*	ns	**	ns
Tusón Cub 62	**	**	**	**	ns
Tusón/Mo44/2/Tusón Cub 75% F3	**	**	ns	ns	ns
Tuxpeño Ven 767	**	**	*	**	*
Yungueño Bov 362	**	**	*	**	*

[a] T_o = peak onset, T_p = peak maximum, T_c = peak conclusion, ΔH = enthalpy.
[b] n = 16 for each population.
[c] Gelatinization range.
[d] *, ** = Significant at the 0.05 and 0.01 levels of probability, respectively; ns = not significant.

genetic variability exists for these traits as well. A wide range of values was observed for each trait (Table I). Mean starch per kernel was 183 mg, with a range of 59 mg in Cateto Sulino Escuro, an early yellow population from the Southern Cone, to 243 mg in Cariaco (Mag 408). Mean kernel weight was 346 mg, with a range of 117 mg in Cateto Sulino Escuro to 456 mg in SLWD-2, a white composite. Mean percentage of starch extract was 53.2%, with a range of 37.2% in Oloton, a late mixed-color population from Central America, to 67.7% in Dente Branco R.G., an early white population from the Southern Cone.

The variability within the same populations for starch thermal properties was also evaluated (Table III). Significant or highly significant variation was found within all populations for T_o, T_p, and T_c except for T_c in Tusón Bai III and Tusón $\times$ (Mo44[2] $\times$ Tusón Cub) 75% F3. The gelatinization range was less variable, with five populations showing no significant variation for this trait. The ΔH was similar within populations, with only six populations showing significant or highly significant variation for this trait.

Among those populations showing significant variation for a trait, Yungueño showed the widest range of variation for T_o (64.1–68.2°C), gelatinization range (11.0–16.8°C), and ΔH (8.8–15.9 J/g) (not shown). Yungueño is an intermediate mixed-color population from the Southern Cone. Cubano Amarillo Duro, an intermediate mixed-color population from the Caribbean showed the widest range of variation for T_p (68.3–71.7°C) (not shown). Cateto Sulino Escuro, an early yellow population from the Southern Cone showed the widest range of variation for T_c (75.4–79.5°C) (not shown).

This study was designed as an attempt to evaluate a broad array of Latin American maize accessions with acceptable agronomic performance (Castillo-Gonzalez and Goodman 1989) for their variation in starch thermal properties and kernel properties. Materials involved in this study are largely typical racial collections, genetically variable for appearance and grain yield. They represent only a fraction of the accessions stored in the Latin American germ plasm banks, and many accessions with unique starch thermal properties may exist.

In our sample of populations, we detected significant interpopulation variability for all starch thermal properties, indicating that a wide range of genetic variation for starch thermal properties existed among and within these populations. We have measured the starch thermal properties of several adapted U.S. corn belt inbreds (data not shown). Compared to the range of variation in these inbreds, the variation for starch thermal properties we detected in these 35 populations is relatively large, but compared to the starch thermal properties of endosperm mutants such as *wx* and *ae*, the range of variation we detected is limited. However, selection for starch thermal properties may be possible within a given population. Since daylength-sensitive tropical maize does not perform well in the the U.S. corn belt, populations with extreme mean values and significant variation for starch thermal properties may be used to integrate their unique starch thermal properties into U.S. maize germ plasm. For example Cubano Amarillo Duro, an intermediate mixed-color population from the Caribbean, has the lowest mean T_o (64.3°C) and T_p (70.1°C) of any population tested (Table I), and it has highly significant genetic variation for these traits (Table III). Further study in this area is needed to determine the heritability and type of gene action controlling starch thermal properties.

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