Gelatinization Properties of Starches from Three Successive Generations of Six Exotic Corn Lines Grown in Two Locations

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ABSTRACT

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The objectives of this research were to evaluate the intra- and interpopulation variability in gelatinization properties of starches from exotic corn lines and their derivatives when grown 1) during two successive years in the same location; and 2) in both temperate and tropical environments. Six novel exotic corn lines (two 100% exotic and four 25% exotic derived from a breeding cross developed by crossing an exotic hybrid with Corn Belt lines) were selected for this research because their starches have significantly different (and potentially useful) thermal properties from those found in starch from normal Corn Belt corn. The S_n ($n = 3$ for 25% exotic lines and $n = 1$ for 100% exotic lines) generations of the six exotic lines were self-pollinated and grown in the winter nursery in Puerto Rico. Two successive generations (S_{n+1} and S_{n+2}) of lines selected for low onset of gelatinization temperature were self-pollinated and grown in the same environment near Ames, IA. To evaluate the effect of environment, the S_{n+2} generation also was self-pollinated and grown in the winter nursery in Puerto Rico. Thermal properties of starches from 10 single kernels from each line were analyzed by using differential scanning calorimetry (DSC) at a ratio of 4 mg of dry starch to 8 mg of distilled water. After subsequent generations, the differences in DSC gelatinization properties between selected kernels within each progeny line narrowed, suggesting increased homogeneity of starch structural properties within each line. Unusual thermal properties were fixed in some progeny lines. Environmental factors also affected the thermal properties of starch and a significant interaction between environment and genotype was observed. These results suggest that introgression of adapted germplasm with useful genes from exotic corn would increase the available genetic variability for starch functionality and allow the development of hybrids with important value-added traits.

Less than one percent of the U.S. germplasm base of corn (*Zea mays* L.) consists of exotic germplasm (Goodman 1985), leading to concerns about corn's genetic vulnerability to changes in environmental and agronomic conditions, and new insect and disease pressures (Crossa and Gardner 1987; Kuckuck et al 1991). The Germplasm Enhancement of Maize Project (GEM), a coordinated and cooperative effort among public and private sectors, was launched with the objective of providing the corn industry with materials developed by using germplasm enhancement of useful exotic germplasm and ultimately improving and broadening the germplasm base of corn hybrids grown by American farmers. Traits targeted for improvement are agronomic productivity, disease and insect resistance, and value-added characteristics (Pollak and Salhuana 1999). Starch represents ≈70% of the dry weight of the mature corn kernels and is the most economically important component. Therefore, further evaluation of the starch quality of GEM materials is essential to fully utilize these materials for food applications.

We currently have developed several corn lines derived from breeding crosses of exotic genotypes with Corn Belt lines. Our research has shown that these new lines can produce starches with improved functional properties, which may have potential applications in the food industry. The desired functional properties of starches from the new developmental lines (Ji et al 2003b) and their possible food applications are listed in Table I. To produce inbred lines from the early generation GEM lines that can be fully evaluated and used commercially, it is necessary to continue to develop and select lines to genetically "fix" the unusual thermal properties, and to evaluate the performance of the selected lines grown under different environments.

Individual properties of starch depend both on the genetic background of the corn and the environment. The effects of genetic background and environment on starch during plant development may be attributable to changes in granule size distribution, chemical structure, crystallinity, organization of the molecules within the granule, and molecular structure of the starch polymers (Fergason and Zuber 1962; Hizukuri 1969; Morrison and Scott 1986; Asaoka et al 1989; Tester et al 1991; Shi et al 1994). The roles of genetic (cultivar and level of breeding) and environmental (year and location) factors in the total variability of starch properties are not known. It may be that different genotypes respond differently to environmental factors.

The objectives of this research were to evaluate the intra- and interpopulation variability in thermal properties of starches from exotic novel corn lines and their derivatives when grown 1) during two successive years in the same location; and 2) in both temperate and tropical environments; and to evaluate whether selecting in the Puerto Rico winter nursery for these traits was possible.

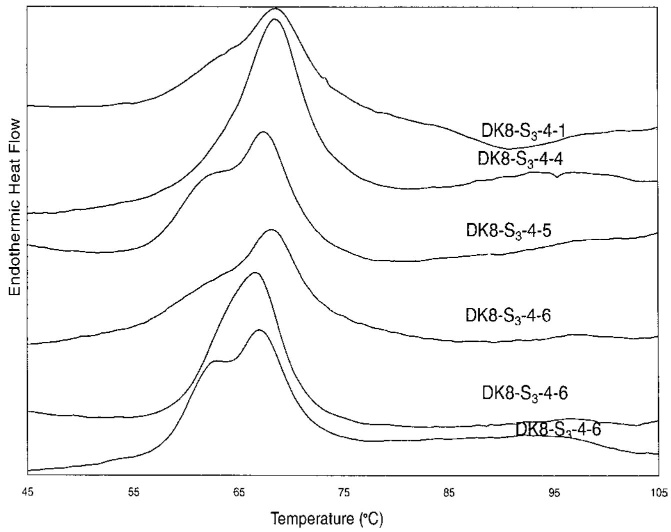

Fig. 1. Gelatinization thermograms of starches from progeny lines of DK8-S_3-4. Samples were analyzed at a ratio of 4 mg of dry starch to 8 mg of distilled water.

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MATERIALS AND METHODS

Materials

Three successive generations (S_n, S_{n+1}, and S_{n+2}) of four selected lines derived from an exotic by adapted breeding cross from the Germplasm Enhancement of Maize (GEM) project and two exotic inbred Plant Introductions were studied (Table II). In the current study, S_n generations of selected lines were self-pollinated and grown in the winter nursery in Puerto Rico in 1996. The S_n designation defines the number of times the line has been self-pollinated, starting with the breeding cross or the Plant Introduction (the S_0 population), in the development of the line (Simmonds 1974). The breeding cross was developed by crossing an exotic three-way hybrid with two commercial inbreds of the Stiff-Stalk heterotic pattern. Two successive generations (S_{n+1} and S_{n+2}) of selected lines were self-pollinated and grown in the same environment near Ames, IA, in 1998 and 1999. To evaluate the effect of environment, the S_{n+2} generation also was self-pollinated and grown in the winter nursery in Puerto Rico in 1999-2000. Grain filling occurred between January and February in Puerto Rico and between June and July in Ames, IA. Puerto Rico has more even temperature year-round and shorter day length than Ames does. In addition, Ames has higher temperature, especially during kernel growth than Puerto Rico does. Ears were harvested at physiological maturity and dried at ≈37.5°C until the moisture content reached ≈12%. All seeds were stored at approximately 4°C and 10% rh until analyzed.

Single Kernel Starch Extraction

Starch was extracted from single kernels by using the method described by White et al (1990) with modifications (Krieger et al 1997). Starch from each of 10 randomly selected kernels from each line was evaluated separately for starch characteristics. After extraction, starch was stored at 4°C until evaluated.

Differential Scanning Calorimetry (DSC)

For DSC analysis, a Perkin-Elmer DSC 7 analyzer equipped with a thermal analysis data station (Perkin-Elmer Corp., Norwalk, CT) was used. Analysis of starch gelatinization was conducted as described by White et al (1990). Starch (≈4.0 mg, dwb) was weighed into aluminum sample pans with 8 mg of distilled water. Samples were heated from 30 to 110°C at a rate of 10°C/min. Thermal transitions for gelatinization were characterized by T_o (onset temperature), T_p (peak temperature), T_c (conclusion temperature), and ΔH (enthalpy of gelatinization). These parameters were calculated directly by the DSC software. All enthalpy calculations were based on the dry starch weight. Retrogradation parameters are not discussed here because significant differences among genotypes and environments were not observed.

Statistical Analysis

The effect of genotype and environment and their interactions on the gelatinization properties of starch of the S_{n+2} generation were analyzed by using an analysis of variance procedure for a mixed model with a nested design for unbalanced data. The model used was:

$$Y_{ijklm} = \mu + En_i + P_j + L_{kj} + (EnP)_{ij} + (EnL)_{ijk} + O_{lijk} + E_{ijklm}$$

In this equation, Y_{ijklm} is gelatinization value of a specific parameter for a single kernel, μ is the overall average, En_i is the "fixed" environmental effect, P_j is the fixed source (S_n) effect, $(EnP)_{ij}$ is the interaction between environment with source, L_{kj} is the random line effect (S_{n+1}, nested within the population), $(EnL)_{ijk}$ is the interaction between the environment with the S_{n+1} line, O_{lijk} is the random progeny line effect (nested within each S_{n+1} line), and E_{ijklm} is the random error. Effects from environment and source were treated as fixed effects here, because only two environments (Ames 1999 and Puerto Rico 1999, winter nursery) and six sources were evaluated (Neter et al 1996a). Because of the unbalanced data, Type III sum of squares was used to test whether the contribution from each item (except μ and E_{ijklm}) in the equation was equal to zero (Neter et al 1996b).

RESULTS AND DISCUSSION

Genetic Variability for S_{n+2} Lines Grown in Ames 1999

The gelatinization properties of starches from 66 S_{n+2} lines from six exotic lines, DK8, DK10, DK14, DK34, PI82, and PI83 grown in Ames 1999, were determined using DSC. The data comprise many pages of values too numerous to print, thus two exotic lines, DK8 and PI82, were selected as examples of the progression of gelatinization properties in these exotic lines with successive generations. The degree of genetic variability of developmental lines can be evaluated quantitatively by measuring variations in starch gelatinization properties with DSC (Brockett et al 1988; Pollak and White 1997; Sanders et al 1990).

TABLE I
Desired Functional Properties of Unusual Starches from New Hybrids and Their Application in the Food Industry

Parameters	Desired Characteristics	Potential Application in Food Industry
Gelatinization temp.	Low onset (< 61°C) and peak (66°C) by DSC[a]	Energy savings, and nutrient and flavor retention during cooking
Viscosity	High peak viscosity (> 200 RVU) by RVA[b]	Good swelling behavior, good thickener, less starch in a formula, formula savings
Pasting properties	High setback value by RVA	Good gelling properties
Gel properties	High firmness by TA[c]	Good gelling properties

[a] Differential scanning calorimetry.
[b] Rapid viscosity analysis.
[c] Texture analysis.

TABLE II
Exotic Breeding Crosses and Exotic Inbred Corn Lines and Their Origins

Exotic Parent[a]	Pedigree for S_n Lines[b]	Source Identification[c]	Origin of Exotic Parent
Exotic breeding crosses			
Ames 23670	DK212T:S0610-8-1-3	DK8-S_3	DeKalb Genetics Hybrid from Thailand
Ames 23670	DK212T:S0610-10-1-3	DK10-S_3	DeKalb Genetics Hybrid from Thailand
Ames 23670	DK212T:S0610-14-1-2	DK14-S_3	DeKalb Genetics Hybrid from Thailand
Ames 23670	DK212T:S0610-34-1-1	DK34-S_3	DeKalb Genetics Hybrid from Thailand
Exotic Inbreds			
PI 186182	PI 186182	PI-82-S_1	Inbred 378 from Uruguay
PI 186183	PI 186183	PI-83-S_1	Inbred 378 from Uruguay

[a] Original corn populations as maintained at the North Central Region Plant Introduction Center, Ames, IA.
[b] Regrown corn ears maintained as lines to preserve a specific starch characteristic; *n* = 3 for exotic breeding crosses and *n* = 1 for exotic inbreds.
[c] Abbreviated source identification.

The thermal properties of starch from different lines exhibited considerable variability. Among 66 S_{n+2} lines (from six sources) analyzed, the mean T_o ranged from 55.8°C (DK8-S_3-4-1, Table III) to 66.7°C (PI83-S_1-7-1), the mean T_p ranged from 66.8°C (PI82-S_1-6-3, Table IV) to 70.0°C (PI83-S_1-7-1), the mean T_c ranged from 71.4°C (DK10-S_3-3-2) to 75.6°C (PI82-S_1-7-1, Table IV), and the mean ΔH ranged from 9.3 J/g (PI83-S_1-1-4) to 12.3 J/g (DK10-S_3-18-4). Only data for DK8 and PI82 are shown in Tables III and IV, respectively. Within each line, a high degree of variability for different gelatinization properties also was observed among the 10 single kernels analyzed. Among the 66 lines analyzed, line DK8-S_3-5-2 showed the greatest variability in T_o, ranging from 51.7–65.1°C with a standard deviation of 3.95 (Table III), and line PI82-S_1-8-2 showed the greatest variability for T_p, ranging from 66.3–70.3°C with a standard deviation of 1.20, and T_c ranging from 71.4–76.1°C with a standard deviation of 1.69 (Table IV). Line DK10-S_3-7-3 showed the greatest variability for ΔH, ranging from 7.9 to 12.3 J/g with a standard deviation 1.7 (data not shown).

Sufficient variability in thermal properties when screening corn germplasm is important in breeding programs aimed at improving starch properties because the outliers reveal genetic materials possessing traits of potential use and make it possible to develop corn lines with unusual thermal properties. Several studies have indicated genetic variability among nonmutant sources of maize by DSC. For example, White et al (1990) observed significant variations for T_o, range of gelatinization (R_G), and ΔH values within and among several genetically variable, open-pollinated populations of maize. Similarly, Li et al (1994) observed variability within several exotic populations, suggesting that selection might be possible within populations to obtain genotypes with specific starch properties. Because the 66 lines in this study were all grown in the same location during the same year, growing conditions and environmental effects on the kernels were similar. Therefore, the significant differences in gelatinization properties suggest that different genetic backgrounds of corn have a major effect on starch gelatinization behavior. The differences in gelatinization properties of starch from various corn lines could be attributed to some structural variations of the starch. It has been suggested that gelatinization temperature represents a measure of starch crystallite perfection and ΔH the amount of crystalline structure (Tester and Morrison 1990). Previous studies defined the structures of these and other corn lines at S_{n+1} (Ji et al 2003a) and S_{n+2} (Ji et al 2003b) generations. Significant differences were observed among their granule size and shape distribution, amylopectin molecular weight distribution, and branch chain length distribution of amylopectin. The variations in these structural properties would lead to the variations in the observed gelatinization properties.

Comparisons Among Three Successive Generations

Tables III and IV list the gelatinization values and the mean, range, and standard deviation (SD) values for starches from derived lines of the exotic breeding cross and the two exotic inbreds over three successive growing seasons (S_n in 1996 at Puerto Rico, S_{n+1} in 1998 at Ames, and S_{n+2} in 1999 at Ames).

In general, each subsequent generation resulted in a narrowing of the differences in gelatinization properties between kernels within each progeny line, suggesting increased homogeneity of starch structural properties within each line. Such a trend of increased homogeneity (or decreased SD of gelatinization properties) was clear following the generations DK8-S_3, DK8-S_3-1, and progeny lines of DK8-S_3-1 (Table III). A decrease in the range of T_o values was found over these three successive generations (S_3 through S_5) of population DK8 (Table III). Among 10 single kernels analyzed for the S_3 generation, the T_o had a range of 6.0°C

TABLE III

DSC Parameters[a] for Gelatinization of Starches from Single Kernels of Selected Corn Lines from DK8

Corn Source[b]	Frequency[c]	T_{oG} (°C)			T_{pG} (°C)			T_{cG} (°C)			ΔH_G (J/g)		
		Mean	Range	SD[d]	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
S_3 generation, grown in Puerto Rico 1996 winter nursery													
DK8-S_3	0/10	66.8	62.4–68.4	1.92	70.7	69.7–71.5	0.67	74.7	73.5–75.5	0.71	12.2	11.3–12.8	0.58
S_4 generation, grown in Ames 1998													
DK8-S_3-1	1/10	63.6	61.5–66.4	1.64	71.4	70.6–72.4	0.49	76.9	76.5–77.6	0.46	12.4	11.7–13.2	0.44
DK8-S_3-2	0/10	65.5	64.7–67.3	1.08	70.2	69.8–70.7	0.32	74.8	74.4–75.9	0.57	11.9	11.3–12.4	0.47
DK8-S_3-3	0/10	64.4	62.3–66.8	1.51	69.9	68.1–70.8	0.85	74.9	73.9–75.4	0.50	12.5	11.4–13.9	0.91
DK8-S_3-4	0/10	66.0	64.1–67.3	1.01	71.1	69.6–72.5	0.78	75.3	73.9–76.1	0.61	12.4	11.5–13.6	0.73
DK8-S_3-5	0/10	65.3	63.8–67.4	1.17	70.1	69.0–71.2	0.73	74.6	74.0–75.7	0.56	12.5	10.8–14.3	1.18
S_5 generation, grown in Ames 1999													
DK8-S_3-1-1	9/10	60.2	59.0–63.2	1.16	68.1	67.5–69.7	0.64	73.6	72.6–74.8	0.69	11.4	10.4–12.6	0.63
DK8-S_3-4-1	10/10	55.8	52.5–57.8	1.54	67.8	67.2–68.7	0.52	73.2	72.3–74.5	0.63	11.6	10.9–12.3	0.45
DK8-S_3-4-2	10/10	57.0	52.8–59.2	2.16	67.6	66.9–68.5	0.54	73.0	72.3–74.3	0.60	11.7	9.46–13.0	1.01
DK8-S_3-4-3	10/10	59.2	55.7–60.6	1.57	68.2	67.3–68.6	0.38	73.1	72.3–73.7	0.42	12.1	11.5–12.8	0.44
DK8-S_3-4-4	10/10	57.5	55.1–59.9	1.72	67.3	66.8–68.2	0.41	72.6	71.9–73.7	0.68	11.2	8.8–12.7	1.25
DK8-S_3-4-5	9/10	58.6	55.1–61.4	1.64	68.0	66.7–69.6	0.92	72.8	71.5–74.6	0.95	12.1	10.7–13.3	0.67
DK8-S_3-4-6	10/10	58.2	55.7–60.6	1.64	67.8	67.0–68.3	0.41	73.4	72.2–75.6	1.11	11.4	9.2–12.4	1.07
DK8-S_3-5-1	9/10	58.5	54.8–62.8	2.39	68.7	67.6–69.9	0.66	73.8	72.9–75.0	0.70	11.2	10.0–12.1	0.67
DK8-S_3-5-2	9/10	56.6	51.7–65.1	3.95	68.3	67.8–68.8	0.34	73.2	72.3–74.2	0.73	10.9	9.23–13.7	1.44
DK8-S_3-5-3	5/10	61.5	58.7–64.1	2.13	67.7	67.0–68.9	0.64	72.8	71.3–74.5	0.97	11.3	9.7–12.5	0.76
S_5 generation, grown in Puerto Rico 1999, winter nursery													
DK8-S_3-1-2	0/10	64.7	61.8–65.7	1.11	69.2	68.7–69.7	0.35	73.8	72.8–75.0	0.62	11.9	10.9–12.5	0.44
DK8-S_3-1-3	0/10	65.2	64.4–66.7	0.70	69.8	69.0–70.8	0.52	74.0	73.0–75.0	0.63	11.7	10.5–12.4	0.59
DK8-S_3-1-4	0/10	66.2	64.2–67.7	1.19	69.9	68.7–71.0	0.74	73.8	72.7–74.8	0.65	12.2	11.4–13.8	0.81
DK8-S_3-1-5	0/10	65.1	63.6–66.3	0.79	69.1	68.2–70.0	0.64	73.4	72.3–74.9	0.81	11.7	10.8–12.8	0.68
DK8-S_3-1-6	0/10	65.0	63.4–66.2	0.93	69.0	68.2–69.8	0.57	73.0	72.0–74.1	0.79	11.2	10.2–12.3	0.75
DK8-S_3-1-7	0/10	66.0	64.0–67.8	1.27	70.1	69.0–71.0	0.61	74.2	73.6–74.7	0.34	12.4	10.8–13.7	1.01
DK8-S_3-1-8	0/10	64.7	63.4–66.2	0.95	69.4	68.7–70.4	0.60	73.8	72.6–74.6	0.69	11.4	9.8–12.8	0.94
DK8-S_3-1-9	0/10	64.5	62.2–66.7	1.33	69.6	68.4–70.3	0.61	73.8	72.5–74.4	0.67	11.6	10.3–13.0	1.03
DK8-S_3-1-10	0/10	69.3	67.9–70.4	1.11	73.0	72.3–74.0	0.62	75.9	75.0–77.1	0.77	11.2	10.1–12.4	0.88

[a] T_{oG}, gelatinization onset temperature; T_{pG}, gelatinization peak temperature; T_{cG}, gelatinization conclusion temperature; ΔH_G, enthalpy of gelatinization.

[a] See Table II for source identification. Number after the dash signifies the ear number for the pedigree of the S_n line.

[b] Number of kernels containing starch with T_o < 61°C/total number of kernels analyzed.

[d] Standard deviation of gelatinization values among 10 randomly selected kernels analyzed within each line.

TABLE IV
DSC Parameters[a] for Gelatinization of Starches from Single Kernels of Selected Corn Lines from PI82

Corn Source[b]	Frequency[c]	T_{oG} (°C)			T_{pG} (°C)			T_{cG} (°C)			ΔH_G (J/g)		
		Mean	Range	SD[d]	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
S_1 generation, grown in Puerto Rico 1996 winter nursery													
PI82-S_1	2/10	64.7	57.6–68.6	3.48	70.0	67.2–72.2	1.49	74.6	70.8–76.9	1.68	11.1	10.3–12.2	0.61
S_2 generation, grown in Ames 1998													
PI82-S_1-1	1/10	64.0	60.4–65.8	1.80	70.4	69.4–71.0	0.45	75.8	75.2–77.3	0.59	10.3	9.2–11.1	0.61
PI82-S_1-2	1/10	63.2	60.4–65.1	1.57	69.5	68.4–70.8	0.74	74.9	73.3–75.8	0.72	10.8	9.6–11.7	0.54
PI82-S_1-3	0/10	66.3	64.1–68.3	1.30	70.9	69.5–72.1	0.84	76.4	75.5–77.4	0.64	· · ·	· · ·	· · ·
PI82-S_1-4	2/10	63.2	60.7–65.3	1.32	69.1	67.4–71.2	1.09	75.7	74.6–77.1	0.66	11.4	10.2–12.2	0.55
PI82-S_1-5	0/10	64.7	62.0–66.5	1.37	69.5	67.2–70.4	0.87	75.3	73.5–77.0	1.13	10.9	8.9–12.5	0.94
PI82-S_1-6	0/10	64.4	62.2–66.5	1.52	69.7	68.5–70.7	0.78	74.5	73.3–76.0	0.97	10.6	10.1–11.4	0.46
PI82-S_1-7	0/10	64.5	62.3–66.4	1.32	69.9	68.7–70.8	0.65	74.6	72.9–75.4	0.77	11.3	9.8–12.1	0.63
PI82-S_1-8	0/10	64.1	61.8–65.1	1.18	69.9	69.1–70.6	0.51	75.1	73.5–76.6	0.93	11.1	10.3–12.2	0.61
S_3 generation, grown in Ames 1999													
PI82-S_1-1-1	2/10	61.5	57.1–63.1	1.83	68.9	68.3–69.5	0.45	73.7	72.9–74.6	0.57	11.3	9.9–12.1	0.68
PI82-S_1-1-2	6/10	60.4	58.0–63.8	1.91	67.4	66.7–68.4	0.63	73.1	72.1–73.9	0.54	10.2	8.5–11.3	0.90
PI82-S_1-2-1	7/10	60.6	58.3–65.1	2.30	68.5	67.8–69.0	0.35	73.2	72.1–73.7	0.52	11.0	9.9–12.3	0.77
PI82-S_1-2-2	6/10	60.9	58.3–63.3	1.70	68.4	67.4–69.8	0.82	73.3	72.6–75.0	0.75	10.7	9.7–11.9	0.67
PI82-S_1-2-3	2/10	62.4	60.2–64.3	1.27	68.5	67.0–69.3	0.63	73.6	71.8–74.5	0.81	10.1	9.0–11.3	0.72
PI82-S_1-2-4	7/10	60.6	58.6–63.0	1.51	67.4	65.9–68.7	0.91	72.4	71.0–73.5	0.85	9.7	8.4–11.7	0.99
PI82-S_1-4-1	5/10	60.4	58.6–62.4	1.33	67.2	66.0–67.9	0.55	73.3	72.6–74.1	0.49	10.1	8.7–11.0	0.62
PI82-S_1-4-2	6/10	60.7	58.6–63.0	1.58	67.8	67.0–68.8	0.61	74.0	73.0–75.5	0.83	10.5	8.8–11.9	1.11
PI82-S_1-4-3	6/10	60.8	58.3–64.6	1.69	68.4	67.3–69.8	0.75	74.3	73.2–75.4	0.96	11.2	9.6–12.3	0.82
PI82-S_1-5-1	1/10	64.4	58.8–67.6	2.86	69.2	67.4–70.7	1.13	73.9	72.7–75.2	0.82	11.0	8.9–12.0	1.05
PI82-S_1-5-2	2/10	62.0	59.8–63.7	1.34	69.1	68.5–69.9	0.47	74.9	74.1–76.1	0.61	10.7	9.9–11.9	0.68
PI82-S_1-5-3	3/10	61.9	58.4–64.8	2.48	68.9	67.9–69.9	0.81	74.4	73.5–75.2	0.63	10.2	8.35–12.6	1.50
PI82-S_1-5-4	6/10	61.1	58.2–64.3	2.80	68.4	67.1–69.1	0.79	74.1	73.1–76.1	1.17	11.1	9.8–12.4	0.94
PI82-S_1-6-1	4/10	62.1	59.7–64.3	1.67	68.6	67.0–69.7	0.73	73.3	72.5–74.3	0.68	10.8	9.7–12.5	0.80
PI82-S_1-6-2	6/10	60.6	59.1–63.0	1.24	67.8	67.1–68.6	0.58	73.8	72.7–74.7	0.64	11.2	10.2–12.5	0.66
PI82-S_1-6-3	9/10	60.3	58.7–61.3	0.77	66.8	66.2–67.7	0.55	72.7	70.8–74.1	1.04	10.7	8.0–12.3	1.23
PI82-S_1-6-4	6/10	60.1	58.5–64.4	1.77	67.4	66.2–69.0	0.90	73.2	72.3–74.0	0.56	11.2	10.3–11.6	0.48
PI82-S_1-7-1	0/10	65.2	61.9–67.0	1.70	70.8	68.9–71.7	0.81	75.6	74.2–76.3	0.63	10.3	9.6–11.2	0.51
PI82-S_1-5-2	0/10	64.7	63.0–66.5	1.04	70.1	68.5–71.7	1.14	74.5	73.3–76.6	1.02	10.1	8.6–12.1	1.02
PI82-S_1-8-2	3/10	62.4	60.4–65.0	1.48	67.9	66.3–70.3	1.20	73.7	71.4–76.1	0.81	10.3	9.2–12.0	0.81
S_3 generation, grown in Puerto Rico 1999, winter nursery													
PI82-S_1-1-3	0/10	64.7	61.8–66.6	1.37	68.5	66.7–77.3	0.93	73.1	72.4–74.5	0.69	10.9	10.4–11.9	0.48
PI82-S_1-1-4	0/10	65.2	63.5–66.3	0.89	69.3	68.3–70.0	0.53	73.5	72.4–74.7	0.63	10.8	8.2–12.3	1.12
PI82-S_1-2-5	0/10	67.3	65.6–69.4	1.37	70.8	69.3–72.5	1.24	74.7	72.8–76.9	1.54	11.0	7.7–13.2	1.7
PI82-S_1-2-6	0/10	67.9	65.5–69.9	1.74	71.6	69.3–73.5	1.51	75.9	73.2–78.0	1.80	10.6	8.0–12.9	1.69
PI82-S_1-4-4	0/10	65.4	64.5–65.9	0.62	69.7	69.5–70.0	0.21	74.0	73.6–74.9	0.63	10.1	9.3–10.6	0.61
PI82-S_1-5-3	0/10	66.1	64.9–67.8	0.88	70.5	69.1–71.7	0.74	75.2	73.7–77.1	1.02	11.0	9.8–12.4	0.73
PI82-S_1-5-4	0/10	65.4	63.3–67.1	1.28	70.6	69.6–71.4	0.53	75.3	74.7–76.8	0.69	11.1	10.3–12.0	0.51
PI82-S_1-5-5	0/10	64.8	62.1–67.4	1.59	70.6	69.3–71.9	0.81	75.1	73.4–76.0	0.77	10.8	7.9–12.3	1.67
PI82-S_1-5-6	0/10	65.6	62.1–68.3	1.98	70.3	69.4–71.4	0.79	75.2	73.7–76.3	0.84	10.5	9.3–11.5	0.73
PI82-S_1-5-7	0/10	66.6	65.0–67.2	0.66	70.9	69.4–71.7	0.64	75.3	73.8–77.1	0.88	11.1	9.2–12.0	0.86
PI82-S_1-5-8	0/10	65.7	61.0–67.6	2.06	71.3	69.8–72.7	0.84	75.5	74.3–76.5	0.80	10.9	8.5–12.5	1.26
PI82-S_1-6-5	0/10	66.6	64.5–69.1	1.59	70.2	68.3–72.6	1.35	74.6	72.4–77.2	1.46	11.2	10.3–11.9	0.57
PI82-S_1-6-6	0/10	65.7	64.2–67.1	0.94	69.6	68.3–70.7	0.67	74.1	72.4–74.7	0.71	11.2	9.5–12.3	0.78
PI82-S_1-6-7	0/10	65.0	63.6–66.6	0.36	69.8	68.7–70.9	0.75	74.8	74.0–75.5	0.54	11.4	10.9–12.9	0.59
PI82-S_1-6-8	0/10	66.6	64.3–67.8	1.13	70.2	69.0–71.1	0.77	74.9	73.6–76.3	0.88	11.3	10.1–12.5	0.81
PI82-S_1-8-3	0/10	65.6	61.1–67.7	1.96	70.3	69.0–71.5	1.01	75.5	73.8–77.0	1.27	9.8	7.8–11.3	1.12
PI82-S_1-8-4	0/10	64.9	62.1–67.5	1.66	69.7	68.1–71.3	0.92	75.0	73.5–76.9	1.06	10.3	9.2–11.3	0.81
PI82-S_1-8-5	0/10	65.6	63.5–67.4	1.18	70.2	68.3–72.2	1.07	74.6	72.4–76.4	1.30	11.3	9.9–12.4	0.75
PI82-S_1-8-6	0/10	68.0	65.8–71.2	1.91	72.5	70.0–74.7	1.61	76.2	74.3–77.8	1.00	10.6	7.5–11.6	1.40
PI82-S_1-8-7	0/10	65.2	63.9–67.7	1.18	70.4	69.7–71.2	0.81	74.8	73.2–77.1	1.33	11.2	9.8–12.2	0.70

[a] T_{oG}, gelatinization onset temperature; T_{pG}, gelatinization peak temperature; T_{cG}, gelatinization conclusion temperature; ΔH_G, enthalpy of gelatinization.

[b] See Table II for source identification. Number after the dash signifies the ear number for the pedigree of the S_n line.

[c] Number of kernels containing starch with $T_o < 61$°C/total number of kernels analyzed.

[d] Standard deviation of gelatinization values among 10 randomly selected kernels analyzed within each line.

and SD of 1.92, whereas for the S_4 generation, ranges of the five progeny lines decreased to 2.6–4.9°C with a decrease in SD to 1.01–1.64. The 10 progeny S_5 lines of DK8-S_3-1 (one grown in Ames and 9 grown in Puerto Rico) showed continued reduction in the range of T_o and SD, with the range decreasing to 2.3–4.5°C, and the SD decreasing to 0.70–1.33. A similar trend of increased homogeneity of gelatinization values over successive generations also was observed for the successive generations from most lines of DK10, DK14, DK34, PI82, and PI83.

The S_3 generation (starting from the S_1 generation) of exotic inbreds in other lines from PI82 and PI83 (data for PI83 in Table IV), regrown in both Ames and Puerto Rico, exhibited great variability for gelatinization temperatures and enthalpy. For the S_3 generation of the PI82 inbred, the greatest variability for T_o occurred among the 10 seeds analyzed from PI82-S_1-5-1, with a range of 8.8°C and SD of 2.86. For the S_3 generation of the PI83 inbred, the greatest variability for T_o occurred among the 10 seeds analyzed from PI83-S_1-4-1, with a range of 9.2°C and SD of 3.55. The reason for these great variabilities might be that inbreds PI82 and PI83 were either not true inbreds or else the trait was not fixed in them.

For some lines, the change in variability of gelatinization properties over successive generations became complicated. Even among progeny lines from the same parent, some progeny lines

TABLE V

Significance of Genotype Effects and Environment Effects and Their Interaction from Analysis of Variance of Starch Gelatinization Properties[a,b]

Source of Variation	df	T_{oG} (°C)	T_{pG} (°C)	T_{cG} (°C)	ΔH_G (J/g)
Environment (E)	1	**	**	**	*
Source (S)	5		*		
E × S	5	*			
S_{n+1} Line (S)	9				
E × S_{n+1} Line (S)	9	**	**	**	
S_{n+2} Line (S_{n+1} Line [S])	64	**	**	**	**

[a] T_{oG}, gelatinization onset temperature; T_{pG}, gelatinization peak temperature; T_{cG}, gelatinization conclusion temperature; ΔH_G, enthalpy of gelatinization.

[b] *, ** Significant at $P < 0.10$ and $P < 0.05$, respectively.

showed greater variability, whereas other progeny lines showed smaller variability than did their parent. For example, for parent line DK10-S$_3$-1 the SD of T_o was 1.27. Among the next generation, the two lines, DK10-S$_3$-1-1 and DK10-S$_3$-1-3, had small SD (1.19 and 1.01, respectively), whereas the two lines DK10-S$_3$-1-2 and DK10-S$_3$-1-4 had greater SD (1.37 and 1.93, data not shown). One possible reason for these differences might be that gelatinization properties of starch are controlled by many genes and their interactions. The line with a small SD might have more homozygosity for genes controlling the trait than the line with a large SD. Another possible reason might be the environmental effect and its interaction with genotype.

Gelatinization thermograms of starch from progeny lines of DK8-S$_3$-4 grown in Ames 1999, show the development of a large shoulder at low temperature (Fig. 1). This type of peak separation shown on the gelatinization thermograms also may contribute to the increased variability of gelatinization values among some progeny lines.

Selection and Verification of a Trait (primarily for low T_o) with Potential Application in the Food Industry

We started the selection described in this study by screening S$_3$ GEM lines using DSC, with the goal to increase the frequency of genes producing starch with $T_o < 61.0$°C in the progeny of selected lines. Before the S$_3$ generation, selection was for agronomic traits and yield. The frequencies of kernels containing starch with a different gelatinization property ($T_o < 61.0$°C) for DK8 and PI82 are listed in Tables III and IV. Unusual thermal properties ($T_o < 61.0$°C) were fixed in some progeny lines of S$_{n+2}$ grown at Ames 1999. For example, one out of 10 kernels of the S$_4$ line DK8-S$_3$-1 contained starch with $T_o < 61.0$°C, whereas this frequency increased to 9 out of 10 kernels in the S$_5$ line of DK8-1-1. Zero out of 10 kernels of the S$_{n+1}$ line DK10-S$_3$-7 contained starch with $T_o < 61.0$°C (with one kernel containing starch with T_o at 61.6°C), whereas this frequency increased to 8 out of 10 kernels in the S$_{n+2}$ line DK10-S$_3$-7-1 (data not shown). In addition, we conclude that selection for these traits using the Puerto Rico winter nursery was successful because the same type of variance occurred in this tropical environment.

Starch Variability Between Two Locations

The average values and ranges of gelatinization parameters (T_o, T_p, T_c, and ΔH) of starch from the S$_{n+2}$ generations of the corn grown at two locations (Ames and Puerto Rico) revealed significant environmental effects and some significant interactions between environment and genotype (Table V). The significant interactions indicate that different genotypes responded differently to environmental factors and that environment affects expression of these traits.

The environment and genotype interactions in this study included environment and source interaction and environment and S$_{n+1}$ line interaction. These significant interactions can be specified from the gelatinization data. For progeny lines of DK10-S$_3$-4, DK10-S$_3$-6, and DK10-S$_3$-10, no significant differences were caused by the effect of environment. On the other hand, for progeny lines of DK10-S$_3$-15 and DK10-S$_3$-18 there was a significant effect of environment on the T_o of starch. Eight out of nine progeny lines of DK10-S$_3$-15 grown in Puerto Rico exhibited lower mean T_o (61.2–63.5°C) than did two progeny lines grown in Iowa (mean T_o 64.4–64.7°C). For DK10-S$_3$-18, one progeny line out of two grown in Puerto Rico exhibited lower mean T_o (61.6°C) than did two progeny lines grown in Iowa (mean T_o 63.0–64.2°C), whereas one progeny line out of two progeny lines of DK10-18 grown in Puerto Rico exhibited higher mean T_o (65.7°C) than did two progeny lines grown in Iowa (mean T_o 63.0–64.2°C) (all data not shown). Similar interactions also were observed when comparing progeny lines of DK34-S$_3$-9 grown at two locations. In contrast, the T_o of starches from progeny lines (S$_{n+2}$ generation) of the exotic accession PI82 grown in the tropical environment were higher than were those grown in the temperate environment.

CONCLUSIONS

The degree of genetic variability among and within developmental lines for gelatinization properties was characterized by analyzing starch gelatinization properties on 10 randomly selected single kernels by using DSC. Large intra- and interline variability was observed and unusual thermal properties were fixed in some progeny lines over two successive generations. Unusual thermal properties ($T_o < 61.0$°C) were fixed in some progeny lines. These results indicate that these lines might be useful for developing corn breeding lines with unusual thermal properties and this technique is useful providing the correct starting material is used. Environmental factors also affected the thermal properties of starch and a significant interaction between environment and genotype was observed for some lines. Gelatinization properties of starch from some lines were not affected by environmental factors, whereas gelatinization properties of starch from some lines were significantly affected by environmental factors in both directions (increased or decreased). Selection and verification of the trait (low T_o) by using the Puerto Rico winter nursery was successful.

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