

Effects of Pollination Method and Growing Location on Starch Thermal Properties of Corn Hybrids¹

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ABSTRACT

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Starch gelatinization and retrogradation properties of corn were studied to determine the effect of controlled (self) pollination versus noncontrolled pollination on analytical determinations, and the potential to eliminate the expensive and time-consuming step of self-pollinating before research screening of corn genotypes. Twenty-four hybrids were grown in two Iowa locations, Story City and Ames. At Story City, all hybrids received three pollination treatments: self-pollination; small-plot, open-pollination (representing corn from small test plots); and large-plot, open-pollination (representing corn from a farmer's field). Self-pollinated and small-plot, open-pollinated corn were grown in replicated two-row plots, whereas large-plot, open-pollinated corn was grown in unreplicated plots of 12.8 m × 8 rows. At Ames, the small-plot, open pollination treatment was not done. Starch was extracted from samples of corn harvested from

each plot, and gelatinization and retrogradation properties were determined using differential-scanning calorimetry (DSC). Hybrids exhibited different starch gelatinization and retrogradation properties. Significant differences ($P \leq 0.05$) in starch gelatinization and retrogradation properties occurred among pollination methods and between locations. Pollination method did not influence gelatinization enthalpy values, but onset temperature values for gelatinization, and range values for retrogradation differed significantly among pollination methods. At Ames, treatments gave different values for retrogradation enthalpy and percentage of retrogradation. Because of differences in some starch characteristics associated with pollination methods, self-pollination is recommended when growing samples in small plots for research purposes.

Open-pollination can be used to improve and develop corn. Breeders use mass selection to improve inherited characteristics such as ear and plant height, maturity, and kernel and ear characteristics. In an open-pollinated field, each ear of corn is pollinated randomly by pollen from nearby corn plants, and fertilization is not controlled. Thus, only the female genetic contribution is known (Gardner 1961).

Since most genes controlling endosperm composition are recessive, open-pollinated fields containing test cultivars need to be isolated from normal dent corn (Zuber and Darrah 1987). Unfortunately, it is impractical to use isolated plantings when small quantities of many different genotypes are needed. If the fertilization process were controlled, however, many different corn genotypes could be planted together. Therefore, self-fertilization techniques were implemented to develop pure, inbred lines in corn and to select for homozygous biotypes (Zuber and Darrah 1987). This process, called recurrent selection, is used to improve corn for traits that are polygenically inherited or to enhance expression of a trait that is controlled by many genes, each having a small effect (Zuber and Darrah 1987). Recurrent selection is accomplished by sibbing, selfing, or crossing lines, followed by selection.

Unfortunately, the process of self-pollinating of corn plants is tedious, labor-intensive, and expensive, even though the outcome is beneficial. Self-pollination requires placing bags on tassels during pollen production and on ear shoots before silk development. At pollination time, bags are shifted from tassels to the ears. Although self-pollination controls gene expression, its usefulness in fixing starch properties of corn, a target of the current investigation, has not been established. The value of using self-pollination as a means to prevent contamination from foreign pollen, i.e., the effect of foreign pollen during pollination on starch properties of the resulting kernels on an ear of a corn plant, has also not been

established. Furthermore, the importance of being able to manipulate and control starch properties is becoming increasingly more evident. Variability in starch thermal properties among different corn hybrids has been observed recently (Pollak and White 1997). Significant differences were noted in differential scanning calorimetry (DSC) parameters of starch among Corn Belt inbreds and exotic inbred lines from Argentina, Uruguay, and South Africa. Reciprocal cross values showed trends suggesting reciprocal differences, indicating that the thermal traits were controlled by many modifying effects. Thus, sufficient variability existed in the germ plasma to warrant further studies on inheritance of these traits and eventual breeding for these properties.

Starch granules begin developing at seven to 10 days after pollination (DAP), and cease developing at 30–35 DAP (Wolf et al 1948, Earley 1952). Thus, starch synthesis by basal endosperm cells does not begin until late in kernel development (Boyer et al 1976). The changes in thermal properties of corn starches during five stages of kernel maturity (12, 18, 24, 30, and 36 DAP) were studied recently using DSC (Ng et al 1997a). Thermal properties of starches during early development were different from those of their mature counterparts, indicating possible differences in the fine structure of starch during endosperm development, caused by variations in chain lengths of the molecules. Other research has shown that kernel starch properties are affected by temperature, soil type, planting date, year, and location (Dunn et al 1953, Juliano et al 1969, Kongseree and Juliano 1972, Lempiainen and Henriksnas 1979, White et al 1991, Ng et al 1997b). Therefore, changes in environments during kernel development may alter the composition of the seeds, including starch.

If kernels obtained from open-pollinated plots had the same starch-thermal properties as kernels obtained from self-pollinated ears, then the self-pollination step would not be needed for producing research samples. Thus, this time-consuming and costly procedure could be eliminated from future inheritance studies of starch thermal characteristics. Furthermore, to interpret results of inheritance studies and to compare data to that of other studies, it is important to know whether other environmental factors, such as location of growth, can influence starch thermal properties of corn. Therefore, the objectives of this research were to: 1) determine the effect of controlled (self) pollination versus noncontrolled pollination on starch thermal properties, 2) determine whether the expensive and time-consuming step of self-pollination before research screening of corn genotypes can be eliminated, and 3) determine

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whether corn grown in different locations differs in starch thermal properties.

MATERIALS AND METHODS

Corn Populations

Twenty-four adapted corn hybrids from similar maturity groups were obtained from MBS Incorporated (Story City, IA) and Pioneer Hi-Bred International, Inc. (Johnston, IA). They were selected to provide as wide a range as possible in grain type and quality within current commercial and experimental hybrids (Table I). Hybrids were planted on May 17, 1995, at Ames, IA, and on May 26, 1995, at Story City, IA. At Story City, hybrids received three pollination treatments: self-pollination; small-plot, open-pollination (representing corn from small test plots); and large-plot, open-pollination (representing corn from farmers' fields). Hybrids grown at Ames were either self-pollinated or large-plot, open-pollinated. Self-pollinated and small-plot, open-pollinated hybrids were grown in two-row plots (6.4 m long), with two replicates arranged in a randomized complete block design. Large-plot, open-pollinated hybrids were grown in plots of 12.8 m \times 8 rows with one replicate. Spacing between rows was 75 cm for all treatments.

The small-plot, open-pollinated treatment was representative of corn grown in research plots. Research fields often contain a variety of corn genotypes grown next to each other in small plots. Large-plot, open-pollinated fields reproduced conditions in a farmer's field with corn genotype per plot.

In each two-row plot, 10 random plants were self-pollinated and harvested. Ten open-pollinated ears were chosen randomly from the remaining plants in two-row plots (small, open-pollinated corn) and from the two middle rows of the large, open-pollinated plots.

Ears from the Ames location were harvested on October 10, 1995, and dried at 37.5°C until moisture content reached 12% (seven days). Ears from the Story City location were harvested on October 14, 1995, and dried at 37.8°C until moisture content reached 12% (two days). Shelled kernels from individual plots were bulked and completely mixed.

Starch Extraction

Starch extraction, including steeping, screening, and drying, was completed using the method of White et al (1990). A tissue homogenizer was used for blending, as recommended and described by Krieger et al (1997). Three separate starch extractions were completed for each treatment (10 random kernels per extraction). Kernels for each of the three extractions were selected by taking a random scoop from the bulk, then counting out the three samples using a seed counter. After extraction, samples were stored in airtight vials before evaluation by differential scanning calorimetry (DSC).

DSC

Starch samples (\approx 4 mg) were weighed in aluminum pans and 8 μ L of distilled water were added. Pans were sealed and allowed to equilibrate for 1 hr at room temperature (21°C). A Perkin-Elmer DSC-7 analyzer (Norwalk, CT) was used to heat starch samples from 30 to 110°C for gelatinization. Initial screening of starch samples showed that heating samples to 110°C was adequate because all starch samples gelatinized at temperatures far below this point. After gelatinization, samples were stored at 4°C for seven days and then heated from 30 to 90°C for retrogradation analysis. The ending temperature of 90°C was considered sufficient because no additional information about the samples was gained at temperatures $>90^\circ\text{C}$. A thermal analysis data station was used to calculate onset temperature (T_o) and enthalpy (ΔH) of starch for both gelatinization and retrogradation. Peak height index (PHI) of gelatinization was calculated from $\Delta H/(T_p - T_o)$, where T_p = peak temperature (Krueger et al 1987). Ranges of gelatin-

ization and retrogradation were calculated as $R_n = 2(T_p - T_o)$ (Krueger et al 1987), and percent retrogradation (%R) was calculated as $(\Delta H \text{ retrogradation}/\Delta H \text{ gelatinization}) \times 100$ (White et al 1989). Only one DSC run was needed for each starch extraction because previous work in our laboratory demonstrated little variation in repeated analyses of the same starch sample (White et al 1990).

Near-Infrared Spectroscopy

Shelled corn from each plot was analyzed with a Tecator Infratec 1221 near-infrared instrument (Silver Spring, MD). Percentages of protein, oil, and starch are reported on a dry-weight basis. Calibrations were performed according to methods of the American Oil Chemists' Society (protein, Ba 3-38 and oil, Ba 4-38 [AOCS 1988]) and the Corn Refiners' Association (starch, G-28 [CRA 1986]).

Statistical Analysis

Proximate analysis data was averaged for all hybrids and treatments. Statistical differences ($P \leq 0.05$) were computed using PROC GLM (SAS Institute, Cary NC). Mean DSC values from three starch extractions (10 kernels per extraction) were compared among pollination methods, locations, and hybrids. Pollination by hybrid and pollination by location interaction effects also were investigated.

Data were analyzed using a randomized block design, with locations and pollination methods as blocks and hybrids as treatments.

RESULTS AND DISCUSSION

Composition of Corn by Near-Infrared Spectroscopy

Table I gives range and mean values for density and percentages of oil, protein, starch, and moisture. Values were typical of normal dent corn at $\approx 72\%$ starch, 9–10% protein, and 4–5% oil, indicating that the corn chosen for this study had compositional characteristics similar to those of corn normally grown in the Corn Belt (White and Pollak 1995).

Effects of Pollination Method and Location on Gelatinization

Table II lists mean starch-gelatinization values for hybrids grown at Ames and Story City. The T_o values for pollination treatments were significantly different at both locations. Values ranged from 66.7 to 68.0°C. Also, T_o values for self-pollinated and large-plot, open-pollinated corn differed between locations. The standard error of the mean (SEM) was greater for the large-plot, open-pollination method than for the self-pollination and small-plot, open-pollination methods perhaps because the large-plot, open-pollination method only had one replicate.

Enthalpy values were not affected by pollination method. Large-plot, open-pollinated corn from the two locations had significantly different ΔH values. But, the ΔH values for self-pollinated corn showed no location differences.

Pollination methods produced different PHI and R_n values only at Story City. The PHI and R_n values were not different between pollination methods at Ames. However, these values for large-plot, open-pollinated corn differed between locations.

Effects of Pollination Method and Location on Retrogradation

Table II also shows mean starch retrogradation values for dent corn hybrids grown at Ames and Story City. Mean T_o values for pollination methods showed significant differences at the Story City location but not at Ames. Small-plot, open-pollinated corn had a lower T_o (43.4°C) than did self-pollinated corn (44.3°C).

The ΔH values and %R for self-pollinated and large-plot, open-pollinated corn differed at the Ames location but not at Story City. The %R for self-pollinated corn at Ames and Story City were different. The R_n (retrogradation) values for pollination methods differed significantly at both locations, with values ranging from 18.0 to 19.8°C.

The differences in retrogradation values varied between locations, depending upon the pollination method being considered. For

example, Story City and Ames locations produced significantly different values for all retrogradation properties for self-pollinated corn. On the other hand, there were no significant differences between the two locations for large-plot, open-pollinated corn.

Location Differences

Differences in starch thermal properties based on location were significant, even though locations were only 24 km apart. Location effects could be due to environmental factors such as soil type or precipitation. The Ames hybrids grew on a soil of a Coland-Spillville-Zook association, whereas Story City corn grew on a soil of a Kossuth-Ottosen-Bode association. A Coland-Spillville-Zook association is characterized as nearly level land with moderately well-drained to poorly drained loamy and silty soils, found on bottom lands. A Kossuth-Ottosen-Bode association is characterized as nearly level to gentle sloping land, which ranges from

well-drained, somewhat poorly drained, to poorly drained, and has silty and loamy soils found on uplands (USDA, Soil Conservation Service, soil survey of Story County, IA, 1984).

Corn hybrids were pollinated in July. Because starch synthesis occurs at seven to 35 DAP (Wolf et al 1948), starch would have been synthesized in July and August. Differences in August precipitation at Ames and Story City might have affected starch synthesis. Ames received 7.68 cm (National Oceanic and Atmospheric Administration [NOAA], Iowa climatological data, volume 106, May-Oct. 1995), whereas Story City had 6.33 cm (Iowa State University Horticulture Station weather summary, May-Oct. 1995). Additional research is needed to determine the effect of these environmental factors, which differ among locations, on starch composition of corn.

Pollination and Hybrid Differences

Because gelatinization and retrogradation properties differed significantly among pollination methods, self-pollination is recommended for producing corn samples for determination of starch characteristics. Even though self-pollination is labor-intensive and expensive, the procedure is necessary to obtain corn with consistent starch functionality within a plot.

Hybrid by pollination and hybrid by location interactions for some retrogradation and gelatinization values were significant, but are not shown because these interactions were not the focus of this study. Such interactions were expected because hybrids behave differently, depending on environmental influences (Thompson et al 1973, Campbell et al 1994). Hybrid by pollination interactions were evaluated statistically, consistent with the objectives of this

TABLE I
Proximate Analysis (%) by Near-Infrared Reflectance (NIR)
of Dent Corn Hybrid Kernels^a

| NIR Parameter | Minimum | Mean | Maximum |
|------------------------------|---------|------|---------|
| Moisture | 8.4 | 9.0 | 9.6 |
| Protein | 8.1 | 9.1 | 9.9 |
| Oil | 3.5 | 4.0 | 4.5 |
| Starch | 70.6 | 71.7 | 73.0 |
| Density (g/cm ³) | 1.23 | 1.27 | 1.30 |

^a Minimum, mean, and maximum values (dwb) for population containing all samples included in the study ($n = 24$).

TABLE II
Effects of Pollination Method and Growing Location of Dent Corn on Gelatinization and Retrogradation Properties of Starch
as Determined by Differential Scanning Calorimetry (DSC)^a

| | Self | Small-Plot, Open | SEM ^b | Large-Plot, Open | SEM ^c |
|---------------------------|---------------------|------------------|------------------|------------------|------------------|
| Gelatinization properties | | | | | |
| T_o (°C) ^d | | | | | |
| Ames | 68.0e* ^e | | (0.232) | 66.7f* | (0.328) |
| Story | 67.0f* | 67.8e | (0.147) | 67.7e* | (0.207) |
| ΔH^f (cal/g) | | | | | |
| Ames | 3.25 | | (0.033) | 3.19* | (0.047) |
| Story | 3.27 | 3.25 | (0.019) | 3.27* | (0.027) |
| PHI ^g | | | | | |
| Ames | 0.78 | | (0.029) | 0.77* | (0.041) |
| Story | 0.77g | 0.91e | (0.026) | 0.85f* | (0.037) |
| R_n^h (°C) | | | | | |
| Ames | 8.6 | | (0.220) | 8.6* | (0.311) |
| Story | 8.8e | 7.3g | (0.219) | 8.0f* | (0.309) |
| Retrogradation properties | | | | | |
| T_o (°C) | | | | | |
| Ames | 42.8* | | (0.573) | 43.9 | (0.810) |
| Story | 44.3e* | 43.4f | (0.478) | 43.8ef | (0.676) |
| ΔH (cal/g) | | | | | |
| Ames | 1.32e* | | (0.062) | 1.10f | (0.087) |
| Story | 1.08* | 1.08 | (0.059) | 1.14 | (0.084) |
| R_n (°C) | | | | | |
| Ames | 19.8e* | | (0.505) | 18.7f | (0.714) |
| Story | 18.3f* | 19.1e | (0.453) | 18.0f | (0.640) |
| % R^i | | | | | |
| Ames | 40.6e* | | (2.311) | 35.1f | (3.268) |
| Story | 33.0* | 33.1 | (1.820) | 35.0 | (2.574) |

^a Means from three starch extractions per experimental unit. Treatments (hybrids) were grown twice (self-pollinated and small-plot, open-pollinated corn) or once (large-plot, open-pollinated corn).

^b Standard error of mean (SEM) for self-pollinated corn at Ames and SEM for the self- and small-plot, open-pollination methods at Story City for each thermal property.

^c SEM for large-plot, open-pollination method at Ames and Story City for each thermal property.

^d Onset temperature.

^e Values for pollination methods followed by the same letter within a row are not significantly different ($P < 0.05$). Values within a column for each DSC parameter followed by * were significantly different ($P < 0.05$).

^f Enthalpy.

^g Peak height index.

^h Range.

ⁱ Percent retrogradation.

study. Interactions were significant only for PHI and R_n values for gelatinization properties in self-pollinated and large-plot, open-pollinated corn.

CONCLUSIONS

Starch gelatinization and retrogradation properties for self-pollinated corn differed from those of small-plot, open-pollinated, and large-plot, open-pollinated corn. These property differences were likely caused by contamination during pollination by other corn having different genetic backgrounds and, thus, different starch characteristics. Therefore, self-pollination is an important and necessary procedure for producing corn samples with reproducible starch characteristics that would be needed to conduct research studies. But, even though pollination methods produced statistically different starch thermal properties, differences were not great enough to be important for practical purposes. Producers concerned with starch quality can grow specialty corn crops (i.e., corn with specific starch quality characteristics) by normal open-pollination procedures.

Differences based on location were significant, even though locations were only 24 km apart. Location effects may be associated with differences in environmental factors such as soil type or precipitation. Reasons for differences in starch thermal properties of corn grown in different locations might be related to differences in development of the corn kernel, as noted previously (Ng et al 1997a). Research studying effects of location on starch composition of corn is needed to ascertain the importance of location differences.

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