Properties of Sugary-2 Maize Starch: Influence of Exotic Background[1]

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ABSTRACT

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Genetic modifiers of maize (*Zea mays* L.) starch thermal properties were examined by differential scanning calorimetry (DSC). Sugary-2 (su_2) kernels from segregating ears were identified based on textural appearance of starches following crosses between an exotic maize accession with the inbred OH43 homozygous for the su_2 allele (OH43su_2). Two exotic maize accessions, PI213768 and PI451692, were used. Germs retained from su_2 kernels were used to produce an F2 population of su_2 plants containing 50% exotic germ plasm. With few exceptions, F2 ears from the populations were homozygous for the su_2 allele. Significant ($P \leq 0.05$) differences were seen between the exotic populations and OH43su_2 for gelatinization onset temperature (T_o), range (RN), enthalpy (ΔH), and retrogradation (%R). The number of DSC values with significant within-population variations was greater among F2 ears within the exotic populations than

among ears within the inbred line OH43su_2. Standard deviations for DSC values were consistently greater for exotic su_2 populations than for those of OH43su_2. Also, the population PI213768su_2 differed greatly from OH43su_2 for mean values of T_o, RN, ΔH, and %R (52.8°C, 13.4°C, 1.5 cal/g, and 34.5%, respectively) when compared to those of OH43su_2 (54.6°C, 10.6°C, 1.3 cal/g, and 29%, respectively). Results from this study indicate that examining the texture of starches from single kernels could be used to identify and develop populations homozygous for the su_2 allele. In addition, the increased variability for DSC values within populations containing 50% exotic germ plasm suggest that genetic modifiers might be used to alter thermal properties and, possibly, functional properties of su_2 starch.

The sugary-2 (su_2) allele in maize (*Zea mays* L.), identified by Eyster in 1934, results in starches with a higher amylose content and a lower birefringence end-point temperature than that of normal corn starch. Differential scanning calorimetry (DSC) thermograms of su_2 starch have a lower gelatinization onset temperature (T_o), gelatinization peak (T_p), and total enthalpy (ΔH) than does normal starch (Inouchi et al 1991a). The su_2 starches also have less retrogradation during storage than do normal starches, as measured by DSC (Inouchi et al 1991b, White et al 1994, Campbell et al 1994). In addition, several patents have resulted on the use of starches from genotypes possessing the su_2 allele, alone or in combination with other mutant genes, because they display unique physical properties (Katz 1991, White et al 1994).

Modifying genes, having quantitative effects, have been shown to interact with the mutant amylose-extender *(ae)* allele, resulting in a wide range of amylose contents (AM). The effects of modifying genes vary depending on the genetic background. For example, AM of segregating F2 kernels from F1 ears derived from 135 dent inbreds crossed to an *ae* genotype ranged from 36.5 to 64.9% (Bear et al 1958). Similarly, starch characteristics of su_2 genotypes vary depending on genetic background. Bear et al (1958) reported a range in AM of 31–42% among ears within a su_2 converted inbred line.

The conversion of maize germ plasm using endosperm mutant genes usually involves an initial cross followed by several generations of backcrossing; the segregating mutant kernels are selected visually (Bear et al 1958). Garwood and Creech (1972) provided a description of kernel phenotypes resulting from single- and multiple-endosperm mutations that can be used to discriminate segregating kernels. But Vineyard et al (1958) reported difficulties in identifying segregating *ae* kernels resulting from crosses with

normal maize inbred lines when endosperm color genes are present. In addition, Bear et al (1958) identified differences in kernel color, degree of translucence, and fullness among maize inbreds possessing the *ae* allele. To overcome difficulties in identifying mutant kernels, Haunold and Lindsey (1964) used a chemical analysis that preserved the embryo in a viable state after crosses with a yellow *ae* stock and Missouri Cassel, a nonmutant white variety. Amylose contents were determined from endosperms of F2 kernels to identify those homozygous for the *ae* allele. The germ was removed to preserve the genetic material of *ae* kernels for inclusion in further breeding studies.

Tracy (1990) emphasized the importance of exploring exotic germ plasm for improving quality traits in maize because "much of the maize grown outside the U.S. is consumed directly by humans and has undergone centuries of selection for flavors, aromas, and textures". Recently, DSC has revealed variations in starch thermal properties among exotic sources of maize germ plasm (White et al 1990, Li et al 1994).

The objectives of this study were to first explore methods for rapidly discriminating su_2 kernels from segregating ears while maintaining the viability of the germ. This method was then used to study the possible contribution that genetic factors from two exotic sources of maize germ plasm, differing in kernel texture and color, make in modifying the expression of su_2.

MATERIALS AND METHODS

Plant Material

Populations of F2 plants homozygous for the su_2 allele containing 50% exotic germ plasm were developed (Fig. 1). In 1992, the accessions PI213768 and PI451692 were used as females in crosses with the inbred OH43su_2 in a breeding nursery near Ames, IA. PI213768 is a Great Plains flour-corn landrace with blue kernels collected in Iowa. PI451692 (Cargill north temperate zone Coroico) is an unimproved population composed of 94% tropical germ plasm with floury to flinty-floury kernels and colors ranging from white to varying shades of yellow to occasional red. These maize accessions were obtained from the North Central Regional Plant Introduction Station located in Ames, IA. Three ears per population were harvested and dried to a moisture content of ~13%.

During the winter of 1992–93, F1 plants from seed of the exotic × OH43su_2 crosses were grown in the greenhouse (two plants/ear). Plants were self-pollinated and F1 ears (producing F2 kernels segregating for su_2) were harvested at physiological maturity.

Identification of su₂ Kernels

In the laboratory, using a modified procedure described by Haunold and Lindsey (1964), an approximately equal proportion of F2 kernels were taken from each ear to screen for homozygous su_2 kernels. F2 kernels were steeped overnight in distilled water and stored at 4°C. After steeping, F2 germs were carefully removed with a razor blade, allowed to dry at room temperature, and stored in aluminum foil. The F2 endosperms were steeped for an additional 48 hr in 0.45% sodium metabisulfite at 50°C and starch was extracted as described by White et al (1990).

Preliminary observations were made on starches from several normal and su_2 inbred lines, including OH43, after drying at ambient room conditions following the starch isolation procedure. The su_2 starches formed hard aggregates upon crushing with a metal spatula, unlike the more floury texture of normal starch (data not shown). The appearance of the air-dried starches from the segregating F2 kernels also fell into the same two texture types (aggregated and floury) after crushing the starches. To determine the extent to which starches classified by texture corresponded to chemical composition of the starches, a subsample of starches from 116 segregating kernels were assayed for AM.

Amylose Determination

The AM of single F2 kernels was determined colorimetrically by dissolving ~5.0 mg of starch in 10 ml of 90% dimethyl sulfoxide containing $6 \times 10^{-3} M$ iodine (Knutson 1986). One milliliter of the dissolved sample was diluted to 9 ml with H_2O, and the absorbance was measured at 600 nm on a spectrophotometer (Hitachi U-2000, Tokyo, Japan). Purified AM was prepared from maize starch as described by Schoch (1942) and used to construct a standard curve.

Embryo Culture

Of 600 F2 kernels screened, 130 su_2 germs were selected, based on whether their starches had an aggregated rather than floury starch texture. In mid-May of 1993, selected embryos were germinated on a growth medium in agar plates containing a modified Murashige and Skoog medium (1962). The medium was prepared with 175 ml of H_2O, 25 ml of Murashige and Skoog basal salt macronutrient solution (M0654, Sigma Chemical Co., St. Louis, MO), 25 ml of Murashige and Skoog basal salt micronutrient solution (M0529, Sigma), 2.5 g of sucrose, 1.75 g of agar, and 0.5 g L-Asparagine adjusted to pH 6.8. Germination occurred under continuous light at 27°C. At the same time, kernels of OH43su_2 were planted in pots containing soil in the greenhouse. After three to four days, germs were removed from the growth chamber and transferred into pots. When plants reached approximately the third leaf stage, they were transferred to the field. Transplants were arranged in a completely randomized design. The populations of F2 plants containing 50% of the genetic materials from PI213768 or PI451692 were designated as PI213768su_2 and PI451692su_2, respectively. A total of 116 F2

transplants (PI213768su_2 and PI451692su_2) were included in the field experiment, in addition to 64 OH43su_2 transplants. Self-pollinations were made for the OH43su_2 and F2 plants, with the exception of several protandrous plants for which pollen was used from another individual within the respective population. F2 ears (producing F3 kernels) were harvested and dried as described previously.

From each su_2 F2 population (PI213768su_2 and PI451692su_2), and for OH43su_2, 14 ears of the best overall quality were selected for DSC.

DSC

DSC analysis was done on an analyzer equipped with a thermal-analysis data station (DSC7, Perkin-Elmer Crop., Norwalk, CT). Analysis of starch gelatinization was conducted as described by White et al (1990). Approximately 4.0 mg (dwb) of starch was weighed into aluminum sample pans, then 8 mg of distilled water was added. Samples were heated from 30 to 102°C at a rate of 10°C/min. DSC parameters recorded for this study included ΔH, T_o, T_p, and RN. The parameters T_o, T_p, and ΔH were given directly by the DSC software. Because the endotherms were symmetrical, the RN was calculated as $2(T_p - T_o)$ according to Krueger et al (1987). Samples were stored for seven days at 4°C and rerun using DSC to determine starch gel retrogradation (%R) as described by White et al (1989). A single DSC run was made per kernel from three kernels per ear.

Statistical Analysis

F-tests were used to determine significant effects among and within genotypes from the ANOVA by using a completely randomized design. Pearson's simple correlations were used to identify correlations between DSC values of F2 kernels produced in the greenhouse and the mean of F2 ears (F3 kernels) grown in 1993 (SAS 1990).

RESULTS AND DISCUSSION

Identification of Mutant Kernels

Samples of F1 ears produced in the greenhouse during 1992–93 are shown in Figure 2. As expected, ears segregated for various
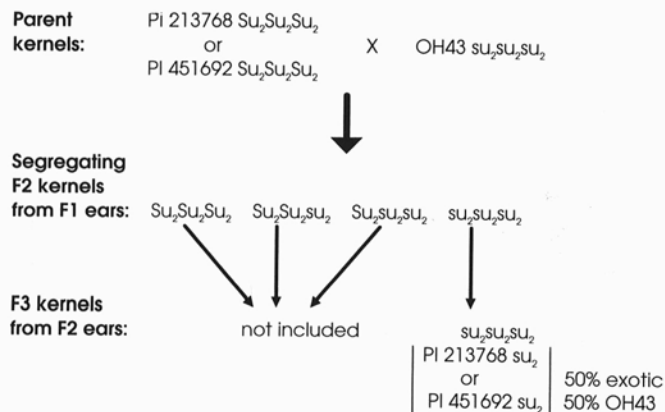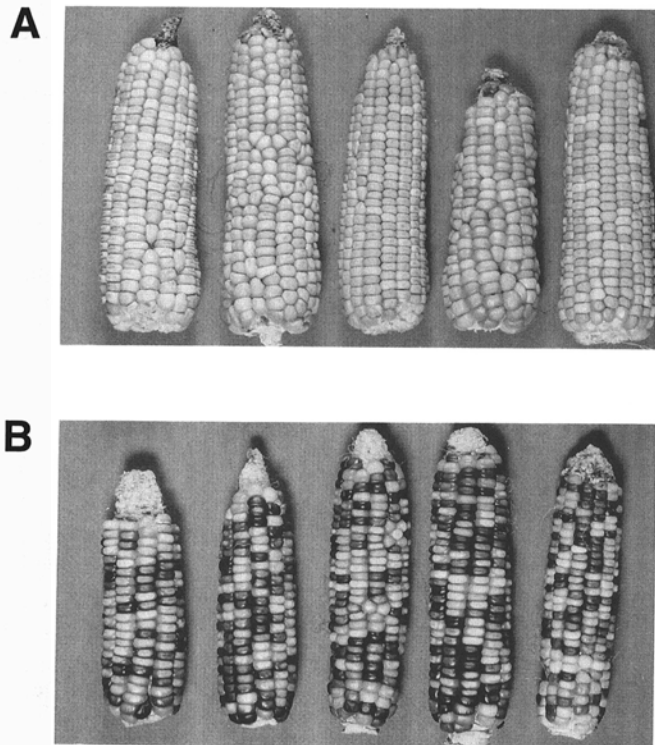
Fig. 2. F1 ears segregating for su_2 maize kernels resulting from crosses between the exotic populations PI451692 **(A)** and PI213768 **(B)** with OH43su_2.

Parent kernels:

PI 213768 $Su_2Su_2Su_2$
or   X   OH43 $su_2su_2su_2$
PI 451692 $Su_2Su_2Su_2$

Segregating F2 kernels from F1 ears:

$Su_2Su_2Su_2$ $Su_2Su_2su_2$ $Su_2su_2su_2$ $su_2su_2su_2$

F3 kernels from F2 ears:

not included

$su_2su_2su_2$
PI 213768 su_2
or | 50% exotic
PI 451692 su_2 | 50% OH43

Fig. 1. Scheme for developing su_2 maize F2 populations (PI213768su_2 and PI451692su_2) from the cross between the maize accessions PI213768 and PI451692 with OH43su_2.

kernel colors and textures, in addition to the su_2 allele. Phenotypic descriptions of endosperm mutants in Corn Belt dent backgrounds (W64A and W23) provided by Garwood and Creech (1972), indicated a slight tarnished and etched appearance of su_2 kernels. Although this description can be used to distinguish su_2 from normal kernels in Corn Belt dent germ plasm, in this study, variations in kernel color and texture did not allow easy recognition of these characteristics.

The su_2 kernels were selected for textural appearance; starches were either aggregated (su_2) or floury (normal). Percentages of AM were compared with samples of a subsample of 116 kernels classified by starch texture (Table I). Mean AM% of starches classified as aggregated was ~10% greater than mean AM% of normal floury starches for both populations. These means are in agreement with previous reports of a 10–15% increase in AM of starches from su_2 genotypes (Shannon and Garwood 1984). Ranges in AM% overlapped between the two starch texture groups among F2 kernels from PI451692 × OH43su_2 F1 ears. This may be the result of either inaccuracies in starch texture classification or AM determination. However, genetic variations in these starch traits within su_2 mutant and normal kernels could possibly account for the overlapping values. For example, Bear et al (1958) observed ranges in AM% in single kernels of 31–42% among genotypes homozygous for su_2. Use of AM% would be expected to be less reliable in discriminating between su_2 and normal genotypes than for discriminating between ae and normal genotypes, in which the minimum value of the range (36.5–64.9%) was greater (Bear et al 1958).

DSC Values Among F3 Kernels

Initially, 16 F2 ears were selected per population. One of the F2 ears from PI213768su_2 and two from PI451692su_2 had at least one F3 kernel that gave rise to DSC thermograms resembling normal or dosage-intermediate starches as described by Campbell et al (1994). The presence of normal and dosage-intermediate starches from F3 kernels on an F2 ear strongly indicated that either the ears were segregating for the su_2 allele as a result of the F2 plants not being in a homozygous condition, or that contamination by foreign pollen may have occurred. These ears were subsequently eliminated from the study. Therefore, 14 ears were selected from each population to be included for DSC analysis.

DSC values for starches from all individual F3 kernels (data not shown) resembled values reported for su_2 genotypes by Inouchi et al (1991a). DSC thermograms also were examined from the originating F2 kernel starches, all of which displayed su_2-type DSC values rather than those typical of normal starch (data not shown). These data support the idea that classification on the basis of starch texture could be used to discriminate su_2 kernels segregating on F1 ears, thus providing a means of identifying mutant su_2 kernels when color and kernel type interfere with the su_2 phenotype. Application of other nondestructive techniques also may be useful in discriminating genotypes homozygous for the mutant su_2 allele. Zaitlin (1993), for example, reported use of a molecular marker assay for the waxy allele, based on polymerase chain reaction (PCR), in the conversion of maize lines. This method allows for the unequivocal identification of plants that are homozygous for the recessive allele.

Variations Among and Within Populations

Significant differences ($P \leq 0.05$) were observed among the su_2 populations of PI213768su_2, PI451692su_2 and the inbred OH43su_2 for T_o, RN, ΔH, and %R as determined from ANOVA (Table II). Both populations significantly differed from OH43su_2 for T_o, RN, and %R. However, only PI213768su_2 differed from OH43su_2 for ΔH. The two populations differed from each other only for RN and ΔH.

Highly significant ($P \leq 0.01$) variations were observed among ears within genotypes for all DSC values with the exception of %R (Table II). When genotypes were examined individually as a source of variation in the ANOVA table, significant variations for DSC values (except %R) occurred among ears within the exotic su_2 populations (PI213768su_2 and PI451692su_2). But for variations within OH43su_2 ears, the level of significance was reduced for T_o and there were no significant differences for RN and ΔH.

Means, ranges, and standard deviations among 14 ears per genotype are compared in Table III. Generally, su_2 F2 ears of the exotic populations had lower T_o and greater RN, ΔH, and %R than did OH43su_2. Ranges and standard deviations of DSC values among su_2 F2 ears for both exotic populations were greater than were those of OH43su_2. These data indicate that the genetically variable nature of the F2 populations from the exotic materials resulted in greater variations for DSC values.

The influence of exotic germ plasm on DSC parameters suggests that modifying genetic factors may play a role in altering expression of the su_2 allele with respect to starch thermal properties. A more thorough screening of exotic sources of maize germ plasm may reveal additional sources of major and minor modifying genes. Several studies have indicated that qualitative and quantitative differences in DSC values reflect variations in starch functional properties (Wang et al 1992, Campbell 1994). Fergason (1994) demonstrated that selection within populations homozygous for the ae allele could be used to effectively increase AM%. Selection based on DSC values within genetically variable su_2 populations may also lead to the development of genotypes with desired starch properties.

Correlation Analysis

To determine whether DSC values of the starch of F2 kernels could be used to predict thermal properties of the F3 kernels on F2 ears, a correlation analysis was conducted between the two sets of data (Table IV). Correlation coefficients were not high for any DSC value. However, the greatest correlation noted for the RN ($r = 0.37$) did approach significance ($P = 0.056$). In a similar study by Haunold and Lindsey (1964), a significant

TABLE II
Levels of Significance for the Effects of Genotype and Ears Within Genotype for su_2 F2 Ears from Exotic × OH43su_2 Crosses and OH43su_2

Source	DF	T_o[a]	T_p[b]	RN[c]	ΔH[d]	%R[e]
Genotype	2	**[f]	NS[g]	**	*	*
PI213768su_2 vs OH43su_2	1	**	NS	**	**	**
PI451692su_2 vs OH43su_2	1	*	NS	**	NS	*
PI213768su_2 vs PI451692su_2	1	NS	NS	**	**	NS
Ears (Genotype)	39	**	**	**	**	NS
Ears (PI213768su_2)	13	**	**	**	**	NS
Ears (PI451692su_2)	13	**	**	**	**	NS
Ears (OH43su_2)	13	*	**	NS	NS	NS
Error	84	...	...	...	...	...
CV%[h]		2.0	1.6	12.4	11.3	22.4

[a] Gelatinization onset temperature.
[b] Gelatinization peak temperature.
[c] Gelatinization range.
[d] Enthalpy of gelatinization.
[e] Retrogradation (ΔH initial run/ΔH rerun after seven days at 4.0°C) × 100.
[f] *, ** = Significant at $P \leq 0.05$ and 0.01, respectively.
[g] Not significant at $P \leq 0.05$.
[h] Coefficient of variation.

TABLE I
Starches from F2 Kernels Resulting from the Crosses Between PI213768 and PI451692 with OH43su_2 Selected for Either Aggregated or Floury Starch Textures and Their Amylose Contents

F1 Population Cross	Starch Texture Type	Number of F2 Kernels	Mean Amylose Content, %	Amylose Range, %
PI213768 × OH43su_2	Aggregated	8	35.8	31.7–41.9
	Floury	50	25.0	22.1–31.7
PI451692 × OH43su_2	Aggregated	16	34.6	28.1–41.6
	Floury	42	24.4	19.1–30.9

TABLE III
Means, Standard Deviations (SD), and Ranges for Differential Scanning Calorimetry Values
Determined from su_2 F2 Ears (PI213768su_2 and PI451692su_2) and OH43su_2

su_2 F2 Population (Exotic × OH43su_2)	Number of Ears	Mean ± SD (Range)				
		T_o [a]	T_p [b]	RN [c]	ΔH [d]	%R [e]
PI213768su_2	14	52.8 ± 1.6 (50.4–55.7)	59.5 ± 1.1 (57.7–61.3)	13.4 ± 1.7 (11.3–16.3)	1.5 ± 0.16 (1.2–1.7)	34.5 ± 4.8 (26.7–47.0)
PI451692su_2	14	53.5 ± 1.3 (50.8–55.9)	59.6 ± 1.0 (57.9–61.8)	12.2 ± 1.3 (9.0–14.3)	1.3 ± 0.21 (1.0–1.6)	32.9 ± 5.1 (26.1–42.2)
OH43su_2	14	54.6 ± 0.8 (53.7–56.2)	59.9 ± 0.8 (59.1–61.6)	10.6 ± 0.7 (9.5–11.9)	1.3 ± 0.05 (1.2–1.4)	29.0 ± 4.1 (21.1–35.3)

[a] Gelatinization onset temperature (°C).
[b] Gelatinization peak temperature (°C).
[c] Gelatinization range (°C).
[d] Enthalpy of gelatinization (cal/g).
[e] Retrogradation (%) (ΔH initial run/ΔH rerun after seven days at 4.0°C) × 100.

TABLE IV
Correlations Between Differential Scanning Calorimetry Values
Determined from su_2 F2 Kernels Harvested During 1992–93 and the
Mean of F3 su_2 Kernels Obtained from F2 Ears Harvested in 1993

DSC Value	Correlation Coefficient
T_o [a]	−0.05
T_p [b]	0.00
ΔH [c]	−0.15
RN [d]	0.37
%R [e]	−0.01

[a] Gelatinization onset temperature (°C).
[b] Gelatinization peak temperature (°C).
[c] Enthalpy of gelatinization (cal/g).
[d] Gelatinization range (°C).
[e] Retrogradation (%) (ΔH initial run/ΔH rerun after seven days at 4.0°C) × 100.

correlation ($r = 0.52$, $P \leq 0.01$) occurred between F2 kernels and F2 ears for amylose content within a genetically variable *ae* population. The authors suggested that selection based on F1 'kernels could be advantageous in selecting desirable high-amylose genotypes in early stages of line development. The lack of significant correlations in this study could be the result of inadequate variability within populations or the extremely different environments in which plants were grown (field vs. greenhouse).

CONCLUSION

The formation of hard aggregates versus a floury texture after drying starches was used as a basis for identifying kernels homozygous for the su_2 allele. This method was effective in creating populations of homozygous su_2 F2 ears containing 50% exotic germ plasm from a total of 32 ears (with the exception of three F2 ears) selected as homozygous su_2. Identification of su_2 kernels in this manner, therefore, is useful when visual identification of mutant phenotypes is masked because of variations in kernel color and texture. In addition, this study provides evidence that populations containing 50% exotic maize germ plasm possess genetic modifiers, as indicated from the increased variances in DSC values of the starches, compared to the variances observed within the inbred line OH43su_2.

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