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**Abstract**

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**Disciplines**

Agriculture | Food Biotechnology | Food Science | Human and Clinical Nutrition

**Comments**

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# Thermal Properties of Starch from Exotic-by-Adapted Corn (*Zea mays* L.) Lines Grown in Four Environments

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**ABSTRACT**

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The effect of four growing environments (two at Ames, IA; one at Clinton, IL; and one at Columbia, MO) on the thermal properties of starch from five exotic-by-adapted corn inbred lines (Chis37, Cuba34, Cuba38, Dk8, Dk10) and two control lines (B73 and Mo17) were studied using differential scanning calorimetry (DSC). The variations in thermal properties within environments were similar for the exotic-by-adapted lines and control lines. Missouri was the warmest environment and generally produced starch with the greatest gelatinization onset temperature ($T_{oG}$), the narrowest range of gelatinization ($R_G$), and the greatest enthalpy of gelatinization ($\Delta H_G$). Illinois was the coolest environment and generally resulted in starch with the lowest $T_{oG}$, widest $R_G$, and lowest $\Delta H_G$. These differences were attributed to higher temperatures in Missouri during grain-filling months either increasing the amount of longer branches of amylopectin or perfecting amylopectin crystalline structure. The Ames 1 environment produced starch with thermal properties most similar to those of Illinois, whereas the Ames 2 environment produced starch with thermal properties most similar to those of Missouri. Ames 2, located near a river bottom where temperatures tend to be warmer, likely had temperatures most similar to those found in Missouri during grain filling.

Nonmutant corn may be developed to contain starch that naturally possesses properties similar to those of chemically modified corn starches. Public and private scientists cooperating with the Germplasm Enhancement of Maize (GEM) project have developed lines from exotic-by-adapted breeding crosses, partly from germplasm foreign to corn races grown in the United States, that may be useful for agronomic, nutritional, and industrial reasons (Pollak 2002). As GEM cooperators, our laboratory routinely develops our own lines plus screens many other GEM lines for starch traits that may be valuable to the starch industry, such as low gelatinization onset temperature ($T_{oG}$) or low percentage of retrogradation (%$R$), and other criteria as described by Seetharaman et al (2001). Ji et al (2002) identified among our exotic-by-adapted corn inbred lines some that exhibit unique properties such low $T_{oG}$ and wide range of gelatinization ($R_G$). Ji et al (2004a) examined the thermal properties of exotic lines grown in Ames, IA, and Puerto Rico and found significant line-by-environment interactions in the thermal properties measured by differential scanning calorimetry (DSC). These results demonstrated that all genotypes do not respond the same to environmental factors. This variation, which is phenotypic, is actually the sum of three components: the effects of line, environment, and line-by-environment interactions (Poehlman and Sleper 1995).

Crops give their highest yield and lowest risk of failure when they are grown as close as possible to their respective temperature optima (Keeling et al 1994). In cereal crops, the optimum temperature for maximum grain yield lies between 20 and 30°C (Chowdhury and Wardlaw 1978). Grain yield, kernel weight, and kernel density were less for corn ears grown at 35°C than for those at 25°C (Lu et al 1996). The reduction in grain weight was likely caused by decreased production of starch because starch accounts for 70% of the dry weight of the grain. Protein content (Denyer et al 1994) and sucrose content (Nicolas et al 1984; Bhullar and Jenner 1986) are affected less by high temperatures than is starch content.

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The effects of high temperature on starch synthesis and yield may result from the elevated heat sensitivity of starch synthase, specifically soluble starch synthase (Denyer et al 1994). The soluble starch synthase has a temperature optimum of 20–25°C (Keeling et al 1993). In wheat heated to 40°C for 2 hr, the soluble starch synthase activity was reduced to 3% of that in the unheated wheat (Keeling et al 1993). Also, amylose content decreased at higher growing temperatures for corn (Fergason and Zuber 1962; Lu et al 1996) and rice (Asaoko et al 1984, 1985; Inouchi et al 2000). However, no differences in amylose content (Goering et al 1957; Tester et al 1991) and amylopectin characteristics (Tester et al 1991) were found in starch from barley grown at different geographical and seasonal conditions.

Others (Boyer and Preiss 1978; Takeda et al 1993) also reported that branching enzyme (BE) activities differed with temperature. BEI, with minor branching activity, preferentially transfers long chains and has a temperature optimum of 35°C. BEIIa and BEIIb, with major branching activity, transfer short chains and have temperature optima of 25 and 20°C, respectively. Lu et al (1996) found that, in general, medium branch-chains were increased and short branch-chains of corn starch were decreased at high development temperatures. Similar results were reported for rice starch (Asaoko et al 1984, 1985; Inouchi et al 2000). Therefore at high grain-filling temperatures, starch would be expected to contain a larger number of longer chains of amylopectin and fewer short branch-chains than at low grain-filling temperatures.

Moisture also affects the grain-filling period. Nicolas et al (1984) found that drought, and drought in addition to high temperatures, reduced the number of cells and starch granules in the endosperm of wheat. Brooks et al (1982) also found that in wheat fewer B-type granules were produced and the size of A-type granules was reduced under water deficit. They also reported that water deficit did not affect the initial grain-filling period but reduced the final dry matter of both wheat and barley as a result of early termination of growth.

Because different environmental factors affect the structural properties of starch, there also may be an effect on the functional properties. White et al (1991) found that starches from corn grown in tropical conditions gave an elevated and narrow $R_G$ when compared with the same populations grown in temperate regions. Lu et al (1996) reported that corn starch developed at 35°C had higher $T_{oG}$ and wider $R_G$ than starch developed at 25°C. The $\Delta H_G$ did not change with elevated temperature. Ng et al (1997) examined the thermal properties of starch from 62 exotic corn inbreds planted in Georgia and Puerto Rico. The starch from Georgia had greater $T_{oG}$, $\Delta H_G$, and peak height index of gelatinization (PHI) than did the starch from corn grown in Puerto Rico. The temperature, being higher in Georgia during the grain-filling period, may have caused perfection of the crystals or raised the chain length of the medium branch-fractions of amylopectin, as reported by Lu et al (1996). Krieger et al (1998) studied corn starch thermal properties from corn grown at two environments, both only 24 km apart. The $T_{oG}$ values were different at both environments, which were attributed to soil and/or precipitation differences.

The purpose of the present study was to examine the thermal properties, using DSC, of exotic-by-adapted corn inbred lines developed from GEM breeding crosses and selected for unusual starch characteristics, that were grown in four different environments in the U.S. Corn Belt. The effect of line, environment, and line-by-environment interactions were examined to further understand the role of environment on the thermal properties of corn starch.

## MATERIALS AND METHODS

### Materials

Corn (*Zea mays* L.) exotic-by-adapted inbred lines developed from GEM breeding crosses were used in this study, along with the public inbred lines B73 and Mo17 as controls. B73 has a Stiff Stalk heterotic pattern, whereas Mo17 has a non-Stiff Stalk heterotic pattern. We developed the exotic-by-adapted inbred lines from breeding crosses made by private GEM cooperators, who crossed exotic populations (DK212T is a tropical three-way commercial hybrid developed by Dekalb Genetics in Thailand) with their proprietary inbreds of the Stiff Stalk heterotic pattern (Pollak 2002). These lines were developed by inbreeding through self-pollination, along with selection for agronomic characteristics and starch thermal properties at each generation. Five inbred generation lines [CHIS775:S1911b-37-1-2-8-7 (Chis37), CUBA164:S1511b-34-1-3-1-11-2 (Cuba34), CUBA164:S1511b-38-1-3-5-13 (Cuba38), DK212T:S0610-8-1-3-4-6-4 (Dk8), DK212T:S0610-10-1-3-6 (Dk10)] were chosen because data generated by our laboratory indicated that these lines possessed unusual desirable thermal properties as detected by differential scanning calorimetry (DSC) (Table I). The Chis37, Cuba34, Cuba38, and Dk8 parent lines were grown during the summer of 2001 in Ames, IA, whereas the Dk10 parent line was grown during the summer of 1998 at the same environment. Unusual desirable thermal properties were previously defined by Seetharaman (2001). Examples include $T_{oG}$ less than 61°C, $\Delta H_G$ less than 9.5 J/g or greater than 14.5 J/g, and $\%R$ less than 20% or greater than 80%. The selected lines were related to lines studied by Ji et al (2002) in our laboratory, in that lines in the previous study were sister or parent lines of the genotypes selected for this study. Cuba34 and Cuba38 were of the same exotic origin, but derived from different self-pollinations ($S_1$ lines) of the breeding cross. The $T_{oG}$ of the Cuba34 and Cuba38 parent lines were similar, 60.8°C and 60.6°C, respectively, but differed in $R_G$, 15.2°C and 12.8°C, respectively. The same derivation scheme applies to Dk8 and Dk10. Both the $T_{oG}$ and $R_G$ of the Dk8 and Dk10 parent lines differed. The data shown in Table I were not previously published but were produced independently in our laboratory.

### Planting Environments

The five inbred lines, plus the two controls, were planted in a randomized complete block design at four environments in the midwestern United States during the summer of 2002 with three blocks at each environment. Rows were the experimental units. Two environments were located in Ames, IA, and were only 9 km apart. The first environment, the North Central Regional Plant Introduction Farm, referred to as Ames 1, had Clarion-Webster-Nicollet association soils, which are formed in loamy glacial till and glacial sediments, moderately drained and permeable, and are found on uplands. The association consisted of 35% Clarion, 22% Webster, 10% Nicollet, and 33% minor soils. It is well-suited for crops if properly drained and erosion is controlled. The second environment, the Iowa State University Hinds Farm, referred to as Ames 2, was near a river bottom and had soils classified as a Coland-Spillville-Zook association. These soils are nearly level, moderately well to poorly drained, loamy and silty, formed in alluvium, and found on bottom lands. The association at Ames 2 consisted of 40% Coland, 32% Spillville, 15% Zook, and 13% minor soils. This association is well-suited for cultivation. The lines also were planted in Columbia, MO, and Clinton, IL. Columbia, MO, soils are classified as Freeburg silt loam, which are formed in silty alluvial sediments, very deep, somewhat poorly drained, and moderately permeable. The Missouri environment also was located near a river bottom, similar to Ames 2. The Clinton, IL, environment has a mixture of two closely related soils: Ipava silty loam (43%) and Sable silty clay loam (68%). Ipava is a very deep, somewhat poorly drained, and moderately permeable soil formed in uplands. Sable is a mesic Typic Endoaquoll, which is a very deep, poorly drained, and moderately permeable soil formed in loess on nearly leveled summits of moraines and stream terraces.

All midwestern fields were planted in May 2002: Ames 1 was planted on May 3, Ames 2 and Missouri on May 22, and Illinois on May 29. Plants in each block were self-pollinated. The Missouri environment was harvested on September 16, Illinois on October 2, Ames 1 on October 7, and Ames 2 on October 22, 2002. Ears were harvested at physiological maturity and dried at 38°C for five days, shelled, and then stored at 4°C and 45% rh until the kernels were needed for analysis.

### Bulk Starch Extraction

Two ears from each row were chosen for analysis. Corn starch was extracted as bulked five-kernel samples from each ear, according to the method of White et al (1990) with modifications by Krieger et al (1997) and Ji et al (2004b). Starch was extracted from each ear in duplicate, and two replicate analyses from each duplicate were analyzed with DSC.

### Differential Scanning Calorimetry (DSC)

Starch (3.50 mg, with 10% assumed moisture content) was weighed into an aluminum pan (Mettler AE 104, Toledo, OH) (White et al 1990). Water was added to the starch sample in a water-to-starch ratio of 2:1; the sample was hermetically sealed and allowed to equilibrate for at least 30 min before DSC analysis. All samples were analyzed using a DSC instrument equipped with thermal analysis software (Perkin Elmer DSC 7, Norwalk, CT) from 30 to 110°C at a rate of 10°C/min. Data parameters collected from the computer included onset temperature ($T_o$), peak temperature ($T_p$), conclusion temperature ($T_c$), and change in enthalpy ($\Delta H$). The enthalpy was calculated on a starch dry weight basis. Also calculated were the range ($R$) ($T_c$ minus $T_p$) and peak height index for gelatinization (PHI) [$\Delta H_G$ (dry basis)/(1/2 × $R_G$)]. A subscript G after a parameter denotes a gelatinization property. Samples were stored at 4°C for seven days to study the retrogradation characteristics. Stored samples were analyzed by DSC from 30 to 90°C at a rate of 10°C/min. The same parameters noted for gelatinization were calculated for retrogradation, except for $R$, and are denoted by a subscript R after the parameter. Percentage of retrogradation ($\%R$) also was calculated from the ratio of $\Delta H_R$ to $\Delta H_G$.

### Statistical Analysis

The effect of environment, line, and their interactions on the thermal properties of starch of inbreds from 2002 was analyzed using an analysis of variance procedure for a mixed model (v. 8.02, SAS Institute, Cary, NC). Fixed factors were environment, line, and line-by-environment interaction. Random effects were included for ear, row, block, extraction, and DSC replicate. Contrast statements were used to determine significant differences ($P < 0.05$) between environments within the same line and DSC parameter.

## RESULTS AND DISCUSSION

Differences between lines for all DSC parameters were significant at no less than $P < 0.001$, while differences between environments were significant at no less than $P < 0.01$, except for $T_{oR}$, which did not vary significantly with respect to environment. The line-by-environment interaction differed for each DSC parameter.

### Climate Variations

Production at the Ames 1 environment was low due to poor growing conditions, including poor soil quality, probably a result of high erosion, control of which is necessary for good crop cultivation for the soil association of Clarion-Webster-Nicollet at Ames 1.

The climate summaries of the three different environments are shown in Table II. In general, Missouri had greater average mean temperatures than did Ames and Illinois during the grain-filling months (July and August). The Ames environments essentially received the same weather patterns, but their difference in planting dates caused them to be exposed to the weather patterns at different stages of ear, therefore starch, development. Ames 2 may have been warmer because it was located near a river bottom and therefore more similar to the temperatures in Missouri, which also was located near a river bottom. Ames 1 and 2 had a lower amount of precipitation in August, a grain-filling month, than did the Missouri and Illinois environments.

### Variations Between Environments for B73

B73 did not perform well agronomically at several environments. Self-pollination at the Illinois and Missouri environments resulted in three complete blocks at both, whereas Ames 2 lost a block. The Ames 1 environment had only one block that produced B73 seed. Lost blocks of lines resulted from a combination of agronomic reasons including poor seed germination, insect infestation, poor seed set, and poor growing conditions.

The $T_{oG}$ at Missouri and Ames 1 were greater ($P < 0.05$) than that at Illinois, which produced B73 starch with lower $T_{oG}$ than did the other environments (Table III). The $T_{oG}$ at Ames 2 was similar to that at Ames 1 but lower than the value at Missouri. The $R_G$ of B73 grown in Missouri was narrower than that at Ames 2 and Illinois, whereas the $R_G$ at Illinois was wider than the $R_G$ at Ames 2 and Missouri. The $\Delta H_G$ at Illinois and Ames 1 were lower than the $\Delta H_G$ at Ames 2 and Missouri. The PHI at Missouri was greater than the PHI at Ames 2 and Illinois.

The $T_{oR}$ of B73 starch did not differ among environments. However, the $R_R$ at Ames 2 was greater than that at Ames 1 and Illinois. The $\Delta H_R$ at Ames 2 was greater than the $\Delta H_R$ at other environments. The $\%R$ at Illinois and Missouri were lower than that at Ames 2.

Statistical tests for interaction revealed that B73 line-by-environment interaction ($P < 0.01$) occurred for $T_{oG}$, $R_G$, $\Delta H_G$, PHI, and $\Delta H_R$, and B73 line-by-environment interaction ($P < 0.05$) occurred for $\%R$ (Table IV). Therefore, for these parameters, the relative outcome depended on the environment of cultivation of B73. The starch from Illinois tended to have lower $T_{oG}$ and wider $R_G$, which indicated less perfect crystallization of the starch (Inouchi et al 1984). These findings could have been a result of lower grain-filling temperatures in Illinois causing the crystallites to be less perfect than those developed at greater temperatures as suggested by Lu et al (1996). However, Ames had temperature patterns

**TABLE I**
**Gelatinization Characteristics and Origin of Exotic Component of Original Parent Lines of Corn**

| | | | DSC Parameters[b] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Line[a] | Abbreviation | Line Origin | $T_{oG}$ (°C) | $R_G$ (°C) | $\Delta H_G$ (J/g) | PHI | $T_{oR}$ (°C) | $\Delta H_R$ (J/g) | $\%R$ |
| CHIS775:S1911b-37-1-2-8-7 | Chis37 | Mexico | **59.7**[c] | 13.5 | 11.6 | 1.7 | 39.7 | 6.4 | 55.1 |
| CUBA164:S1511b-34-1-3-1-11-2 | Cuba34 | Cuba | **60.8** | 15.2 | 10.9 | 1.4 | 41.6 | 5.3 | 49.2 |
| CUBA164:S1511b-38-1-3-5-13 | Cuba38 | Cuba | **60.6** | 12.8 | 12.5 | 2.0 | 42.8 | 5.8 | 46.4 |
| DK212T:S0610-8-1-3-4-6-4 | Dk8 | Thailand | **59.4** | 14.0 | 11.4 | 1.6 | 42.2 | 5.6 | 49.0 |
| DK212T:S0610-10-1-3-6 | Dk10 | Thailand | 65.3 | **9.4** | 11.7 | 2.5 | 42.9 | 5.8 | 49.5 |

[a] Exotic corn inbreds derived from Germplasm Enhancement of Maize (GEM) project exotic-by-adapted breeding crosses; inbreds and data produced independently in our laboratory.

[b] $T_{oG}$, gelatinization onset temperature; $R_G$, range of gelatinization temperature; $\Delta H_G$, change in enthalpy of gelatinization; PHI, peak height index [$\Delta H_G/(R_G/2)$]; $T_{oR}$, retrogradation onset temperature; $R_R$, range of retrogradation; $\Delta H_R$, change in enthalpy of retrogradation; $\%R$, percent of retrogradation ($\Delta H_R/\Delta H_G \times 100\%$).

[c] Values in bold type are considered unique characteristics as defined by Seetharaman et al (2001), except for the $R_G$ of Dk10, which is not considered unique by those characteristics but has a value that is lower than the other samples.

**TABLE II**
**Mean Monthly Temperatures (°C) and Total Precipitation at Three Locations**

| Location (2002) | Month | Maximum | Minimum | Average | Total Precipitation (cm) |
|---|---|---|---|---|---|
| Ames, IA[a] | May | 21.6 | 8.6 | 15.1 | 10.2 |
| | June | 29.4 | 17.3 | 23.3 | 8.4 |
| | July | 31.1 | 19.4 | 25.2 | 11.7 |
| | August | 27.8 | 16.4 | 22.1 | 12.2 |
| | September | 26.2 | 12.7 | 19.4 | 3.0 |
| | October | 12.9 | 3.3 | 8.1 | 30.7 |
| Clinton IL[b] | May | 21.3 | 8.6 | 14.9 | 17.7 |
| | June | 29.3 | 17.5 | 23.4 | 9.9 |
| | July | 31.4 | 19.4 | 25.4 | 10.3 |
| | August | 28.9 | 17.7 | 23.3 | 24.3 |
| | September | 27.6 | 13.3 | 20.4 | 4.2 |
| | October | 16.5 | 5.2 | 10.8 | 7.2 |
| Columbia, MO[a] | May | 22.7 | 10.4 | 16.6 | 27.0 |
| | June | 30.0 | 19.0 | 24.5 | 8.8 |
| | July | 32.6 | 21.1 | 26.9 | 9.9 |
| | August | 31.2 | 20.2 | 25.7 | 19.8 |
| | September | 28.4 | 15.9 | 22.2 | 4.3 |

[a] National Climatic Data Center (www.ncdc.noaa.gov: Asheville, NC).

[b] Central Golden Harvest Research (Clinton, IL).

similar to those of Illinois and therefore the results between the two environments in Ames and the environment in Illinois should be similar if only temperature were considered. These expected results did not occur, perhaps because of differences in soil type, precipitation differences, or interactions between soil types, precipitation, and temperature within the Illinois and Ames environments. Missouri, however, had the highest average temperatures during the grain-filling period and resulted in B73 starch with the greatest $T_{oG}$ and narrowest $R_G$. This finding was partly consistent with results reported by Lu et al (1996), who found that starch from corn developed at higher temperatures (35°C) had greater $T_{oG}$ and wider $R_G$. Ng et al (1997) also found starch developed at higher temperatures had greater $T_{oG}$, $\Delta H_G$, and PHI. This increase may have resulted from an increase in the medium branch-chains of amylopectin caused by the higher temperature optimum of BEI or the perfection of crystallites at higher temperatures (Ng et al 1997). The Ames 2 environment also produced B73 starch with greater $\Delta H_R$ and $\%R$ than that grown in the other environments, which may have been caused by the formation of more fractions of longer amylopectin chains that were able to recrystallize during refrigerated storage.

### Variations Between Environments for Mo17

Only two blocks of Mo17 were succesfully produced in Illinois, whereas the Mo17 replicates were complete at all other environments. The $T_{oG}$ of Mo17 were in the general order Ames 2 = Missouri > Ames 1> Illinois, and Ames 2 > Ames 1 and Illinois at $P < 0.05$ (Table III). The $T_{oG}$ at Illinois was lower than the $T_{oG}$ at

**TABLE III**
**Thermal and Retrogradation Properties of Five Corn Lines and Two Controls Grown in Four Environments[a]**

| Line | Environment[b] | DSC Parameters[c] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $T_{oG}$ (°C) | $R_G$ (°C) | $\Delta H_G$ (J/g) | PHI | $T_{oR}$ (°C) | $\Delta H_R$ (J/g) | %*R* |
| B73 | Ames 1 | 65.8ab[d] | 8.4a–c | 12.6b | 3.0a–c | 41.9a | 5.6bc | 45.0ab |
| | Ames 2 | 66.2b | 9.0b | 13.3a | 3.0b | 42.1a | 6.9a | 50.6a |
| | Illinois | 63.7c | 10.2a | 12.6b | 2.5c | 41.0a | 5.7c | 44.5b |
| | Missouri | 67.2a | 7.7c | 13.2a | 3.4a | 41.0a | 6.1b | 46.3b |
| Mo17 | Ames 1 | 67.7b | 8.5b | 12.9c | 3.1b | 41.4b | 6.5b | 50.1a |
| | Ames 2 | 69.0a | 8.7b | 14.0a | 3.2ab | 42.6a | 7.3a | 53.0a |
| | Illinois | 65.4c | 10.5a | 13.5b | 2.6c | 40.7b | 6.5b | 49.4a |
| | Missouri | 68.7ab | 8.3b | 14.0a | 3.4a | 41.3b | 6.3b | 44.6b |
| Chis37 | Ames 1 | 64.3bc | 9.5b | 12.3b | 2.6ab | 40.9ab | 6.2b | 52.5a |
| | Ames 2 | 66.0ab | 9.9b | 13.3a | 2.7a | 42.3a | 7.1a | 51.9a |
| | Illinois | 62.8c | 11.5a | 12.4b | 2.2b | 40.7b | 5.3c | 42.9b |
| | Missouri | 66.8a | 9.2b | 13.1a | 2.9a | 42.0a | 6.0b | 46.2ab |
| Cuba34 | Ames 1 | 61.6c | 14.4a | 11.7b | 1.6c | 40.9b | 5.1b | 42.5b |
| | Ames 2 | 64.4b | 11.3c | 12.5a | 2.2b | 42.2a | 6.2a | 49.2a |
| | Illinois | 62.2c | 13.1b | 11.8b | 1.8c | 40.8b | 5.3b | 44.2bc |
| | Missouri | 65.9a | 10.1d | 12.6a | 2.5a | 41.3ab | 5.9a | 47.5ac |
| Cuba38 | Ames 1 | 65.9a | 9.1a | 12.5b | 2.8a | 41.1b | 5.8bc | 48.3b |
| | Ames 2 | 65.8a | 9.3a | 12.9a | 2.8a | 42.4a | 6.8a | 52.9a |
| | Illinois | 64.5b | 9.0a | 12.6ab | 2.8a | 40.5b | 5.6c | 45.4b |
| | Missouri | 66.0a | 9.4a | 12.9a | 2.8a | 41.5ab | 6.0b | 47.1b |
| Dk8 | Ames 1 | 63.3c | 10.3a | 12.0b | 2.4b | 40.9b | 5.6c | 46.3b |
| | Ames 2 | 64.7ab | 10.6a | 13.1a | 2.5ab | 42.8a | 6.6a | 50.4a |
| | Illinois | 64.2bc | 9.9a | 12.1b | 2.5ab | 40.5b | 5.7c | 47.5ab |
| | Missouri | 65.7a | 9.7a | 13.3a | 2.8a | 41.2b | 6.2b | 46.7a |
| Dk10 | Ames 1 | 64.3c | 10.1a | 12.4b | 2.5b | 40.7b | 6.1b | 49.2ab |
| | Ames 2 | 65.7ab | 9.7ab | 13.2a | 2.7ab | 42.2a | 6.8a | 52.1a |
| | Illinois | 64.7bc | 8.8b | 12.8a | 2.9a | 40.6b | 6.0b | 47.1b |
| | Missouri | 66.5a | 9.2ab | 13.3a | 3.0a | 43.5a | 6.4ab | 48.8ab |

[a] Exotic corn inbreds: B73, Mo17, CHIS775:S1911b-37-1-2-8-7 (Chis37), CUBA164:S1511b-34-1-3-1-11-2 (Cuba34), CUBA164:S1511b-38-1-3-5-13 (Cuba38), DK212T:S0610-8-1-3-4-6-4 (Dk8), DK212T:S0610-10-1-3-6 (Dk10).
[b] Ames 1 and Ames 2 (each 9 km apart); Clinton, IL; and Columbia, MO.
[c] DSC parameters are defined in Table I.
[d] Values followed by different letters are significant at $P < 0.05$ for a DSC parameter within one set of locations for a line.

**TABLE IV**
**Significance of Line Source by Environment Interaction Effects on Each Line from Analysis of Variance of Starch Gelatinization Properties[a–d]**

| Line | DSC Parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| | $T_{oG}$ (°C) | $R_G$ (°C) | $\Delta H_G$ (J/g) | PHI | $T_{oR}$ (°C) | $\Delta H_R$ (J/g) | %*R* |
| B73 | ** | ** | ** | ** | ns | ** | * |
| Mo17 | ** | ** | ** | ** | ** | ** | ** |
| Chis37 | ** | ** | ** | ** | * | ** | ** |
| Cuba34 | ** | ** | ** | ** | * | ** | ** |
| Cuba38 | * | ns | ns | ns | ** | ** | ** |
| Dk8 | ** | ns | ** | ns | ** | ** | ns |
| Dk10 | ** | ns | ** | * | ** | ** | * |

[a] B73, Mo17, CHIS775:S1911b-37-1-2-8-7 (Chis37), CUBA164:S1511b-34-1-3-1-11-2 (Cuba34), CUBA164:S1511b-38-1-3-5-13 (Cuba38), DK212T:S0610-8-1-3-4-6-4 (Dk8), DK212T:S0610-10-1-3-6 (Dk10).
[b] Ames 1, Ames 2, (each 9 km apart), Clinton, IL, and Columbia, MO.
[c] Defined in Table I.
[d] *, ** Indicates significance at $P < 0.05$ and $P < 0.01$, respectively; ns = not significant.

all other environments. The $R_G$ at Illinois was greater than the $R_G$ at the other environments. The $\Delta H_G$ at Ames 2 and Missouri were greater than the $\Delta H_G$ at other environments. The PHI values were in the general order Missouri > Ames 2 > Ames 1 > Illinois, and Missouri > Ames 1 and Illinois at $P < 0.05$.

The $T_{oR}$ and $\Delta H_R$ of Mo17 grown at Ames 2 were greater than the $T_{oR}$ and $\Delta H_R$ of Mo17 grown at the other three environments. The $\%R$ at Missouri was lower than $\%R$ at other environments.

Significant interactions ($P < 0.01$) between Mo17 and environment were found for the $T_{oG}$, $R_G$, $\Delta H_G$, PHI, $T_{oR}$, $\Delta H_R$, and $\%R$. Similar to the B73 starch, lower $T_{oG}$ and wider $R_G$ resulted at Illinois compared with the other environments, likely indicating a less perfect crystalline structure of the amylopectin (Inouchi et al 1984). Again, this difference was probably a result of lower temperatures at the time of starch development. Similar to B73 starch, the Missouri environment produced Mo17 starch with greater $T_{oG}$, narrower $R_G$, and greater $\Delta H_G$, which could indicate more perfect crystallization of the amylopectin strands or more crystallization overall and may have been a result of higher mean average temperatures during the grain-filling period. However, the Ames environments had weather similar to Illinois, but did not produce results similar to that environment or similar to each other. This latter difference may be a result of the actual environment of Ames 2, which was near a river bottom, likely causing higher temperatures than actually reported for Ames from the National Climatic Data Center (www.ncdc.noaa.gov) and producing Mo17 with starch that had a greater $T_{oG}$, $\Delta H_G$, $T_{oR}$, $\Delta H_R$, and $\%R$ than from the Ames 1 environment, indicating more perfect crystals. Also, the two environments had different types of soil, as noted above, which may have affected the results. The Ames 1 environment had poor growing conditions, including poor soil quality. The Ames 2 environment soil, of better quality than the Ames 1 soil, may have interacted with the environment, causing these differences in starch quality.

**Variations Between Environments for Chis37**

Chis37 did not perform well agronomically due to late silk dates, thereby interfering with pollination. The Illinois and Missouri environments produced incomplete replicates for analysis, whereas Ames 2 lost one block and Ames 1 lost two blocks.

Chis37 was chosen as a line of interest because its starch previously was reported to have low $T_{oG}$ (59.7°C) (Table I). In the present study, progeny seed only partly retained the properties, particularly $T_{oG}$ and $R_G$, of the original line (Table III). The $R_G$ decreased from 13.5°C in the parent line to an average of 9.5°C for the current generation. The Illinois environment, however, produced Chis37 starch with an $R_G$ of 11.5°C, which was closer to that of the parent line. The Illinois environment also produced Chis37 starch with a $T_{oG}$ of 62.8°C, which was the closest of the progenies to the $T_{oG}$ of the parent line.

The parent Chis37 was produced during the summer of 2001 at a third Iowa environment (Iowa State University Agronomy and Agricultural Engineering Environment). This environment was ≈10 km from Ames, IA, in Boone County and had soils that were Canisteo-Clarion-Nicolet associations. The association had 29% Canisteo, 27% Clarion, 14% Nicollet, and 30% minor soils. This type of soil is very good for cultivation, being characterized as loamy, nearly level to moderately sloping, poorly drained to well-drained, and found on uplands. The temperature was relatively similar to the temperature during the summer of 2002 during the grain-filling months. There was considerably less precipitation, however, during July and August of 2001 in Ames (4.2 and 6.8 cm, respectively) than July and August of 2002 in Ames (11.7 and 12.2 cm, respectively). The lesser precipitation and different environment may have affected the differences in thermal properties of the parent and succession lines.

None of the 2002 environments resulted in starch with $T_{oG}$ as low or $R_G$ as wide as these values from the Chis37 parent line grown in 2001. One explanation for this finding is that that the traits are genetically complex, and genes affecting the parameters were still segregating, producing ears with kernels that were not completely homogenous in thermal properties. This segregation may then have caused the progeny grown in summer 2002 to produce starch that did not completely mimic the starch from the parent lines due to genes that controlled starch production with major effects and numerous modifier genes with small effects as suggested by Campbell et al (1995b). This suggestion is somewhat unlikely, however, because the Chis37 progeny were highly inbred. By way of explanation, Table I displays the full name of each line. Each number after a dash is a generation of self-pollination. With each self-pollination, homozygous genes increase by 25%. Thus, Chis37 of 2002 is 99% homozygous because it had been self-pollinated six times. Therefore, it is more likely that the phenotypic outcome of Chis37 was affected more by environment and the line-by-environment interaction than by genotypic variations alone (Table IV).

The $T_{oG}$ of starch from Chis37 grown at Missouri was greater than that from Illinois and Ames 1, and the $T_{oG}$ at Illinois was less than the $T_{oG}$ from Missouri and Ames 2 (Table III). The $R_G$ at Illinois was greater than the $R_G$ at the other three environments. The $\Delta H_G$ at Ames 2 and Missouri were greater than the $\Delta H_G$ at Illinois and Ames 1. The PHI at Ames 2 and Missouri were greater than the PHI at Illinois.

Retrogradation results showed that the $T_{oR}$ of starch from Chis37 grown at Ames 2 and Missouri were greater than the $T_{oR}$ at Illinois. The $\Delta H_R$ at Ames 2 was greater than the $\Delta H_R$ at other environments. The $\%R$ at Ames 1 and Ames 2 were greater than the $\%R$ at Illinois.

A significant interaction ($P < 0.01$) existed between the Chis37 line and environment of cultivation for $T_{oG}$, $R_G$, $\Delta H_G$, PHI, $\Delta H_R$, and $\%R$ (Table IV), and an interaction between Chis37 and environment ($P < 0.05$) also existed for $T_{oR}$. Similar to the Mo17 and B73 results, Chis37 starch grown at Illinois had lower $T_{oG}$, wider $R_G$, and lower $\Delta H_G$, whereas the Chis37 starch from Missouri had greater $T_{oG}$, narrower $R_G$, and greater $\Delta H_G$. Again, these differences may be a result of higher temperatures in Missouri than in Illinois during the grain-filling period. The Ames 1 environment produced starch similar to that from the Illinois environment, whereas the Ames 2 environment produced starch similar to that of Missouri. Similar to the results for B73 and Mo17, the Ames 2 environment tended to produce Chis37 starch with the greatest $\Delta H_R$, $T_{oR}$, and $\%R$. The differences between Ames 1 and Ames 2 may be caused by differences in temperature, with Ames 2 being warmer because of its environment in a river bottom. Differences in soil type and quality may also have affected the results.

**Variations Between Environments for Cuba34**

Cuba34 performed very well agronomically with complete replicates at all environments. Cuba34 was chosen as a line of interest because its starch previously had a low $T_{oG}$ (60.8°C) (Table I). Starch from Cuba34 at Ames 1 resulted in $T_{oG}$ of 61.6°C and a broad $R_G$ of 14.4°C (Table III), which were similar to those of the starch from the parent line.

The $T_{oG}$ at Missouri was greater than the $T_{oG}$ at all other environments (Table III). The $R_G$ at Ames 1 was greater than the $R_G$ at other environments and the $R_G$ at Missouri was the lowest. The $\Delta H_G$ at Ames 2 and Missouri environments were greater than the $\Delta H_G$ at Ames 1 and Illinois. The PHI at Missouri was greater than the PHI at Illinois and Ames 1.

The $T_{oR}$ at Ames 1 was greater than the $T_{oR}$ at Illinois and Ames 1. The $\Delta H_R$ at Ames 2 and Missouri were greater than the $\Delta H_R$ at Ames 1 and Illinois. The $\%R$ at Ames 2 was greater than the $\%R$ at Illinois and Ames 1.

Significant interactions ($P < 0.01$) were found between Cuba34 line and environment for $T_{oG}$, $R_G$, $\Delta H_G$, PHI, $\Delta H_R$, and $\%R$, and interactions ($P < 0.05$) also were found for $T_{oR}$ (Table IV). Similar to the results of the genotypes just discussed, starch from Cuba34 grown in Illinois and Ames 1 had lower $T_{oG}$, wider $R_G$, and lower $\Delta H_G$ than starch from Cuba34 produced in Missouri and Ames 2. For retrogradation results, the Ames 2 environment again produced starch with greater $T_{oR}$, $\Delta H_R$, and $\%R$ than Cuba34 starch from other environments. Again, the differences between Missouri and Illinois may be a result of higher temperatures in Missouri than in Illinois. The phenotypic variations between Ames 1 and Ames 2 may have been caused by differences in soil type and quality of growing conditions and also the possibility of higher temperatures at Ames 2 as a result of its environment near a river bottom.

**Variations Between Environments for Cuba38**

The Cuba38 corn line performed well agronomically with complete blocks obtained at all five environments. It was chosen as a line of interest because its starch exhibited a low $T_{oG}$ (60.6°C) and moderately wide $R_G$ (12.8°C) (Table I). No progeny planted at the four environments exhibited these interesting thermal properties of the parent Cuba38 line (Table III). The $R_G$ of starch from the progeny were narrower (9.2°C) than the starch of parent line Cuba38. The parent Cuba38 line (Table I) was grown in Ames during the summer of 2001 at a different environment than in the present study. As noted in the discussion about the Chis37 line, the different environment and climate during 2001 may have affected the results. Also as previously noted, the Cuba38 line, like the Chis37 line, also had been self-pollinated six times, making the genes 99% homogenous, thus the phenotypic variations in starch properties between parent Cuba38 and progeny Cuba38 starches were most likely a result of the effects of environment and of line-by-environment interaction rather than genotypic effects alone.

The $T_{oG}$ at Illinois was less than the $T_{oG}$ at the other three environments (Table III). The $R_G$ did not differ among the four environments. The $\Delta H_G$ at Missouri and Ames 2 were greater than the $\Delta H_G$ at Ames 1, whereas the $\Delta H_G$ at Illinois did not differ from $\Delta H_G$ at the other environments. The PHI at the four different environments were not different from each other.

The $T_{oR}$ at Ames 2 was greater than that at Ames 1 and Illinois. The $\Delta H_R$ at Ames 2 was greater than that at other environments. The $\Delta H_R$ at Illinois was lower than that at Ames 2 and Missouri. The $\%R$ at Ames 2 was greater than that at other environments.

There was a significant interaction ($P < 0.01$) between Cuba38 line and growing environment for $T_{oR}$, $\Delta H_R$, and $\%R$, and an interaction ($P < 0.05$) also was found for $T_{oG}$ (Table IV). The $T_{oG}$ at Missouri was greater than the $T_{oG}$ at Illinois, which is similar to the results for B73, Mo17, Chis37, and Cuba34. The Ames 1 and 2 environments, however, were similar to each other and to Missouri, with respect to $T_{oG}$. The $T_{oR}$, $\Delta H_R$, and $\%R$ were generally greater for starch produced at the Ames 2 environment than for starch from the other environments, which is similar to the other lines previously discussed. Fewer Cuba38 starch parameters were affected by environment than for the previous genotypes; however, no environment produced Cuba38 starch that retained the thermal properties of the parent line. These results suggest either an overall interaction between the 2001 environment and 2002 environments, or continued genetic segregation. As previously mentioned, this latter suggestion is not likely. Probably the phenotypic variations from year to year were caused by variations in environment and in line-by-environment interactions.

**Variations Between Environments for Dk8**

Dk8 performed well agronomically, resulting in only one missing block at the Illinois environment. The later generations produced starch that only partly retained the original unique property of $T_{oG}$ 59.4°C (Tables I and III). The Dk8 seed planted in the present study came from parents produced in the summer of 2001 at the same environment as the Chis37, Cuba34, and Cuba38 parents. Again, the Dk8 progeny from the summer of 2002 was the seventh inbred generation, suggesting that phenotypic variation between the parent and progeny starch thermal properties were likely a result of environmental effects and line-by-environment interaction effects rather than genotypic variation alone.

The $T_{oG}$ at Missouri was greater than the $T_{oG}$ at Illinois and Ames 1 (Table III). The $T_{oG}$ at Ames 1 was lower than the $T_{oG}$ at Ames 2 and Missouri, and the $R_G$ of Dk8 starch did not differ among the four environments. The $\Delta H_G$ at Ames 2 and Missouri were greater than the $\Delta H_G$ at Illinois and Ames 1. The PHI at Missouri was greater than the PHI at Ames 1.

The $T_{oR}$ and $\Delta H_R$ at Ames 2 were greater than these values at the other four environments. The $\Delta H_R$ at Ames 2 was greater than that at the other three environments. The $\%R$ at Ames 2 and Missouri were greater than the $\%R$ at Ames 1.

Significant interactions ($P < 0.01$) were found between the Dk8 line and environment for $T_{oG}$, $\Delta H_G$, $T_{oR}$, and $\Delta H_R$ (Table IV). Dk8, like Cuba38, was less affected by environment than the other lines. However, no progeny retained the unique properties of the parent line like Cuba38, which was likely a result of an overall environment effect between summer 2001 and summer 2002 in Ames, IA.

Similar to results with the genotypes previously discussed, Dk8 starch tended to have greater $T_{oG}$ and $\Delta H_G$ when grown in Ames 2 and Missouri than when grown at Ames 1 and Illinois. Also similar to other results, the Ames 2 environment produced Dk8 starch with greater $T_{oR}$ and $\Delta H_R$ than did the other environments.

**Variations Between Environments for Dk10**

Dk10 seed was obtained from only one block in Missouri, whereas all other environments produced three full blocks of Dk10. Dk10 was chosen as a line of interest because although it was a sibling of Dk8, its starch had gelatinization properties that were more typical of traditional starches, with a $T_{oG}$ of 65.3°C and a narrow $R_G$ (9.4°C) (Table I). The $T_{oG}$ and $R_G$ of successions produced in the present study were similar to those of the parent line starch, with $T_{oG}$ of 64.3–65.8°C and $R_G$ of 8.6–10.1°C (Table III).

The $T_{oG}$ at Missouri was greater than those at Ames 1 and Illinois. The $R_G$ at Ames 1 was greater than the $R_G$ at Illinois. The $\Delta H_G$ at Ames 1 was lower than that at the other three environments. The PHI at Illinois and Missouri were greater than the PHI at Ames 1.

The $T_{oR}$ at Ames 2 and Missouri were greater than those at Ames 1 and Illinois. The $\Delta H_R$ at Ames 2 was greater than that at Ames 1 and Illinois, and $\%R$ at Ames 2 was greater than that at Illinois.

Significant interactions ($P < 0.01$) occurred between the Dk10 line and environment for $T_{oG}$, $\Delta H_G$, $T_{oR}$, $\Delta H_R$ and $\%R$, and interaction ($P < 0.05$) also was found for PHI and $\%R$. The Dk10 starch from Missouri and Ames 2 had greater $T_{oG}$ and $\Delta H_G$ than did starch from corn grown in Illinois and Ames 1 and, similar to other genotypes, was likely a result of differences in temperatures, soil types, and quality. Also, similar to the other lines, Ames 2 produced starch that had greater $T_{oR}$, $\Delta H_R$, and $\%R$ than did starch from all other environments except for Missouri, whose Dk10 starch had greater $T_{oR}$.

## CONCLUSIONS

The results in this study are relatively consistent with the results of previous studies (White et al 1991; Lu et al 1996; Ng et al 1997). The differences in DSC parameters have been attributed to the impact of temperature on enzyme activity within the kernel, with a rise in temperature increasing the amount of longer branches of amylopectin or perfecting the amylopectin crystalline structure. Evidence from the other environments, however, suggests that soil type and quality also may affect the thermal properties of starch from these inbred lines. The Ames 1 and 2 environments produced starch with thermal properties that were consistently different from each other. The Ames 2 environment was located in a river bottom, which is generally warmer, and the environments had different soil types. The temperature difference may have caused the results of the Ames 2 environment to be more similar to that of the Missouri environment, the warmest of all environments in this study. The significant variations between the Ames environments may also have been a result of differences in soil type and quality. The Ames 1 environment, with poorer quality soil of the Clarion-Webster-Nicollet association, produced starch with thermal properties generally similar to those from Illinois. The Ames 2 environment, with better quality soil of the Coland-Spillville-Zook association, generally produced starch with thermal properties similar to those of the starch from Missouri. Starch from the Ames 2 environment also consistently had greater $T_{oR}$, $\Delta H_R$, and $\%R$ than did starch from the other three environments in the Midwest. These results may be a result of increased numbers of longer amylopectin chains in the Ames 2 corn starches that caused a stronger network during retrogradation.

Previously, related lines were successfully developed that retained unique thermal characteristics with further selfing (Ji et al 2004a). The advanced generations in this study only partly retained the interesting thermal properties present in their parent lines. It is not likely that the genes were still segregating, causing these differences, because the inbreeding level of the lines should have resulted in a high degree of homogeneity (99%). The most likely scenario is that the phenotypic variations of the lines were affected by environment and line-by-environment interactions.

Both Cuba lines had excellent agronomic performance in this study. The estimated yields were even higher than that of the control lines, B73 and Mo17, suggesting the use of Cuba lines for agronomic improvement in addition to unusual starch thermal traits.

Overall, a number of factors can affect the thermal properties of corn starch from a particular growing environment, including temperature, precipitation, soil type, and growing conditions. In the current study, the strongest relationships were between temperature and soil type. There also were highly significant interactions between the growing environment and line, thereby complicating the prediction of the effect of a growing environment on the thermal properties of corn. Future studies could include controlled growing environments in a greenhouse to further elucidate the effects of temperature, precipitation, and soil type on the thermal properties of starch from these exotic lines, with an additional focus on understanding the complicated inheritance of unusual starch traits in these exotic-by-adapted inbreds.

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