Characterization of sugary-1 (su-1) sugary enhancer (se) Kernels in Segregating Sweet Corn Populations

Don R. La Bonte and John A. Juvik
Department of Horticulture, University of Illinois, Urbana, IL 61801

Abstract. A single-kernel, sugar analysis technique was used to study the genetic relationship between morphological and metabolic traits previously associated with expression of the sugary enhancer (se) endosperm mutation in a su-1 sweet corn (Zea mays L.) background. Analysis of sucrose and total carotene content in su-1 kernel populations segregating for se showed that light-yellow kernel color was a reliable phenotypic indicator for kernels homozygous for the se gene. High levels of kernel maltose was not always indicative of su-1 se kernels in mature (55 days after pollination) kernel populations. Characteristic high levels of percent moisture in su-1 se kernels at 28 and 35 days post-pollination were identified as an expression of high sugar content. Kernels homozygous for su-1 se were also found to weigh less at maturity than su-1 Se kernels, and se was found to be partially expressed in a heterozygous condition.

Traditional sweet corn cultivars have the endosperm carbohydrate fraction modified by the sugary-1 (su-1) mutation. Although kernels of su-1 cultivars have a very desirable, creamy texture at eating maturity, they contain about half the total sugar content of “super sweet” cultivars (Cameron and Teas, 1954; Creech, 1965; Creech, 1968; Laughnan, 1953). Endosperm carbohydrates of super sweet cultivars are modified by mutations such as shrunken-2 (sh-2), brittle (bt), and brittle-2 (bt-2), or the combination of the mutants amylose extender (se), dull (du), and waxy (wx) (Creech, 1968). In addition to consumer preference for the higher sugar levels, improved postharvest storage properties are also associated with high sugar content (Garwood et al., 1976; Flora and Wiley, 1974).

Super sweet cultivars retain higher levels of sugar and moisture for a longer period after harvest than traditional su-1 cultivars (Garwood et al., 1976; Soberlaske and Andrew, 1978; Warm et al., 1971). One disadvantage of super sweet cultivars is the texture is not as creamy as that found in su-1 cultivars (Gonzales et al., 1974, 1976). Kernel creaminess depends on high levels of phytoglycogen, a water-soluble starch (Gonzales et al., 1976).

The sugary enhancer (se) gene is a recessive modifier of the su-1 endosperm mutation (Ferguson et al., 1978). When homozygous, the se allele increases total sugar levels in su-1 kernels to levels comparable to those in sh-2 kernels without a reduction in phytoglycogen (Gonzales et al., 1974; Gonzales et al., 1976). Hence, su-1 se kernels contain the desirable textural characteristics of su-1 kernels and the high levels of sugar found in super sweet cultivars. Desirable moisture and sugar retention during postharvest storage are also associated with su-1 se kernels (Carey et al., 1982; Gonzales et al., 1976).

Unlike other endosperm mutations, su-1 kernels homozygous for se are not always phenotypically different from su-1 Se kernels in segregating populations. Two phenotypic traits, lighter yellow kernel color and slower dry-down (Gonzales et al., 1974), are associated with kernels homozygous for se, but we have observed that these traits are variable in expression, depending on the genetic background and kernel maturity. This lack of a readily identifiable trait has limited breeders’ ability to incorporate se into elite germplasm.

Our objective was to more fully develop the relationship between metabolic and morphological traits associated with se gene expression, and to determine whether these traits facilitate identification of individual su-1 se kernels in segregating populations.

Materials and Methods

Plant materials. Three inbred maize lines IL451b (su-1 Se), IL678a (su-1 Se), and IL677a (su-1 se) developed at the Univ. of Illinois Agricultural Experiment Station were used in this study. Both su-1 Se inbreds were crossed with IL677a to generate F1 seed using IL677a as the female. F1 seed was planted and selfed to generate F1 seed. Rows of 25 seeds of each of the inbreds and hybrids were planted on the Dept. of Horticulture’s Vegetable Research Farm in South Urbana in a randomized block design with four replicates. Six or seven ears in each row were self-pollinated, bagged to prevent contamination, and dated. An additional six or seven ears of IL677a in each replicate were pollinated with pollen from IL678a to create F1 seed. Ears were harvested at 21 (eating maturity), 28, 35, and 42 days after pollination (DAP), immediately frozen in liquid N2, and then stored in a freezer at –80°C for later analysis. Additional ears from each replicate were harvested at 55 DAP, dried in a forced-air oven at 33°C for 72 hr, and stored in the laboratory as mature-dry kernel samples.

Extraction and analysis of maize kernels. Individual kernels were removed from the frozen immature ears, weighed, freeze-dried, reweighed, placed in 1.5-ml plastic microcentrifuge tubes, and ground into a powder with a Phillips screwdriver. Mature dry kernels were weighed and ground in a rotary mill with a 20-mesh screen. Ground powder (100 to 200 mg) from a mature-dry kernel or the entire freeze-dried kernel was placed in a microcentrifuge tube and extracted four times with 1.0-ml por-
tions of 95% ethanol on a rotary-evaporator at 70°C for 15 min per extraction. After each extraction, the vials were centrifuged for 10 min at 8000 × g. The supernatant fluids were brought up to a 5.0-ml volume with ethanol.

The sugars contained within a 50-µl aliquot of each extract were converted to trimethylsilyl (TMS) derivatives in the sample for 10 min at 8000 × g. After each extraction, the vials were centrifuged. The supernatant fluids were brought up to a 5.0-ml volume with ethanol.

Adsorption of total carotene in ethanol extracts was determined using a Zeiss PMQ II spectrophotometer. Adsorption values were converted to a dry-weight basis (mg total carotene/g dry kernel weight) by comparison with a set of Sudan I (phenylazo-2-napthol) 95% ethanol standards. Quantification is based on 0.04 mm of Sudan I =2.35 mg total carotene/liter at 436 nm in an acetonitrile solution (Association of Official Analytical Chemists, 1984). The exchange of ethanol for acetone: isopropyl alcohol did not significantly alter the slope of the standard solution set. A typical adsorption spectra between 400 and 450 nm for carotenoids (Davies, 1976) was observed (La Bonte, 1988).

Effect of se gene expression on total carotene content and sucrose (Expt. 1). An experiment was conducted to determine the extent of variation in total kernel carotene content between su-I Se inbreds and a su-I se inbred, between ears of the same inbred, and between kernels from the same ear. A seeded ear from each of the four replicates of the inbreds IL677a (se), IL451b (Se), and IL678a (Se) at 21 DAP were used. Six kernels were selected from each ear for single-kernel analysis. The total sums of squares from an analysis of variance (ANOVA) was partitioned into component sums of squares for genotype, ear-to-ear within a genotype, and kernel-to-kernel within an ear. Means and ss for total carotene represents a sum of all kernels for each inbred.

To determine the relationship between se gene expression and kernel color, 95 kernels were randomly selected from a single ear of the F1 kernel population (IL677a × IL678a) self, for single-kernel analysis at 21 DAP. Individual kernels were classified visually as either light or dark yellow before extraction and quantification of sucrose and total carotene. These two kernel classes were subjected to X analysis to determine whether expected segregation ratios would be obtained. Means were separated by the F test. A multivariate analysis was used to solve a quadratic equation with total carotene and sucrose as variables. The solutions from the quadratic equation were then used to develop 95% confidence ellipses.

To determine whether endosperm heterozygous for these gene effects endosperm sucrose, 25 kernels were randomly selected from individual ears harvested at 21 DAP of IL678a, IL677a, and their F1 (IL677a × IL678a), for single-kernel analysis. The triploid maize endosperm arises from the union of two polar nuclei contributed by the female and one nucleus contributed by the male. Dosage for se varies from zero for IL678a, two for F1 kernels (IL677a as female), to three for IL677a. Data were subjected to ANOVA and means were separated by Fisher’s LSD.

Effect of se gene expression on kernel maltose and weight (Expt. 2). To determine the relationship between se gene expression, mature kernel dry weight, and maltose level, 50 kernels from a single ear were randomly selected from the mature dry F1 kernel population: (IL451b × IL677a) self, for single-kernel analysis. Individual kernels were classified visually as either light or dark-yellow. Kernel dry weight represents the weight of the whole kernel after drying in a forced-air oven at 33°C for 72 hr. Data were subjected to ANOVA and means were separated by the F test. Development of 95% confidence ellipses, used in the study concerning kernel dry weight, was described previously.

Effect of se gene expression on percent moisture (Expt. 3). To determine the relationship between se gene expression and percent moisture, 151 kernels were randomly selected from an F1 kernel population from a single ear of (IL677a × IL678a) self harvested at 35 DAP for single-kernel sugar analysis. Differences in percent moisture content between su-I Se and su-I se kernels are greatest at 35 and 42 DAP (La Bonte, 1988). Percent moisture is a measure of the proportion of the total kernel weight due to water content, and is calculated from the following equation: percent moisture content = (fresh kernel weight – dry kernel weight)/fresh weight × 100. Data were subjected to regression analysis at P > 0.05, and means were separated by the F test.

Results and Discussion

Carotene and sucrose contents. Kernels of the su-I se inbred, IL677a, possessed a significantly lower amount of total carotene than kernels of the su-I Se inbreds, IL451b and IL678a (Table 1). The primary source of the total variability in kernel total carotene comes from genetic differences between the two inbreds in each comparison. Sources of variation due to ears-within-inbreds and kernels-within-ears accounted for a smaller proportion of the total variation in total carotene. Only ear-to-ear variation within inbreds for the comparison involving kernels of IL677a and IL678a was significantly different.

The proportion of variation in total carotene attributed to the genotype of the kernel is similar to that observed for sucrose in a previous comparison of IL677a and IL451b (Juvik and La Bonte, 1988). Together, these results indicate that kernels homozygous for se might be identified by single-kernel analysis in segregating plant populations based on total carotene content and sucrose, particularly when segregation is occurring on an individual ear.

The 95 kernels selected from an F1 kernel population at 21 DAP fit a significant X distribution (X2 = 0.032, 0.50 > P > 0.20) for a 3:1 segregation ratio for se (3 Se: 1 se) based on kernel color (Table 2). The light-yellow kernel class had 63% less total carotene than the dark-yellow kernel class. These data

| Mean total carotene concn (mg g-1 dry wt) and ss | Inbreds | Ears (inbreds) | Kernels (ears) |
|-----------------------------------------------|--------|---------------|----------------|
| IL677a                                        | 0.244  | 0.056         | 0.404          |
| IL678a                                        | 0.244  | 0.056         | 0.445          |
| IL451b                                        | 0.096  | 0.060         | 0.060          |

Table 1. Total carotene in inbred kernels of IL677a, IL451b, and IL678a with relative contribution of each source of variation to the total variation in total carotene 21 days after pollination.

*Percent of total variation uniquely due to genotype (inbred), ears within inbreds, and kernels within ears.

*Level of significance for inbreds, ears within inbreds, and kernels within ears are presented as ns when P > 0.05, and as * when P < 0.001.

154 J. Amer. Soc. Hort. Sci. 115(1):153-157. 1990.
substantiate the use of subjective visual color determination as a means of selection for variation in endosperm carotene content. These same two kernel classes differed significantly in sucrose content. Light-yellow kernels contained 65% more sucrose than dark kernels (Table 2).

These results are further exemplified in 95% confidence ellipses that divide the population into two kernel classes based on sucrose and total carotene (Fig. 1). One class contains kernels within relatively low levels of sucrose and high levels of total carotene. These kernels were visually identified as dark yellow and likely are homozygous or heterozygous for the partially dominant \(\text{Se}^+\) allele. The other class contains kernels that are high in sucrose and low in total carotene. These kernels were identified as light yellow and are likely homozygous for \(\text{se}\). The overlap between the confidence intervals produces a third class of kernels, intermediate in sucrose. The intermediate level of sucrose may represent expression of \(\text{se}\) in a heterozygous condition. Previous work suggested \(\text{Se}\) is not completely dominant over \(\text{se}\) (Ferguson et al., 1979; La Bonte and Juvik, 1987a). Expression of \(\text{se}\) in a heterozygous condition is clearly demonstrated in a comparison of kernels with varying doses of \(\text{se}\) in the endosperm (Table 3). F1 kernels (\(\text{se} \text{ Se}\)) are significantly higher in sucrose than kernels of the inbred IL678a (\(\text{Se} \text{ Se}\)) and significantly lower than the inbred IL677a (\(\text{se} \text{ se}\)).

Several kernels (Fig. 1) fall outside the ellipsoidal intervals. One kernel is low in both total carotene (dark-yellow) and sucrose. These kernels may simply represent either random outliers or recombinant types in which a crossover may have occurred between the \(\text{se}\) gene and a gene affecting carotene production. The latter hypothesis is a possibility because the biochemical pathways producing carotenoids and carbohydrates are distinct. Various white endosperm mutants in maize have enzymatic deficiencies in pathways leading from precursor molecules to cyclic carotenoids (Robertson et al., 1978). Cyclic carotenoids, such as \(\beta\)-zeacarotene and \(\alpha\)-carotene, are the principal carotenoids found in frozen yellow maize endosperm (Lee et al., 1981). In contrast, endosperm carbohydrate mutations in maize consist primarily of enzymatic deficiencies involved in the formation of starch and phytoglycogen from mono- and disaccharides (Shannon and Garwood, 1984).

A nondestructive method is proposed as a means of determining the appropriate hypothesis explaining the relationship between \(\text{se}\) and kernel color. Embryos can be excised from kernels and cultured (Green and Phillips, 1975) on modified (low-salt) Linmaier and Skoog basal medium (Linmaier and Skoog, 1965). The sucrose and total carotene in the excised endosperm can be quantified. Seedlings from developing embryos can then be transplanted and grown to maturity. Single-kernel analysis of selfed seed from these transplants would indicate the presence or absence of \(\text{se}\). The development of a dark-yellow \(\text{su}^+\text{l se}\) stock would confirm the linkage hypothesis. Such a stock would be desirable to the sweet corn processing industry.

Table 2. Chemical variation in individual kernels visually classified as light (L) or dark (D) yellow from \(F_2\) \(\text{se}^{-1}\) kernel populations segregating for the \(\text{se}\) gene.*

| Kernel maturity* | F2 pedigree | No. kernels | Total carotene (mg g\(^{-1}\) dry wt) | Sucrose (mg g\(^{-1}\) dry wt) | Maltose (mg g\(^{-1}\) dry wt) | Kernel dry wt (g) | Moisture content (%) |
|-----------------|-------------|-------------|--------------------------------------|---------------------------------|-------------------------------|-------------------|----------------------|
| 21 DAP IL677a x IL451b | 95 | 1.77* 0.281 | 71* | 46 | 3.7* 2.1 | NA NA | 72* 70 |
| 35 DAP IL677a x IL678a self | 151 | 0.148* 0.246 | 37* | 24 | 9.4* 5.7 | NA NA | 62* 39 |
| Mature-dry seed IL451b x IL677a self | 50 | NA NA | 48* | 33 | 0.71* 0.05 | 0.20* 0.25 | NA NA |

*Means with an asterisk represent paired comparisons between light and dark-yellow kernels significantly different at \(P < 0.05\).

Table 3. Sucrose content for inbreds and \(F_1\) kernels (25 for each genotype) from individual ears containing various doses of \(\text{se}\) in the endosperm at 21 days after pollination.

| Genotype | Gene dosage in endosperm | Mean sucrose concn (mg g\(^{-1}\) dry wt)* |
|----------|-------------------------|----------------------------------------|
| IL677a   | se se se                | 76 a                                   |
| IL677a x IL678a | se se Se               | 71 b                                   |
| IL678a   | Se Se Se                | 47 c                                   |

*Means followed by the same letter are not significantly different based on LSD test at \(P = 0.05\).
industry in the light of consumer preference for dark-yellow kernels of canned or frozen corn.

**Kernel maltose and dry weight.** Significantly more maltose was observed in mature-dry, light-yellow F2 kernels than in dark-yellow kernels (Table 2). As in the previous experiment, light-yellow kernels also contained significantly higher levels of sucrose. Although most individual kernels followed these same trends for sucrose and maltose (Fig. 2), there were some light-yellow kernels that contained high levels of sucrose, even in comparison to other light-yellow kernels, but no detectable level of maltose. This observation and previous data (Carey et al., 1982) suggests that higher concentrations of maltose may not always be found in se kernels. Several hypotheses explain the presence of high levels of sucrose and low levels of maltose. First, if elevated maltose is a pleiotrophic expression of se, this amplification may be better observed in kernels at 28 or 35 DAP and not in mature-dry kernels. Maltose content in mature-dry kernels may be more sensitive to environmental variations since levels of this compound in kernels of IL677a have been found to vary dramatically from one year to the next (Carey et al., 1982). Second, segregation of other genes in the genetic background of the inbreds in this study may influence levels of maltose accumulation. Kernel maltose accumulation may represent the expression of a gene linked to se. The embryo rescue procedure described in Expt. 1 could be used to clarify further the relationship between se and maltose.

Significantly lower kernel dry weight was observed in light-yellow kernels than in the dark-yellow kernels (Table 2, Fig. 3). Light-yellow kernels weighed 21% less than dark-yellow kernels. The 95% confidence ellipses contain the majority of kernels classified as either light or dark yellow with respect to sucrose and kernel dry weight (Fig. 3). The apparent cause of low kernel dry weight is not readily identifiable. Warm et al. (1980) identified differences in dry kernel weights and weights of both embryos and endosperm in comparisons of su-1 and super sweet cultivars (sh-2, ae du wx). Super sweet cultivars tended to have lower kernel dry weights, endosperm, and embryo dry weights. The se gene, to a certain degree, decreased kernel starch content (Gonzales et al., 1976). Further investigations are required to substantiate this result. Breeders could use kernel dry weight as a means of selecting su-1 se kernels, but poor seed vigor has also been associated with low kernel dry weight (Warm, 1980).

**Moisture content.** A significantly higher percentage of moisture was observed in light-yellow than in dark-yellow kernels (Table 2). Regression analysis indicates two distinct slopes fitting a linear regression model for kernels that are light- and dark-yellow (Fig. 4). Ferguson et al. (1979) suggested that moisture retention is an osmotic effect associated with the high levels of soluble carbohydrates (sugars and phytoglycogen) found in kernels homozygous for se. The concomitant increase in percent moisture content with sucrose observed in these data strengthens this hypothesis. Although the majority of kernels follow this trend, several kernels are relatively low in sucrose and high in percent moisture. These kernels could have compensated for lower sucrose by having higher levels of phytoglycogen as a result of expression of genes other than se in the segregating population.

In conclusion, individual su-1 se kernels in segregating populations can be identified based on metabolic and morphological traits. The sucrose content of a kernel is the direct phenotypic expression of se and, as such, provides the most reliable means of identifying su-1 se kernels. In contrast, elevated kernel maltose content is not always associated with enhanced sugar levels. Selection based on costly and time-consuming sugar analysis is impractical for breeders. A preferential means of identifying su-1 se kernels is their light-yellow appearance. Selection of su-1 se kernels based on moisture content (differential dry-down of
kernels) at 35 or 42 DAP is also suitable, but requires an added step of marking kernels before harvest, whereas selection based on color can be made after harvest of mature-dry seed. Selection based on moisture content would be necessary if environmental or genetic effects diminish differences in color between su-1 se

and su-1 Se

kernels or a dark-yellow su-1 se

line were developed and incorporated into breeding programs. The ability to identify individual su-1 se

kernels in segregating populations has been facilitated by mapping of the chromosomal location of the se

gene (La Bonte and Juvik, 1987b). Using the phenotypic selection criteria described above in conjunction with maize B-A translocation stocks (Beckett, 1976) these genes have been mapped in the maize nuclear genome (unpublished data). This step is important in the development of a genetic marker that could more readily identify su-1 se

kernels.

Literature Cited

Association of Official Analytical Chemists. 1984. Official methods of analysis. 13th ed. Assoc. Offic. Anal. Chem., Washington, D.C.

Beckett, J.B. 1976. B-A translocations in maize. J. Hered. 69:27-36.

Cameron, J.W. and J.H. Teas. 1954. Carbohydrate relationships in developing and mature endosperm of brittle and related maize genotypes. Amer. J. Bot. 41:50-55.

Carey, E.E., A.M. Rhodes, and D.B. Dickinson. 1982. Postharvest levels of sugars and sorbitol in sugary enhancer (su se) and sugary maize (su Se). HortScience 17:241–242.