Dry-Milling and Fractionation of Transgenic Maize Seed Tissues with Green Fluorescent Protein as a Tissue Marker

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ABSTRACT

The efficiency of fractionating cereal grains (e.g., dry corn milling) can be evaluated and monitored by quantifying the proportions of seed tissues in each of the recovered fractions. The quantities of individual tissues are typically estimated using indirect methods such as quantifying fiber or ash to indicate pericarp and tip cap contents, and oil to indicate germ content. More direct and reliable methods are possible with tissue-specific markers. We used two transgenic maize lines, one containing the fluorescent protein green fluorescent protein (GFP) variant S65T expressed in endosperm, and the other containing GFP expressed in germ to determine the fate of each tissue in the dry-milling fractionation process. The two lines were dry-milled to produce three fractions (bran-, endosperm-, and germ-rich fractions) and GFP fluorescence was quantified in each fraction to estimate the tissue composition. Using a simplified laboratory dry-milling procedure and our GFP-containing grain, we determined that the endosperm-rich fraction contained 4% germ tissue, the germ-rich fraction contained 28% germ, 20% endosperm, and 52% nonendosperm and nonembryo tissues, and the bran-rich fraction contained 44% endosperm, 13% germ, and 43% nonendosperm and nonembryo tissues. GFP-containing grain can be used to optimize existing fractionation methods and to develop improved processing strategies.

The corn (*Zea may*s L.) kernel is composed of pericarp, tip cap, embryo, and endosperm tissues. Each tissue has different physical properties, chemical compositions, and cellular structures. Being rich in lipids, corn germ is a source of edible corn oil. The endosperm is rich in starch and protein and is used in various food products such as flakes, grits, meal, or flour, and in ethanol production. The pericarp is rich in fiber and is primarily used as cattle feed. More recently, the corn kernel has been used for producing recombinant proteins, for example industrial enzymes, by targeting the protein to one tissue and using fractionation to obtain a recombinant protein-rich fraction for more efficient subsequent extraction and purification (Kusnadi et al 1998; Yildirim et al 2002). The value of grain can be increased by fractionation because separation of the tissues allows each tissue to be used for a specific purpose to which it is well suited.

Dry-milling results in three major fractions: a germ-rich fraction, an endosperm-rich fraction, and a bran-rich fraction. The separation of maize tissues is never perfect, resulting in fractions containing different proportions of different tissues, and the degree of contamination among fractions greatly affects the value of the fractionated product. A variety of processing parameters can be optimized (tempering time, mill type and speed, feed rate, and sieve screen sizes), and the optimal parameters depend on the type of corn used, the type of available equipment, and the required fraction purity, particle size, and yield. To optimize these parameters, however, it is important to know the tissue composition of each fraction. For this purpose, a tissue-specific marker would be very useful.

An ideal tissue-specific marker would be one that is easy to measure and is homogeneously distributed within a specific tissue and confined to that tissue. Such a marker would enable fractionation efficiency to be easily and reliably determined and enable the milling process to be adjusted to produce fractions that are as pure as possible or meet other product specifications for purity. For germ tissue, current methods use oil content and oil yield as markers to track germ recovery during dry-fractionation processes (Yildirim et al 2002). Because the germ is composed on average of 33% oil (Watson and Ramstad 1987) and typically contains ≈85% of the total oil in the corn kernel, estimations of germ content in various fractions can be made. This is the best marker currently available for quantifying germ, but it is not perfect because small amounts of oil are present in the endosperm and pericarp as well. In wheat, the bran is tracked using crude fiber content as a marker (Pomeranz 1987), but fiber is not reliable as a marker in corn because it is present in all tissues, with 2.7% of the endosperm being fiber and 8.8% of the germ being fiber (Watson and Ramstad 1987). Biochemical markers such as phenolic acids have also been used as tissue-specific markers (Antoine et al 2004) in wheat, but this marker is present in all cell walls and cannot be used for tracking tissues in corn.

The natural markers that have been used previously present problems of tissue specificity and their relative levels in tissue may be subject to variation due to environmental and genetic factors. Therefore, we set out to develop transgenic plants containing an exogenous marker designed to accumulate in different tissues of the corn kernel. Green fluorescent protein (GFP) has been well characterized as a marker protein and is frequently used in biological systems (Ehrhardt 2003). In our previous work (Shepherd et al 2008), we used three seed storage protein promoters to develop transgenic corn plants containing GFP in maize seed tissues. The transgenes were based on the *globulin-1* (Glb1) promoter, which is primarily active in germ tissue, but also has activity in the endosperm and aleurone tissue, and the 27 kDa γ-zein promoter, which is active only in endosperm tissue. The distribution of GFP in the grain of transgenic plants containing these constructs was determined. Grain containing the zein promoter constructs contained GFP only in endosperm tissue, while grain containing the Glb1 promoter construct had 73% of the GFP in the embryo and 27% of the GFP in the endosperm (Shepherd et al 2008). Because GFP is much easier to quantify than other compounds used to determine tissue composition, it may be useful for characterizing grain fractionation.

The objective of the present study was to determine whether GFP produced in grain using seed storage protein promoters can be used as a tissue-specific marker in milling experiments. Our approach was to subject GFP-containing seeds to a simplified laboratory dry-milling and fractionation procedure to determine the partitioning of GFP in each fraction. This information was then used to determine the amount of each seed tissue in each dry-milled fraction.

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MATERIALS AND METHODS

Grain Production

Transgenic plants expressing the S65T variant of GFP were developed as previously described (Shepherd et al 2008). The genetic background of the plants used in the present study was predicted to be 87.5% B73 and 12.5% A188, based on the pedigree of the plants. Transgenic plant lines were designated as 27zn, 19zn, or Glb1 according to the promoter used to regulate GFP transcription. Transgenic plants and nontransgenic B73 were grown in the same nursery, near Ames, IA, in 2005. Plants were hand-pollinated and individual ears were picked at harvest and allowed to dry to ≈12% moisture before shelling by hand. Ears used were segregating for the GFP transgene with approximately a 3:1 negative ratio.

Dry-Milling

GFP-containing kernels from the 27zn and Glb1 transgenic lines, as well as kernels from the B73 inbred line that lacks GFP, were cleaned of insects, broken and damaged kernels, and tempered in a sealed bag for 2.5 hr by adding water to achieve 21% moisture. The tempered corn was fractionated using a laboratory Beal-type drum degermer according to the flow diagram shown in Fig. 1. Mill settings were the same as the optimal settings for a lipase-containing corn (Vignaux et al 2004). Germ-, endosperm-, and bran-rich fractions were weighed and used for analytical tests.

About 15 mg of each dry-milled fraction was spread evenly on small sections of weigh boats and GFP fluorescence was observed with an Olympus SZH10 microscope (Leeds Precision Instruments, Minneapolis, MN) fitted with 485-nm excitation and 535-nm emission filters under 4× magnification.

Hand-Dissection

About 100 g of whole kernels from each of the 27zn and Glb1 transgenic lines and 100 g of whole kernels from the B73 inbred line was soaked overnight in water and dissected by hand into pericarp, embryo, and endosperm tissues. The recovered tissues were dried for two days and then ground to fine consistencies. Germ, endosperm, and bran fractions were weighed and analyzed.

Analytical Procedures

Ground whole kernels and endosperm-, germ-, and bran-rich fractions from the dry-milling and hand-dissection experiments were analyzed for moisture, protein, crude fat, and GFP contents, and mass balances and recoveries of GFP were calculated. Whole kernels and fractions were ground with a Wiley mill equipped with a #40-mesh screen. The moisture contents were determined in triplicate according to Approved Method 44-19 (AACC International 2000). Crude free-fat contents were determined in duplicate on all fractions according to Approved Method 30-25 (AACC International 2000). Mass-balances were determined on a moisture-free basis.

GFP Measurement

Active GFP content was determined by fluorometry in a black 96-well plate. GFP-containing fractions (10 mg) were placed each well of the plate and fluorescence was measured in a 96-well spectrofluorometer (Tecan, Mannedorf/Zurich, Switzerland) using 485-nm excitation and 535-nm emission wavelengths.

Calculation of Dry-Milling Efficiency

The separation of maize tissues using dry-milling processes is not perfect, and the result is endosperm-, germ-, and bran-rich fractions. To determine separation efficiency achieved using our laboratory dry-milling process, the amount of each seed tissue present in each fraction was calculated. Each fraction was comprised of a percentage of endosperm tissue (X), a percentage of germ tissue (Y), and a percentage of other tissues, including pericarp and tip cap (Z). The percentages X, Y, and Z differ in each fraction, but for each fraction

$$X + Y + Z = 100\% \quad (1)$$

Because GFP expression was confined nearly exclusively to either the germ or endosperm tissue, we used GFP yield and the known tissue composition of the starting material determined by hand-dissection (Shepherd et al 2008) to calculate the values of X, Y, and Z for each fraction. For example, to calculate the amount of endosperm tissue (X) in the germ-rich fraction, we started with the percentage of endosperm tissue in the starting material. Because several of the grain samples were fractionated this way in

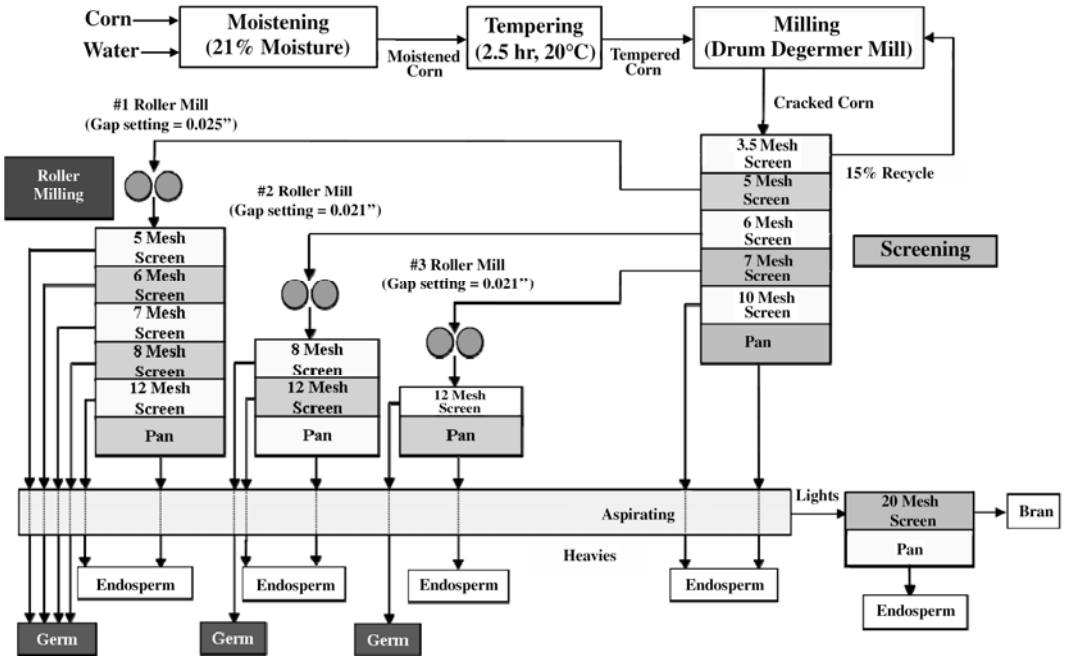

Fig. 1. Simplified laboratory dry-milling procedure for corn with green fluorescent protein (GFP) and B73 inbred line as a control.

the study, we used the average value. We multiplied this average value of percentage of endosperm tissue in the starting material by the endosperm-specific 27zn GFP yield in the germ-rich fraction from the 27zn grain from Table I. This quantity was then divided by the mass percentage of the germ-rich fraction from dry milling to determine *X*. As before, because several fractionations were conducted, we used the average mass percentage of the germ-rich fractions from Table I. For example

$$X = \frac{(\text{Endosperm \% in starting material})(\text{GFP yeild in 27zn germ-rich fraction})}{(\text{Mass \% of germ-rich fraction})} \quad 2$$

$$X = \frac{(83)(3.6)}{(15)} = 20.0 \quad 3$$

where *X* was the percentage of endosperm tissue in the germ-rich fraction, average mass percentage of endosperm tissue (Shepherd et al 2008) and GFP yield of the germ-rich fraction of 27zn grain, and average mass percent of germ-rich fraction from dry milling were taken from Table I.

We used a similar equation to calculate the percentage of germ tissue in the germ-rich fraction, but this calculation was complicated by the fact that, unlike our endosperm tissue marker, our germ tissue marker was not completely tissue-specific. Using data from a previously reported conservative sampling experiment (Shepherd et al 2008), we determined that the 73% of the GFP in the transgenic Glb1 corn was present in the germ, while 27% of the GFP was in the endosperm. The value of *Y* was calculated using an equation similar to that for *X*, but to calculate *Y*, the equation included the percentage of the total GFP that was present in the germ of Glb1 grain as the correction factor to account for GFP expression in nongerm tissues

$$Y = [(\text{Germ \% in starting material})(\text{GFP yield in Glb1 germ-rich fraction} - (X)(\text{fraction of Glb1 GFP in endosperm})]/[(\text{mass \% of germ-rich fraction})(\text{fraction of Glb1 GFP in germ})] \quad 4$$

$$Y = \frac{(12)[47.7 - (20)(0.322)]}{(15.3)(0.678)} = 47.7\% \quad 5$$

Y was the % of germ tissue in the germ-rich fraction. Average mass % of germ-rich fraction was calculated from hand-dissected kernels (Shepherd et al 2008) and average mass percent of germ-rich fraction from dry-milling and the average GFP yield in the germ-rich fraction from the Glb1 grain were taken from Table I.

The mass in each fraction that was not accounted for by germ or endosperm tissue was presumably composed mainly of pericarp and tip cap tissue, and the mass percentage that this tissue represented was calculated for each fraction as

$$\%\ \text{Remaining tissue in germ-rich fraction} = 100\% - X - Y \quad 6$$

This calculation determined the amount of tissue in the germ-rich fraction that was not endosperm- or embryo-tissue-based on GFP yield. *X*, *Y*, and *Z* were calculated for each fraction to get the percentages of germ, endosperm, and pericarp tissue in each fraction.

Statistical Analysis

One experiment tested the effect of the transformation event on the composition and yields of the dry-milled fractions. The treatment (transformation event) was randomized and tested in duplicate. Analysis of variance (ANOVA) was independently performed for each fraction by the general linear model (GLM) procedure (v.9.10, SAS Institute, Cary, NC) to test the hypothesis that mean values were not significantly different when corn derived from different transformation events was used. When the effect of a factor was significant, Tukey's multiple range tests were used to differentiate treatment means at the 95% significance level.

RESULTS

Dry Fractionation

Separation efficiency of dry milling is the proportion of each fraction comprised of the desired tissue. We set out to use GFP as a tissue maker that would allow milling efficiency to be determined. Dry fractionation was performed on grain from Glb1 and 27zn transgenic lines containing GFP expressed from the Glb1 promoter, which is very active in the embryo, and the 27 kDa zein promoter, which is very active in the endosperm, respectively. We included B73 in this experiment as a normal corn control (Fig. 2).

TABLE I
Mean Composition of Dry-Milled Fractions of Transgenic Lines 27zn and Glb1 with B73 Inbred Line as the Control[a]

Fractions and ID	Mass Yield (%)	Oil Content (% db)	Oil Yield (%)	Protein Content (% db)	Protein Yield (%)	GFP RFU	GFP Yield (%)
Whole kernel							
27zn	100	4.5	100	10.9	100	30,300	100
Glb1	100	4.4	100	11.2	100	5,850	100
B73	100	3.7	100	13.0	100	0.0	100
Avg	100						
Endosperm-rich							
27zn	74.3	2.1	34.2	10.3a	70.5	28,303a	93.1a
Glb1	75.0	1.9	33.4	10.8b	72.6	1,935b	33.5b
B73	82.9	2.1	47.3	12.1c	76.9	0.00c	0.00c
Avg	77.0						
Germ-rich							
27zn	16.3a	16.9	61.3a	15.3a	22.9	1,080b	3.6a
Glb1	16.2a	17.0	62.9a	14.3a	20.6	2,885a	47.7b
B73	12.5b	22.9	44.3b	21.5b	11.8	0.0c	0.0c
Avg	15.3						
Bran-rich							
27zn	7.4	2.0	3.30	10.1	6.90	1,273a	4.2a
Glb1	8.4	2.0	3.80	10.2	7.70	605b	8.8b
B73	6.9	2.6	4.80	11.8	6.20	0.0b	0.0c
Avg	7.8						
Total							
27zn	98.1		98.9	na	100a	na	100a
Glb1	99.6		100	na	90.0b	na	90.0a
B73	96.9		96.4	na	95.0b	na	0.0b
Avg	98.2						

[a] Values followed by the same letter within the same section of a column are not significantly different ($P < 0.05$).

Fractionation of B73 resulted in more endosperm-rich fraction and less germ-rich fraction. As observed in a hand-dissection experiment (Shepherd 2008), B73 had a different kernel shape than the two transgenic lines, and this characteristic affected its dry-milling mass yields. The resulting fractions were characterized for oil, mass, protein, and GFP yields (Table I). Within each fraction, significant differences ($P < 0.05$) between the three grain samples were observed for GFP concentration and GFP yield, and between the protein content of different grain samples in the endosperm-rich and germ-rich fractions. Significant differences were also observed between grain samples for mass yield in the germ-rich fraction. The oil content of all three B73 grain fractions was significantly different from those of the two transgenic lines.

We compared oil content and yield in each fraction between the lines in the study. Oil content has been traditionally used to identify germ-rich fractions. Fractions with oil yields higher than mass yield have been bulked to become the germ-rich fraction. Our results show that there were no significant differences in oil contents within a given fraction among corn types. B73 tended to produce a germ-rich fraction with higher oil content (≈23%). However, oil yield in the germ-rich fraction was the lowest for this corn due to the smaller mass of this fraction. The oil yield in the endosperm-rich fraction of B73 was higher than that of the transgenic lines. Using oil as a marker for germ tissue, we would conclude that the endosperm-rich fraction of B73 contained more germ than the same fractions of the two transgenic lines.

Protein contents were significantly higher in the germ- and endosperm-rich fractions of B73 compared with the transgenic corn. This was consistent with the higher protein content of the B73 whole kernels (13.0%) compared with the protein contents of

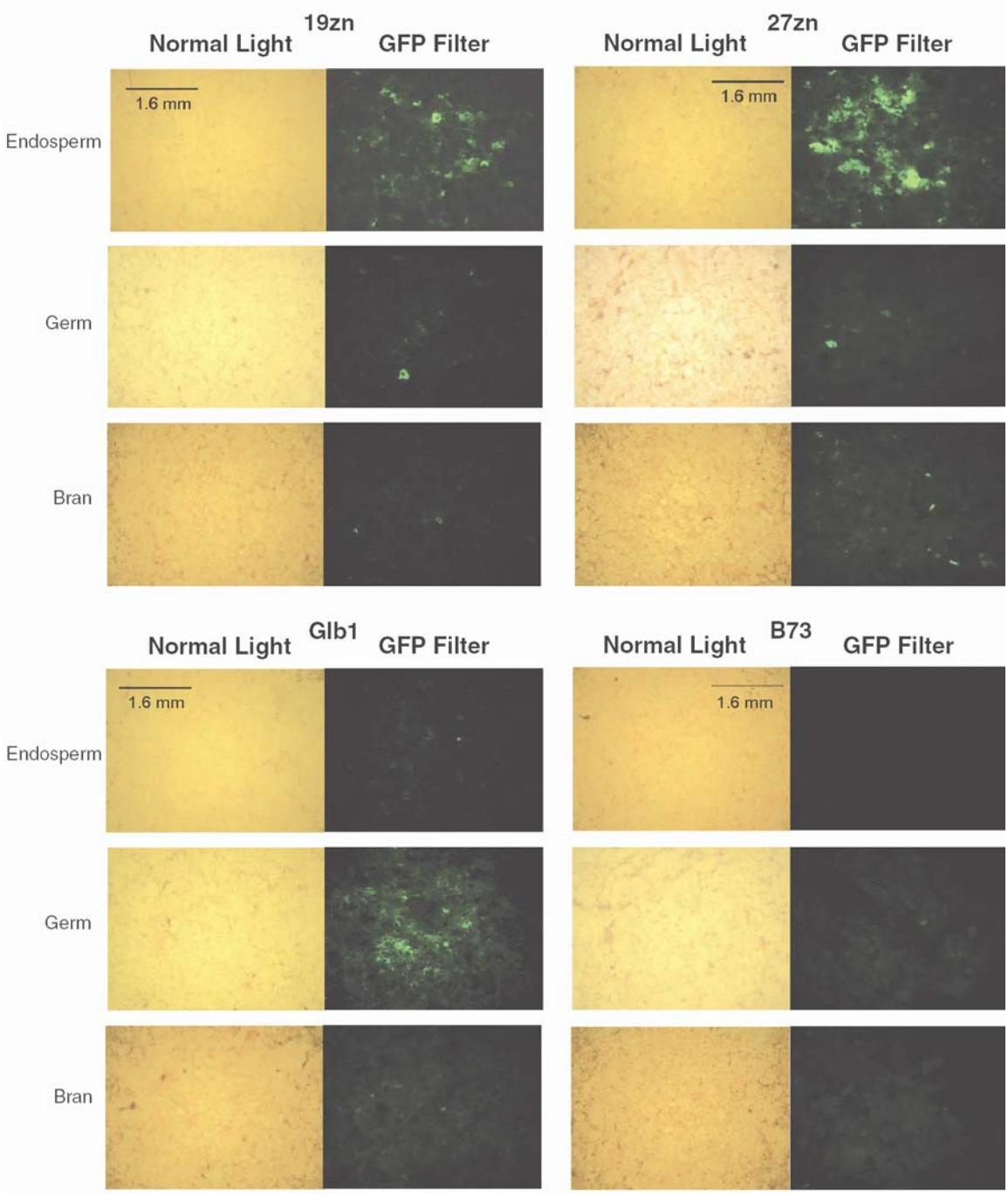

Fig. 2. Visualizations of transgenic corn and B73 inbred line control after dry-grind and fractionation procedure. Endosperm-, embryo-, and bran-rich fractions of the 19zn, 27zn, and Glb1 transgenics and B73 inbred line under normal and UV light.

27zn (10.9%) and Glb1 (11.2%). The mill settings were kept the same throughout the entire experiment, so the differences in fractionation observed between B73 and the three transgenic lines were likely due to the more spherical shape of the B73 kernels.

All fractions recovered after milling from the two transgenic lines contained GFP. In both lines, the highest GFP concentration was found in the fractions expected based on the tissue preference of the promoter used in the GFP construct. For example, the GFP concentration in the endosperm-rich fraction of the transgenic line containing the endosperm-preferred promoter was ≈15× higher than the endosperm-rich fraction from the transgenic line containing the embryo-preferred promoter. The majority of the GFP for the 27zn event was recovered in the endosperm-rich fraction as expected, with lower amounts in the germ- and bran-rich fractions. In the Glb1 event, the largest amount of GFP was recovered in the germ-rich fraction but considerable amounts were also found in the endosperm-rich fraction (33.5%) and bran-rich fraction (8.8%). Essentially all of the GFP was recovered when GFP was expressed in the endosperm and 90% of the GFP was recovered when GFP was expressed in the germ.

Visual Observation of Fluorescence in Dry-Milled Fractions

An advantage of using GFP as a tissue-specific marker is that it is visually detectable, allowing visual characterization of the fractions and confirmation of the quantitative results. To visually confirm the distribution of GFP determined by fluorescence measurements, we examined the dry-milled tissues after fractionation. The fractions were weighed, ground, and spread evenly for viewing under a microscope fitted with fluorescent filters. All images were taken at the same fluorescent filter settings (Fig. 2). Visual observation agreed with the distribution of GFP content shown in Table I. The images show that the GFP fluorescence level was highest in the 27zn endosperm-rich fraction and in the Glb1 embryo-rich fractions. Low levels of fluorescence were observed in the germ-rich fractions of the 27zn event and endosperm of the Glb1 event, which suggested possible contamination of these fractions with GFP-containing tissues. The nontransgenic B73 control showed no visually detectable fluorescence. These results are in agreement with the quantitative results presented in Table I.

Homogeneity of the samples was also scrutinized in the present work. In viewing GFP fractions under a microscope fitted with fluorescent filters, we observed heterogeneous distribution in the endosperm-rich fractions (Fig. 2). Regions of intense fluorescence were observed, possibly due to the presence of larger hard endosperm pieces. GFP in ground germ-rich fractions was more evenly distributed based on visual data (Fig. 2).

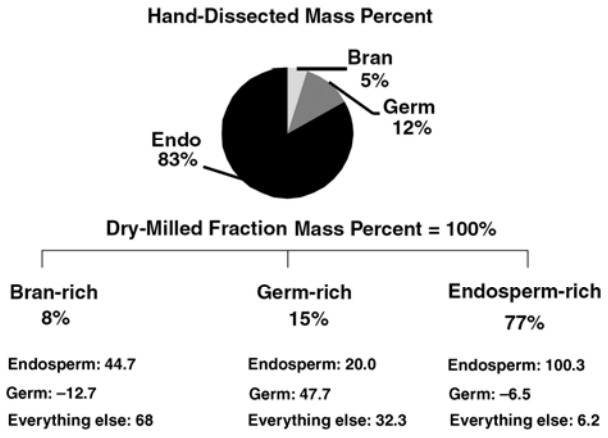

Fig. 3. Mass percent of hand-dissected fractions followed by mass percent of dry-milled fractions. Bottom pie charts represent % tissue composition of bran-, germ-, and endosperm-rich fractions calculated from mass percent and green fluorescent protein (GFP) yield.

Tissue Compositions of Dry-Milled Fractions

The main objective of this study was to measure the separation efficiency of the dry-milling process. By using GFP as a tissue marker, it was possible to estimate the tissue composition of each fraction (Fig. 3). This calculation was based on the actual tissue distribution of each grain sample, which was obtained from a previously reported hand-dissection experiment (Shepherd et al 2008). Because we required data from several fractionations to determine fractionation efficiency, we used the average values of all fractionations as the tissue percentages. The tissue specificity of GFP expression is nearly perfect in the 27zn grain but it is not as good in the Glb1 grain. To account for the low level GFP expression outside of the embryo in Glb1 grain, the distribution of GFP in the grain tissues of each construct was also needed for this calculation. This distribution was determined previously by conducting a conservative sampling experiment (Shepherd et al 2008).

Because the same protein was used for the endosperm marker and the embryo marker, it was necessary to mill the grain carrying each marker separately and use the average mass percentages of the fractionations in our calculations. As a result of using values that were averaged across all fractionations, the fractionation efficiencies presented in Fig. 3 represent averages across the experiment rather than the efficiencies of a given fractionation.

Using GFP as a tissue-specific marker enabled us to calculate the tissue composition of each dry-milled fraction. A consequence of using the mass percentages averaged across several fractionations with the GFP content of one fractionation to calculate the tissue content of each fraction is that the germ content of the bran- and endosperm-rich fractions are negative values. Since the minimum this value can be is zero, the magnitude of these negative values give a measure of the error in our method. In spite of this error, it is clear that the endosperm-rich fraction is quite pure and that endosperm tissue is the main contaminant of each of the other fractions.

DISCUSSION

A perfect tissue marker must fulfill several characteristics: 1) have a known tissue distribution, preferably with a strong tissue bias; 2) be relatively homogeneous in distribution throughout the tissue; and 3) be easy to quantify. In the grain used in this study, the distribution of GFP in the different grain tissues is known and each line has a strong bias for a single tissue (Shepherd et al 2008). Homogeneity of the starting material was examined and it is clear that GFP is not perfectly homogeneous within a tissue but it may be as good as or better than other tissue-specific markers. GFP can be quantified more easily than other tissue markers by taking direct measurements of the fluorescence of grain fractions. A standard curve using fluorescence units can be converted to protein content because GFP fluorescence is directly related to protein amount (Southward and Surette 2002). Previous reports have quantified GFP fluorescence in plants (Niwa et al 1999; Remans et al 1999). While GFP fluorescence measures only the active GFP present in the cell, and inactive GFP would not be accounted for, GFP fluorescence was directly related to the amount of GFP present (Richards et al 2003). Measurement of transiently expressed GFP in plant tissues was reported by visual confirmation (Tee et al 2003) and by image analysis (Furtado and Henry 2002). Thus, GFP meets these criteria and is a good tissue marker.

In this study, transgenic maize lines that expressed GFP in seed tissues were used to measure the efficiency of fractionation procedures. Two transgenic maize lines were used; one contained GFP in the endosperm (27zn). The other transgenic line (Glb1) contained GFP mainly in the embryo. By fractionating these grain samples and measuring the fluorescence of each fraction, we were able to estimate the proportion of each tissue present in each dry-

milled fraction. This analysis showed that the endosperm-rich fraction obtained by our laboratory dry-milling procedure was more pure for the intended tissue than were the germ-rich and bran-rich fractions. The laboratory dry-milling procedure used was developed based on oil contents of the fractions. Therefore, mill settings were chosen so that the endosperm-rich and bran-rich fractions contained a minimal amount of oil, and the germ-rich fraction contained a maximal amount of oil. Optimization did not include measuring the pericarp content in the bran-rich fraction because no marker was available to do so. Furthermore, as observed with B73, tissue separation was influenced by corn kernel shape and because the dry-milling process was optimized using a setting developed for more typical corn, it was perhaps not optimal for our transgenic corn containing GFP. It may be possible to optimize this procedure for better kernel tissue separation using the data from this study.

The results from the laboratory dry-milling of the GFP-containing corn could also be useful as a model for recombinant protein production in corn. Targeting recombinant protein in a specific tissue has been a long-used strategy to concentrate the target protein before the purification steps to increase efficiency of recovery and decrease the cost of downstream processing. We showed that 47.7% of the germ-targeted GFP could be recovered in 15% of the total seed mass, corresponding to a concentration of ≈3× GFP relative to whole grain. It is possible to obtain a more pure sample by optimizing the process for the specific corn. We also showed that it was possible to recover ≈90% of the GFP protein in 77% of the total seed mass when the protein was expressed in the endosperm. This means that GFP was concentrated by a factor of 1.2 relative to its concentration in whole grain. This result was in agreement with a similar study done with corn containing recombinant dog gastric lipase (Vignaux et al 2004). Lipase-containing corn was subjected to the same laboratory dry-milling process as in the present study and showed that the production of an endosperm-rich fraction made up of 70% of the total mass contained 89% of the total recombinant lipase. The dry-milling process used with lipase-containing corn was optimized using oil content as a marker and resulted in less endosperm-rich fraction with lower oil recovery than the transgenic corn used in this work.

The amount of fluorescence in each tissue was determined previously for the transgenic lines by a conservative sampling technique (Shepherd et al 2008). The 27zn promoter had exclusive activity in the endosperm. The Glb1 promoter had activity mostly in the embryo, with some activity in the endosperm tissue. Therefore, to determine fractionation error, it was necessary to correct for the expression of GFP in endosperm tissue of the Glb1 transgenic lines. While this complicates the calculation of the tissue composition of each fraction, the degree of tissue specificity is probably higher than other components that have been used as tissue specific markers. It may be possible develop a better embryo maker with a promoter with a higher degree of embryo specificity.

In the present study, we confirmed that GFP can be used as a tissue marker to evaluate corn dry-milling procedures. Endosperm-targeted GFP was produced and recovered in an endosperm-rich fraction. Embryo-targeted GFP was recovered less efficiently than endosperm-targeted GFP, resulting in a germ-rich fraction that contained large proportions of other tissues. Optimizing the dry-milling process to recover higher proportions of the protein is necessary to decrease the loss and increase the efficiency of tissue fractionation. Process definition and corn type influence corn kernel tissue separation, and optimization may be necessary with new types of corn due to differences in physical properties of the grain. In the present study, we showed that the GFP-containing transgenic corn can be helpful in that respect.

CONCLUSIONS

Our main objective was to test the ability of GFP to be used as a tissue-specific marker protein for use in evaluating corn dry-milling and fractionation procedures. We have shown that GFP can be processed and recovered during dry milling. The amount of GFP in each fraction can be quantified and used to calculate the tissue composition of dry-milled fractions. Our work represents an encouraging first attempt to use biotechnology to optimize corn fractionation.

ACKNOWLEDGMENTS

We would like to thank Steve Fox for assistance with dry-milling our GFP-containing corn. Names are necessary to report factually on the available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may be suitable. CTS, NV, and JMP were supported by the USDA-CSREES special appropriation "Plant Biotechnology Iowa".

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Erratum

On September 3, 2008, modifications were made to Equations 4 and 5 and Figure 3 of this article.

The authors supplied the following information concerning this article: There was an error in the formula for calculating the amount of germ (%) in the germ-rich fraction (*Y*). To correctly account for GFP expression in nongerm tissues, Equation 4 should be:

$$Y = [(\text{Germ \% in starting material})(\text{GFP yield in Glb1 germ-rich fraction} - (X)(\text{fraction of Glb1 GFP in endosperm})]/ [(\text{mass \%of germ-rich fraction})(\text{fraction of Glb1 GFP in germ})]$$

where the % germ in the starting material is the average of the hand-dissection values in Table V of our previous article "Green Fluorescent Protein as a Tissue Marker in Transgenic Maize Seed," *Cereal Chemistry* 85(2):188-195; the % GFP yield in Glb1 germ is from Table I of this article; X is the % of endosperm in the germ-rich fraction as calculated in Equations 2 and 3 of this article; the % Glb1 GFP in endosperm was determined in the conservative sampling experiment in (Table VI) of our previous article; the % of Glb1 GFP in germ is from the same table; and the mass % of the germ-rich fraction is from Table I of this article.

The changes in the tissue content of the fractions generally still support the conclusions of the original article, with the purest fraction being endosperm and the germ-rich and bran-rich fractions being contaminated with endosperm. The negative values illustrate problems with mass closure that are likely derived from averaging values across several grain fractionations and a failure to completely recover GFP when expressed in germ.