Mutagenesis Breeding for Increased 3-Deoxyanthocyanidin Accumulation in Leaves of Sorghum bicolor (L.) Moench: A Source of Natural Food Pigment

Carloalberto Petti,† Rekha Kushwaha,‡ Mizuki Tateno,† Anne Elizabeth Harman-Ware,‡ Mark Crocker,‡ Joseph Awika,§ and Seth DeBolt*†‡

†Plant Physiology, Department of Horticulture, Agricultural Science Center North, University of Kentucky, Lexington, Kentucky 40546, United States
‡Center for Applied Energy Research, University of Kentucky, 2540 Research Park Drive, Lexington, Kentucky 40511, United States
§Department of Soil and Crop Science, Texas A&M University, 2474 TAMU, College Station, Texas 77843, United States

ABSTRACT: Natural food colorants with functional properties are of increasing interest. Prior papers indicate the chemical suitability of sorghum leaf 3-deoxyanthocyanidins as natural food colorants. Via mutagenesis-assisted breeding, a sorghum variety that greatly overaccumulates 3-deoxyanthocyanidins of leaf tissue, named REDforGREEN (RG), has been isolated and characterized. Interestingly, RG not only caused increased 3-deoxyanthocyanidins but also caused increased tannins, chlorogenic acid, and total phenolics in the leaf tissue. Chemical composition of pigments was established through high-performance liquid chromatography (HPLC) that identified luteolinidin (LUT) and apigeninidin (APG) as the main 3-deoxyanthocyanidin species. Specifically, 3-deoxyanthocyanidin levels were 1768 μg g⁻¹ LUT and 421 μg g⁻¹ APG in RG leaves compared with trace amounts in wild type, representing 1000-fold greater levels in the mutant leaves. Thus, RG represents a useful sorghum mutagenesis variant to develop as a functionalized food colorant.

KEYWORDS: Sorghum bicolor, anthocyanin, flavonoids, lignin, mutant, food colorant, 3-deoxyanthocyanidin

INTRODUCTION

Phenolics are synthesized in plants from the phenylpropanoid pathway (PPP) and comprise a wide range of essential metabolites with important implications in food quality and human health. The PPP starts with phenylalanine, which is deaminized by phenylalanine ammonia-lyase (PAL) to form cinnamic acid.¹ The pathway follows a series of metabolic branchpoints that can result in the biosynthesis of lignins, flavonoids, isoflavonoids, or anthocyanins. Flavonoids and anthocyanins are two of the most common products resulting from the PPP. These metabolites are associated with multiple biological functions in plants, including antioxidant activity,² defense,³,⁴ and developmental regulation.⁵,⁶

In Sorghum bicolor (L.) Moench (sorghum), the most common anthocyanidin types are the 3-deoxyanthocyanidins, primarily luteolinidin (LUT) and apigeninidin (APG), and their glycosylated derivatives,⁷ known as 3-deoxyanthocyanins. The 3-deoxyanthocyanidins are rare in nature and are of increased interest as natural food colorants due to their better stability to food-processing conditions than the more common anthocyanins found in most food plants.⁸,⁹ Despite their potential as natural food ingredients, commercially viable sources of the 3-deoxyanthocyanidins have not been identified. High concentrations of the 3-deoxyanthocyanins are reported in black sorghum bran;⁹ however, the bran tissue is a limited component of the total plant biomass. In addition, the 3-deoxyanthocyanidin pigments are relatively difficult to extract from bran tissue due to their strong association with cell wall material. Recent findings demonstrate that the more abundant plant tissues, leaves and sheaths, of sorghum may be more viable sources of the 3-deoxyanthocyanidins for commercial applications.⁸,¹⁰ Sorghum has long been known to accumulate 3-deoxyanthocyanins in leaf tissue as a localized response to pathogen attack.¹¹ However, such accumulation is unpredictable and has limited commercial potential. Genetic mechanisms to induce 3-deoxyanthocyanin accumulations in sorghum leaf tissue provides a more promising opportunity to enhance pigment yield.

The PPP metabolic grid is highlighted by branch-specific regulation and specification in a temporal and spatial manner. A genome-wide mutagenesis strategy was employed in sorghum to generate mutations, and we subsequently screened this population for tissue-specific coloration. A mutant identified in this screen was REDforGREEN (RG), which as reported herein displays enhanced accumulation of 3-deoxyanthocyanidin pigments specifically in leaf tissue.

MATERIALS AND METHODS

Sorghum Mutagenesis Population. Ethyl methanesulfonate (EMS) was utilized to generate a mutagenesis population in S. bicolor (L.) Moench var. Della. Approximately 12000 seeds were exposed to EMS (0.75%) for 12 h followed by neutralization. Seeds were sown on soil-less media (SunGro, Bellevue, WA, USA) supplemented with Osmocote (3 g/kg; Scotts Co., Maryville, OH, USA). Plants were grown in a glasshouse (24 °C) for 6–8 weeks before they were...
transplanted to the field. Plants were visually screened for red coloration phenotype. Target RG mutant had the inflorescence bagged upon emergence for self-pollination. RG M1 lines were bagged and followed by single-seed descent and progeny phenotypically scored for leaf coloration.

**Morphological Characterization of RG.** Phenotype was examined over the course of three generations. Three discrete zones are defined in the RG leaf, the green zone (no red), the transition zone where green and red tissues are present, and the red zone, which comprises the red area of the leaf (no green, phase 3). Chlorophyll content was measured\(^\text{12} \) (\( n = 4 \) leaf disks per leaf from \( n = 10 \) plants).

**Spectrophotometric Analyses.** Extraction and pigment quantification were carried out on fully expanded RG and wild type leaves (dried for 2 days at 37 °C). Each biological sample comprised the same physiological and ontological leaf type from multiple plants (\( n = 10 \)), and four biological pool replicates were used. For the determination of total pigments, sample (10 mg) was extracted in acidified methanol (1 mL HCl/100 mL) and centrifuged, and supernatant absorbance measured at 480 nm as previously described.\(^\text{3} \) 3-Deoxyanthocyanidin content was estimated as luteolinidin equivalents (\( e = 31000 \)), because it was the dominant pigment in the samples. Total phenolic were measured as ferulic acid equivalents as previously described.\(^\text{13} \) Condensed tannins were estimated using the vanillin–HCl method described by Butler et al.\(^\text{14} \) Chlorogenic acid was determined as described.\(^\text{15} \)

**HPLC and LC-MS Analysis.** Analysis of 3-deoxyanthocyanidin composition in sorghum leaf tissue was performed using a HPLC-DAD (Agilent 1200 system) as previously described.\(^\text{8} \) A Kinetex C18 75 × 4.6 mm i.d. column (Phenomenex, Torrance, CA, USA) thermostated at 40 °C was used; UV–vis spectra were collected between 200 and 700 nm. LC-MS analysis was performed by a reverse phase quadrupole time-of-flight (Q-TOF) mass spectrometer equipped with an Agilent Jet Stream electrospray ionization (ESI) ion source with continuous introduction of reference mass compounds: 121.050873, 922.009798 (positive ion mode) (QTOF MS/MS) on an Agilent 1200 LC stack interfaced with an Agilent 6530A (Agilent Technologies, Santa Clara, CA, USA). Samples (5 μL) were separated using an Acquity BEH Shield RP-18 analytical column (1.7 μm 2.1 × 150 mm, Waters Corp., Milford, MA, USA) maintained at 40 °C. Sample analysis was replicated. The MS/MS data were used to infer the anthocyanidin species. Quantification of the 3-deoxyanthocyanidins was achieved by comparing HPLC peak areas at 480 nm to those obtained using pure standards (apigeninidin, O-methylapigeninidin, 5,7-di-dimethyloctuloinidin, and 5,7-di-dimethyloctuloinidin). The 3-deoxyanthocyanidin standards were purchased from AlscAChin (Strasbourg, France); all standards were at least 97% pure.

**Dihydroflavonolreductase (DFR) Activity.** Plant samples were homogenized in liquid N\(_2\) and total protein extracted.\(^\text{16} \) Protein concentration was measured and standardized between samples using the Bradford method; the DFR activity was measured according to the method in ref 17. The absorbance due to cyanidin in the butanol solvent at 550 nm (absorption coefficient = 34.7)\(^\text{18} \) was normalized for the 550 nm absorbance due to chlorophyll at 600 nm (absorbance at 550 nm – absorbance at 600 nm). Control used heat-inactivated enzyme. (\( n = 3 \) biological pooled samples each comprising five leaves.)

**Statistical Analysis.** Statistical analyses consisted of Student’s t test or one-way ANOVA with a post hoc Tukey test and performed using PRISM4 (Graphpad, La Jolla, CA, USA) (significance established at \( P < 0.05 \)).

### RESULTS

**Identification of REDforGREEN (RG).** Within an M1 generation of chemically mutagenized \( S. \) bi\( o \)\( l \) var. \( L. \) Mediterranea, the RG mutant was identified from approximately 12000 M\(_1\) individuals on the basis of distinct red coloration of lamina tissue throughout development, which carried through to subsequent M\(_2\) and M\(_3\) generations. Self-pollination of the M\(_1\) RG selection resulted in 761 M\(_2\) generation individuals. These inherited the trait in a nearly 3:1 ratio of red coloration of lamina (RG) to wild type (green lamina) (\( 567:194, \chi^2 = 0.0557, df = 1, P = 0.4067 \)), which would be expected for a dominant allele. Furthermore, the single wild type segregant failed to inherit the trait in subsequent generations. Two subsequent backcrosses were made between the red coloration mutant RG and wild type \( S. \) bi\( o \)\( l \) var. \( D. \) (referred to throughout as wild type). Each backcross was carried through to the M\(_2\) generation and the inheritance examined. Interestingly, the heterozygous individuals failed to carry lighter lamina pigmentation than homozygous RG, suggesting the RG allele in the heterozygote was fully penetrant. The progeny of the M\(_2\) were examined to determine homozygosity of the allele and the homozygote used in the third backcross. Both rounds of backcrossing resulted in 3:1 red versus green lamina coloration, consistent with single gene dominant inheritance of the target allele. The third backcross RG line was used during mutant characterization.

Between 3 and 5 days post-germination RG displayed no distinguishable difference from the sorghum wild type seedling. Onset of pigmentation was evident from the cotyledon and each true leaf lamina thereafter (Figure 1). Upon the leaf completing expansion, light-driven pigmentation accumulation initiated at the leaf apex and progressed to the base, termed basipetal accumulation (Figure 1A–N). Subsequent basipetal progression of red coloration occurred in all lamina during the plant life cycle (Figure 1). Visual examination indicated that coloration was predominantly interveinal (Figure 1B,C). As a general trend, basipetal red/purple coloration of lamina began after cellular expansion had plateaued. The red/purple coloration therefore progressed in a hierarchical manner (with the earliest emerged leaves being fully red, and newly emerged and expanding leaves being green). This phenotype led to a continuum of green terminal leaves and red fully expanded leaves. Red/purple leaf coloration extended partially into the leaf sheath (shown in Figure 1L–N). The process of complete red/purple coloration of the lamina took progressively longer (2 days for leaves 1–3 to 21 days for leaves subtending the inflorescence).

**Spectrophotometric Determination of Pigment and Related Polyphenols.** To estimate the pigment accumulation, we spectrophotometrically quantified (as luteolinidin equivalents, LUTE\(_{eq}\)\(^\text{7} \)) pigments in whole lamina at various temporal points during leaf development (leaves 1–2, 3, 5, and 7; Figure 2A). Fully pigmented RG leaves were compared with leaves from a wild type plant at the identical spatial and temporal ontology. Results demonstrated (1) that pigments accumulated until reaching a plateau level (Figure 2A) and (2) a >20-fold LUTE\(_{eq}\) increase in the RG leaf material compared with wild type, with a maximum value of 10.05 mg g\(^{-1}\) (Figure 2A). The LUTE\(_{eq}\) levels observed in the RG leaves were much higher than those reported for high-pigment black sorghum grains\(^\text{7} \) and are economically relevant when current anthocyanin pigment yield from commercial sources, such as red cabbage, are considered.\(^\text{19} \)

Measurements of total phenolics, condensed tannins, and chlorogenic acid content were also completed in RG and wild type leaves (Figure 2B–D). Results showed that the RG accumulated between 2- and 6-fold greater total phenolics than wild type (\( P < 0.05 \); Figure 2B). Condensed tannins also were up to 4-fold higher in the RG leaf material when compared with wild type leaf (\( P < 0.05 \); Figure 2C). As a general trend, total phenolics and tannins positively correlated with the LUTE
pigments, thus suggesting a coordinated metabolic response in the RG mutant leaves. In agreement with this trend, chlorogenic acid content was also higher in the RG mutant versus the wild type, with the red leaf being up to 7-fold greater and the RG-green being up to 2-fold greater than the wild type (Figure 2D). The presence of other polyphenols generally helps to stabilize the pigments via condensation reactions or copigmentation;20,21 thus, the coaccumulation of the non-pigmented polyphenols may improve functionality of the 3-deoxyanthocyanins from the leaf tissue. Additionally, there is a potential for the RG leaves to serve as a source of bioactive dietary polyphenols.

Chemical Characterization of Pigment Composition.

The specific composition of accumulated pigments was investigated using HPLC and LC-MS. We identified LUT as the dominant 3-deoxyanthocyanidin compound in the RG leaf, accounting for 68% of resolved 3-deoxyanthocyanin peaks, followed by apigeninidin (APG) (16%), and 7-O-methyl-luteolinidin (13%) (Table 1; Figure 3). Results demonstrated 1768 μg g\(^{-1}\) dw LUT was present in RG leaves compared to trace amounts in wild type (Table 1). Low levels of 7-O- and 5-O-glucosides and diglycosides of LUT and APG, as well as 7-O- and 5-O-glucosides and diglycosides of O-methyl derivatives of LUT and APG, were also detected (Figure 3). In addition, dimers of LUT and its 7-O-methyl derivative were also detected at low levels (Figure 3); such dimers (apigenin- or luteolin-flavone dimer) were recently reported as highly stable compared to the monomeric 3-deoxyanthocyanins.8 In general, LUT and its derivatives accounted for >80% of total 3-deoxyanthocyanins, whereas APG and its derivatives accounted for the rest (Table 1). The 3-deoxyanthocyanin composition of the RG was generally comparable to the typical composition observed in sorghum grains derived from purple plant phenotypes.22 Thus, the potential performance of these pigments as natural food colorants should be equivalent to those previously reported.7–23

LC-MS data revealed the presence of significant quantities of various mono- and diglycosides of the flavones luteolin and apigenin (Table 2). These flavones are common components of sorghum grain and tissue;24 however, the extensive degree of glycosylation with glucose, galactose, and rhamnose observed in this study is not typically reported for sorghum. Interestingly, the relative proportions of the glycosides of luteolin and apigenin seemed to match the relative proportions of LUT and APG pigments, yet the luteolin glycosides were much more predominant. The higher proportion of LUT family pigments and corresponding luteolin-based flavones compared to APG/
apigenin family of compounds has been reported for purple sorghum plant phenotype. 22 Red plant sorghum phenotypes, on the other hand, express the opposite effect, with the APG/apigenin compounds dominating. 22, 24 In general, accumulations of most of these compounds were much higher in the RG compared to wild type, following the overall trend observed for the other phenolics.

Quantification of Seed Bran Pigments. The sorghum seed bran accumulates 3-deoxyanthocyanidins in the red, brown, and black varieties. 7, 25−27 S. bicolor Della is a “brown” variety displaying accumulation of 3-deoxyanthocyanidin. However, no increase in the pigment content or in total phenolics was observed between the seed bran of RG and wild type (pigments: RG, 48 ± 8.6 μg g−1 seed; WT, 51 ± 6.2 μg g−1 seed), consistent with visual observations and the RG leaf specific accumulation of anthocyanins in the broad sense.

DFR Activity Supports Accumulation of 3-Deoxyanthocyanidins in RG. DFR catalyzes the reduction of dihydroflavonols to leucoanthocyanidins 26 and the conversion of naringenin to apiferol. 27 The DFR products are substrates for downstream steps in both branches of the PPP that lead to 3-deoxyanthocyanidins and 3-hydroxyflavonoids. Consistent with greater LUT and APG accumulations in RG (Tables 1 and 2), DFR activity (Figure 4) was significantly higher in the RG compared to wild type.

Table 1. Composition of the Major 3-Deoxyanthocyanidins from Sorghum Leaves of the RG and Wild Type (WT) (Micrograms per Gram, Dry Weight)

<table>
<thead>
<tr>
<th>RT&lt;sup&gt;a&lt;/sup&gt; (min)</th>
<th>LUT</th>
<th>APG</th>
<th>7MeOH LUT</th>
<th>5MeOH</th>
<th>7MeOH APG</th>
<th>DiMeOH LUT</th>
<th>DiMeOH APG</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG</td>
<td>6.7</td>
<td>10.7</td>
<td>11.7</td>
<td>14.7</td>
<td>17.4</td>
<td>19.0</td>
<td>19.7</td>
<td>2612</td>
</tr>
<tr>
<td>WT</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>RT, HPLC retention time. <sup>b</sup>LUT, luteolinidin; APG, apigeninidin; 7MeOH, 7-O-methyl; 5MeOH, 5-O-methyl; DiMeOH, 5,7-dimethyl.

Table 2. Mass Spectrometric Data Obtained for Luteolin-Based Flavone Glycoside Peaks as They Appeared in Chromatograms from both Wild Type (WT) and RG Sorghum Samples

<table>
<thead>
<tr>
<th>m/z</th>
<th>retention time</th>
<th>aglycon</th>
<th>parent ion, other diagnostics</th>
<th>flavone glycoside</th>
<th>biomass, relative abundance&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.54</td>
<td>271</td>
<td>595, 433, 397</td>
<td>luteolinidin-diglucoside</td>
<td>WT, RG</td>
<td></td>
</tr>
<tr>
<td>7.38</td>
<td>271</td>
<td>595, 433</td>
<td>luteolinidin-diglucoside</td>
<td>RG &gt; WT</td>
<td></td>
</tr>
<tr>
<td>7.39</td>
<td>287</td>
<td>611, 449</td>
<td>luteolin diglucoside</td>
<td>RG &gt; WT</td>
<td></td>
</tr>
<tr>
<td>7.47</td>
<td>287</td>
<td>449, 329, 299</td>
<td>luteolin 7-O-glucoside</td>
<td>RG &gt; WT</td>
<td></td>
</tr>
<tr>
<td>7.82</td>
<td>287</td>
<td>449</td>
<td>luteolin 5-O-glucoside</td>
<td>RG &gt; WT</td>
<td></td>
</tr>
<tr>
<td>8.06</td>
<td>287</td>
<td>449</td>
<td>luteolin 7-O-galactoside</td>
<td>WT, RG</td>
<td></td>
</tr>
<tr>
<td>8.34</td>
<td>287</td>
<td>595, 449</td>
<td>luteolin 5-O-glucoside</td>
<td>WT, RG</td>
<td></td>
</tr>
<tr>
<td>8.64</td>
<td>287</td>
<td>449</td>
<td>luteolin 5-O-galactoside-O-rhamnoside</td>
<td>RG &gt; WT</td>
<td></td>
</tr>
<tr>
<td>9.09</td>
<td>287</td>
<td>595, 449</td>
<td>luteolin 5-O-galactoside-O-rhamnoside</td>
<td>RG &gt; WT</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>“>” indicates peak area for extracted ions in the chromatograms was greater in one pooled sample over another. Not all peaks in the chromatograms were identified.
protein in tissues and compared to the wild type (WT) green tissues. (identifiable natural food colorants,9 but the bran yield remains low relative to other parts of the plant. In this work we have sought to zone of RG red leaf tissue than in wild type (0.36 nmol h−1 mg protein−1). Notably, despite a lack of anthocyanin in the green zone of RG, detectable DFR activity was observed, which was not the case for wild type leaves, suggesting early onset of precursor accumulation (Figure 4).

Pigment Progression Is Paralleled by Total Chlorophyll Reduction. The large and progressive increase in 3-deoxyanthocyanidins in the RG mutant led us to ask if in parallel there was an alteration in the chlorophyll content. It was found that no significant changes in total chlorophyll occurred in the green and transition zone, but a significant reduction in total chlorophyll was evident in the red zone (Figure 5A). A lower proportion of chlorophyll a versus chlorophyll b was observed (Figure 5B,C). Thus, total chlorophyll levels were not impeded in RG until the fully red leaf stage.

■ DISCUSSION

The suitability of sorghum 3-deoxyanthocyanin pigments as potential natural food colorants has been established in prior studies (pH stability and antioxidant).23,28 Prior work has identified dark bran sorghum lines as possible sources for natural food colorants,9 but the bran yield remains low relative to other parts of the plant. In this work we have sought to identify a sorghum plant (via mutagenesis-assisted breeding) with increased concentration of 3-deoxyanthocyanin in leaf tissue. Leaves are vastly more abundant than bran and can be easily stored and harvested using existing technology. RG displayed intense red-purple coloration of the normally green lamina tissue (Figure 1). The RG mutant accumulated a >1000-fold increase in 3-deoxyanthocyanidin structures LUT and AP (and variants thereof, Figure 3) in leaves. In addition, we identified by LC-MS/MS a range of glycosylated flavones, which also contribute to the complex phenolic fingerprint of the sorghum RG leaf. The RG mutant also caused an antithetic leaf-stem accumulation–depletion of lignin.19 Increased aglycosylated flavones, 3-deoxyanthocyanin, and lignin suggest that the RG locus simultaneously influenced multiple branches in the phenylpropanoid pathway.

In our measurements, RG leaf material contained up to 10.1 mg of pigment per gram of dry leaf tissue. This concentration compares favorably to popular commercial sources of anthocyanin pigments, such as red cabbage, on a dry weight basis.19 Because most commercial sources of anthocyanin colorants are succulent tissues high in moisture and enzyme activity, and thus having poor storability (e.g., purple carrots, sweet potatoes, and red cabbage), the opportunity to add sorghum leaf tissue to the mix seems interesting. Moreover, sorghum leaves are very thin and easy to dehydrate for transportation and/or long-term storage.

Many variables would influence the production of pigment from the RG mutant, and the agronomic appropriateness of the crop was not part of the current work scope. However, drawing rough estimates from agronomic data such as density of planting (typically, between 20000 nonirrigated and 60000 irrigated plants per acre), leaf yield (approximately 70:30 to 50:50 ratio of stem to leaf), and biomass per acre (between 6 and 13 tons per acre), the yield of pigment would be approximately between 27 and 45 kg/acre (or 67−111 kg/ha). This again compares favorably to pigment yield from, for example, red cabbage, of 17−98 kg/ha.19 Additional advantages of using a high pigment accumulating sorghum variety (RG) to obtain 3-deoxyanthocyanins are that it displays C4 photosynthesis, which confers drought tolerance. A row crop alternative may also offer cost-effective planting and harvesting compared with below-ground crops (purple-fleshed sweet potato and carrots, which are limited to water and soil type constraints). However, it is important to note that the 3-deoxyanthocyanins are not replacements for anthocyanins, but are complementary to them, because each class of pigments displays distinctly

![Figure 4](image-url)

Figure 4. Estimation of the dihydroflavonolreductase (DFR) activity in RG leaves. The DFR activity was determined in green and red leaf tissues and compared to the wild type (WT) green tissues. (n = 3 biological pooled sample each containing 5 leaves.) Significance (P < 0.05) is indicated by a star (∗).

![Figure 5](image-url)

Figure 5. Chlorophyll estimation in RG and WT leaves: (A) total chlorophyll content for the RG, measurements made on red, green, and transition zone and compared to WT leaves at an equal developmental stage; (B, C) chlorophylls a and b were estimated as in (A). A star (★) indicates significant difference between the columns (four leaf disks for each leaf, leaf number = 6). (P < 0.05, ANOVA with a post hoc Tukey test.)

dx.doi.org/10.1021/jf405324j J. Agric. Food Chem. 2014, 62, 1227−1232
different hues and would thus work in different applications or as blends to achieve expanded hues for water-soluble plant-based food colorants.

**AUTHOR INFORMATION**

**Corresponding Author**
*(S.D.) Phone: (859) 257 8654. Fax: (859) 257 2934. E-mail: sdebo2@uky.edu.*

**Funding**
This work was supported by Grant NSF EFRI-0937657 (S.D., M.C.) and by the U.S. Department of Energy (DOE) under Award DE-FG36-08GO80043. We gratefully acknowledge the financial support of the U.S. Department of Agriculture National Institute for Food and Agriculture, Biomass Research and Development Initiative Grant 2011-10006-30363 (M.T., S.D.) and Hatch KAES13-17.

**Notes**
The authors declare no competing financial interest.

**REFERENCES**