Variation of Starch Granule Size in Tropical Maize Germ Plasm[1]

M. R. CAMPBELL,[2] J. LI,[3] T. G. BERKE,[4] and D. V. GLOVER[2,5]

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ABSTRACT

Granule size is an important characteristic of starch that can influence its functional and wet-milling extraction properties. Genetic variation for granule size has been found among maize (*Zea mays* L.) genotypes possessing single gene mutations affecting starch synthesis. In a previous study, 35 tropical and semitropical maize populations were examined for starch thermal properties. The purpose of this study was to evaluate these same populations for starch granule diameter using a laser light scattering technique. Highly significant ($P \leq 0.01$) differences were seen among the populations for mean granule diameter. Granule mean and mode diameter ranges were 16.2–18.2 µm and 14.2–16.8 µm, respectively. Although ranges in mean granule diameter were not as large as those seen among mutant maize genotypes, they were greater than those of a group of normal (nonmutant) hybrids. Highly significant differences were also seen among ears within the populations. With few exceptions, mean granule diameter values varied significantly ($P \leq 0.05$) within each of the populations, while the mode values varied to a lesser extent. NAL-TEL AM.T.B GUA220, an early maturing population from Central America, had the largest granule mean diameter (18.2 µm) and the highest gelatinization onset (T_o), peak (T_p), and conclusion (T_c) temperature. A correlation analysis between mean and mode granule diameter values and thermal properties (T_o, T_p, and T_c) revealed several highly significant positive correlations.

Starch granule diameter recently has been found to be an important component for food and industrial applications of starches. For example, small granules (2.0 µm) extracted from the grain of amaranth (*Amaranthus* L.), as well as those prepared by acid treating corn starch followed by ball milling, can be used as fat substitutes because the sizes are similar to those of lipid micelles (Daniel and Whistler 1990, Jane et al 1992). Other applications where starch granule size is important include the manufacturing of degradable plastic films (Lim 1992) and carbonless copy paper (Nachtergade and Nuffer 1989).

Previous studies have shown that starch thermal properties may reflect differences in starch granule size. Knutson et al (1982) found that the gelatinization onset temperature (T_o), range of gelatinization (RN), and enthalpy (ΔH) of amylomaize genotypes increased with decreasing granule size, based on granule size classes developed after a sedimentation procedure. Normal (nonmutant) and waxy (*wx*) maize (*Zea mays* L.) starches exhibited little change in differential scanning calorimetry (DSC) thermograms, except for a slight broadening and flattening of the endotherms for smaller granules. Stevens and Elton (1971) compared size classes of wheat (*Triticum aestivum* L.) starches by DSC and found that the gelatinization peak temperature (T_p) for small granules was ≈3.0°C greater than that of large granules, which was confirmed by the loss of birefringence of smaller granules at a higher temperature.

In maize, genetic variation in starch granule size occurs as a result of single gene mutations that influence starch synthesis. Brown et al (1971) studied various maize genotypes at 21 days postpollination and found that normal starch granules sizes ranged from 7.99 to 8.53 µm in diameter. Starch from a maize genotype possessing the soft starch mutation (*h*) had the largest diameter (9.96–9.45 µm), while the genotype sugary-1 and sugary-2 (*su1* and *su2*) had the smallest diameter (2.56–2.98 µm). There is also some indication that starch granule size may be under polygenic control. Wilson (1994) identified significant ($P \leq 0.05$) additive genetic effects from a generation means analysis of both normal and *h* corn-belt dent inbreds. Wilson suggested that enough genetic variation was present among public inbred lines to develop genotypes with the desired starch particle sizes.

The primary objective of this study was to evaluate variation for starch granule size among and within 35 tropical and semitropical normal maize populations by using a laser light scattering technique. In addition, particle size data was compared to DSC values reported in a previous study (Li et al 1994) to determine a relationship between starch particle size and thermal properties.

MATERIALS AND METHODS

Thirty-five tropical and semitropical normal maize populations were evaluated for starch granule size. These populations represented several Central and South American regions and varied widely in endosperm color and maturity. Starches from these maize populations were previously evaluated for thermal properties by DSC. Details of growing conditions and of the starch extraction procedure are described by Li et al (1994). A single starch sample was isolated from the bulk of 10 kernels per ear and from 16 ears per population. Determination of starch granule diameter was accomplished using a particle size analyzer (CIS-100, Galai Instruments, Islip, NY). Starch samples were prepared for particle size analysis according to Wilson et al (1994) in which ≈30–40 mg of starch was suspended in 75 ml of deionized water. Ten drops of an iodine stock solution (20.7 g of KI/L and 4.14 g of I_2/L) diluted to 4% were added to define the surfaces of the individual starch granules. Samples were then suspended for 10 min by a magnetic stir bar set to a high speed to disrupt starch agglomerates and to avoid settling of the granules. After 10 min, a portion of the suspension was placed immediately into a plastic cuvette with a small magnetic stir bar and inserted into the particle size analyzer. Each sample was subjected to duplicate 5-min data acquisitions using an acquisition range of 0.5–40.0 µm. In most cases, a minimum granule count of 500,000 was achieved. An aqueous suspension of a sample containing polymer microspheres (20.49 ± 0.20 µm) was used as a standard (Duke Scientific, Palo Alto, CA) and run following every 10 starch samples to monitor fluctuations, if any, in the particle size analyzer. The distribution of starch granule diameter was represented on a volume density basis, i.e., the projected volume of particles (assuming a sphere) found at a given diameter. The particle size analyzer was inter-

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[2]Department of Agronomy, Purdue University, West Lafayette, IN.
[3]Department of Agronomy, Huazhoug Agricultural University, Wahau, Hubei, Peoples Republic of China.
[4]Asian Vegetables Research and Development Center, AVRDC, P.O. Box 42, Shanhua, Taiwan 741.
[5]Corresponding author.

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faced with a computer software program that directly calculated starch granule diameter mean (based on the volume moment) (Allen 1981) and modes (location of the histogram peak) (CIS-series, Galai). The mode diameter was also recorded because it is a measure of the most frequent size class and affected less by skewed distributions when compared to the mean. An analysis of variance was conducted for the mean granule diameter to test for interpopulation effects (differences among populations) and intrapopulation effects (differences among ears within the populations) (SAS 1990). Mean separation for significant ($P \leq 0.05$) F-tests were conducted by the Duncan's multiple range test for both mean and mode values (Snedecor and Cochran 1967). Correlation analysis was used to determine relationships between starch granule mean and modes and thermal properties (Snedecor and Cochran 1967).

TABLE I
Analysis of Variance for Mean Starch Granule Diameter for 35 Tropical and Semitropical Normal Maize Populations

Source	Degrees of Freedom	Mean Squares (μm)
Population	34	8.4**[a]
Plant population	525	1.8**
Error	560	0.7
CV%[b]		4.9

[a] ** = Significant at the 0.01 level of probability.
[b] Coefficient of variation.

TABLE II
Populations and Mean Particle Size Statistics (Mean and Mode) for 35 Tropical and Semitropical Maize Populations[a]

	Particle Size Statistic (μm)	
Population	Mean	Mode
Amarillo Salvadoreño Composite	16.3	14.7
Canilla (P.G.)/Mo44/2/Canilla	16.6	14.7
Cariaco Cor 338	17.5	15.9
Cariaco Mag 408	17.5	16.8
Cariaco Ven 408	16.2	15.4
Cateto Assis Brasil RGS XIV	17.4	15.3
Cateto Sulino Escuro Urg IV	17.9	15.6
Cateto Sulino Urg IV	16.3	13.9
Chandelle Ven 460	16.8	14.8
Costeño Atl 314	16.3	14.9
Costeño Atl 328	16.4	15.5
Costeño Cor 320	17.3	15.6
Costeño/Mo44/2Costeño Atl 75% F3	16.2	14.7
Cuban Flint Cub 63	17.1	15.2
Cubano Amarillo Duro Esu 904	16.2	14.6
Cubano Dentado Bov 585	17.1	14.8
Cubano Tusón Ecu 542	16.4	14.2
Dente Branco Paulista SP V	16.5	15.6
Dente Branco R.G. RGS X #	16.8	16.0
Early Caribbean Mar 9	17.2	15.1
NAL-TEL AM.T.B GUA 220	18.2	15.7
Negrito/Mo44/2/Negrito Mag 75%	16.8	15.8
Olotón Gua 383	17.2	16.1
Perla Lim 13	16.3	14.7
Puya/Mo44/2/Puya Mag 322 75% F3	16.9	15.6
Semi-Dentado RGS XV	16.7	15.3
Short Tuxpeño Planta Baja	16.6	15.4
SLWD 1236	17.3	15.6
SLWD-2 1235	16.9	14.9
Tepecintle Gua 597	17.1	15.6
Tusón Bai III	16.6	14.9
Tusón Cub 62	16.4	14.5
Tusón/Mo44/2/Tusón Cub 75% F3	16.2	14.8
Tuxpeño Ven 767	16.4	14.7
Yungueño Bov 362	16.9	15.2
Mean	16.8	15.2
Walter-Duncan least significant difference ($P < 0.05$)	0.7	0.6

[a] $n = 16$.

RESULTS AND DISCUSSION

A highly significant ($P < 0.01$) population and plant population effects indicated that differences exist for mean starch granule diameter among and within the 35 populations (Table I). The low coefficient of variation (0.04%) observed for the 20.49 μm polymer microsphere standard indicated that minimal or no fluctuations in the particle size analyzer occurred over the duration of the data acquisition (data not shown). Mean and mode diameters for the 35 populations are shown in Table II. The population NAL-TEL AM.T.B GUA220 had the greatest mean diameter (18.2 μm), while the population Cariaco Mag 408 had the greatest mode diameter (16.8 μm). Four of the populations had the lowest mean value (16.2 μm), while Cubano Tusón Ecu 542 had the lowest mode value (14.2 μm).

TABLE III
Significance Levels from F-tests for Intrapopulation Variability of Starch Diameter Mean and Mode for 35 Tropical and Semitropical Maize Populations[a]

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

[a] $n = 16$
[b] *, ** = Significant at the 0.05 and 0.01 levels of probability, respectively.
[c] ns = Not significant.

TABLE IV
Pearson's Correlation Coefficients Between Starch Thermal Properties and Starch Granule Diameter Mean and Mode for 35 Tropical and Semitropical Maize Populations

	Thermal Properties[a]				
Diameter	T_o	T_p	T_c	T_c–T_o[b]	ΔH
Mean	0.46**[c]	0.48**	0.39**	–0.31	–0.40*
Mode	0.37	0.29	0.36*	–0.20	0.23

[a] T_o = peak onset, T_p = peak maximum, T_c = conclusion, ΔH = enthalpy.
[b] Gelatinization range.
[c] *, ** = Significant at the 0.05 and 0.01 levels of probability, respectively.

Although the ranges in granule mean are not large when compared to those for mutant starches 21 days postpollination (Brown et al 1971), they are greater than those described by Wilson et al (1994), where mean diameter value range among 10 normal corn-belt hybrids was only 14.1–15.1 µm.

Note that the population NAL-TEL AM.T.B GUA220 (an early maturing population from Central America with the greatest mean diameter) had the greatest T_o and T_p (Li et al 1994). This suggests a possible relationship between starch granule size and thermal properties. The mean starch granule diameter observed for NAL-TEL AM.T.B GUA220 (18.2 µm) was relatively larger when compared to the overall mean of the populations (16.8 µm). This is similiar to the larger granule diameters reported for hybrids homozygous for the recessive *h* allele. (15.91) when compared to normal (14.7 µm) (Wilson 1994). It is possible, therefore, that the *h* allele, or another single gene mutation affecting particle size, may be present in the NAL-TEL AM.T.B GUA220 population.

Tests for intrapopulation variability for each population are shown in Table III. Significant intrapopulation effects from the analysis of variance inidicate the presence of genetic variation among the 16 ears sampled per population for starch granule size, suggesting a potential for population improvement through selective breeding. Significant intrapopulation variation was observed for the populations with respect to starch granule size, with the exception of seven populations for mean and 15 for mode diameters. One population (Tusón/Mo44/2/Tusón Cub 75% F3) did not show intrapopulation variability for any of the particle size values. This same population also displayed the least amount of variation for thermal properties as shown by Li et al (1994).

A correlation analysis was conducted between starch granule size data and thermal properties for the starches (Table IV). Highly significant positive correlations were seen between measured diameters and DSC values (T_o, T_p, T_c). Although the coefficients are not large, they do suggest that larger particle diameters may result in gelatinization endotherms shifting to higher temperatures. The positive correlation between particle size and T_o, T_p, and T_c found in this study are in contrast to studies by Knutson (1982) and Stevens and Elton (1971), who found greater DSC values with smaller particle sizes. Other small but significant positive correlations were seen between granule diameter mean and ΔH and between the diameter mode and T_o and T_c.

Results from this study suggest that, as with thermal properties, the tropical populations may be an additional source of genetic variation for starch granule size. A few populations, however, appear to lack enough genetic variation needed for certain breeding strategies, such as recurrent selection. Selection within genetically variable populations or incorporation of tropical germ plasm into adapted corn-belt material may yield genotypes with desired starch properties. The association between starch granule size and gelatinization properties remains unclear. Further studies using populations exhibiting more extreme differences in granule diameter are required to establish whether granule size influenced gelatinization properties rather than some other structural feature of the granule.

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