Received: 11 November 2021 | Revised: 4 February 2022 | Accepted: 9 February 2022

DOI: 10.1002/cche.10536

RESEARCH

Toward bioeconomy of a multipurpose cereal: Cell wall chemistry of sorghum is largely buffered against stem sugar content

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Abstract

Background and Objectives: Sorghum (*Sorghum bicolor* L. Moench) is a multipurpose crop with high potential for the bioeconomy. Ten sweet, grain and dual-purpose sorghum genotypes were compared with respect to sugar-related traits and cell wall composition.

Findings: The dual-purpose hybrids Ganymed, Zerberus, and Tarzan performed better with respect to plant height, cane weight, and juice volume, but the sweet genotype KIT1 produced the highest sugar concentration (°Brix) in the stem. Analytical sugar data demonstrate genotypic differences in terms of sugar composition and concentrations, with sucrose being dominant. The monosaccharide composition of the stem cell wall polysaccharides showed surprisingly little genotypic variability. Variations in contents of lignin and cell wall-bound hydroxycinnamate monomers were moderate.

Conclusions: Sweet, grain and dual-purpose sorghum genotypes differ widely in terms of sugar-related morphological parameters but are comparable with respect to their cell wall chemistry.

Significance and Novelty: The use of sorghum as a bioeconomy crop has mainly focused on the extraction of sugar for bioethanol production. However, besides cell wall polymers, the potential usage of hydroxycinnamates as platform molecules for the chemical industry may improve the valorization of the residues after sugar extraction. This application appears to be fairly independent of genotype, further increasing the potential of sorghum for the bioeconomy.

KEYWORDS

bioeconomy, cell wall chemistry, hydroxycinnamic acids, lignin, sorghum genotypes, sugar contents

Adnan Kanbar and Daniela S. Schäfer contributed equally to this study.

1 | INTRODUCTION

Sorghum is considered the world's fifth-most important cereal crop, which is based on the fact that it allows for multipurpose use as food, animal feed, source of bioethanol, and other industrial applications (Cuevas et al., 2015; Tesfaye, 2017). As sorghum is capable of C_4 photosynthesis, it can convert carbon dioxide very efficiently into biomass, further promoting its multipurpose versatility and bioeconomy potential (Irving, 2015). In contrast to other bioeconomy crops, sorghum provides several sources for the production of ethanol, including stem juice, bagasse, and grains (Wang et al., 2008). However, grains and preferentially also the juice should primarily be used for food, leaving the lignocellulosic bagasse biomass for the production of ethanol and (aromatic) platform compounds for the chemical industry or, alternatively, as animal feed or as a substrate for biogas production.

Sorghum varieties are classified into four major types based on their primary uses: sweet, grain, feed, and high biomass types (Murray et al., 2009; Shakoor et al., 2014). Grain sorghum, which stores carbohydrates preferentially as starch in seeds (Dicko et al., 2006), has a dry stem and is a popular, gluten-free staple crop in Africa and China (Dicko et al., 2006; Felderhoff et al., 2012). In contrast, due to its high contents of easy-to-extract sugar in the stem, sweet sorghum is well suited for plant-based bioethanol production (Disasa et al., 2018), and also to produce syrups for food use. Sweet sorghum is a natural variant of grain sorghum with thicker stems that serve as a primary sink tissue for synthesized sugars during flowering and translocation into seeds during seed filling (Rao et al., 2013). Large efforts have been made by breeders to exploit such variability between sweet and grain sorghum for developing dual-purpose sorghum varieties combining high grain yield and high sugar content in the stem juice to develop a novel (energy) crop that is able to thrive on marginal lands, which allows circumventing the "fuel versus food" conundrum (Prasad et al., 2007; Tsuchihashi & Goto, 2004; Vinutha et al., 2014). Also, sweet and dual-purpose sorghum genotypes are discussed as a feedstock for second-generation bioethanol production and, more generally, as lignocellulosic feedstocks in temperate areas such as Germany due to its ability to produce high biomass and sugar under adverse conditions (Smith & Buxton, 1993; Windpassinger et al., 2015).

Previous research investigating the accumulation of sucrose in sweet and grain sorghum stems indicated that sucrose accumulation begins with the start of the reproductive phase (Kanbar, Flubacher, et al., 2021; Lingle, 1987). Sorghum accumulates sugar preferentially in the central internodes, whereas the basal internodes accumulate more stem weight (Kanbar, Flubacher, et al., 2021). Compared to the grain types, sweet genotypes double or even triple the sugar concentration in all internodes (Kanbar et al., 2021). Sucrose is the main soluble sugar of sorghum, accompanied by glucose and fructose (Chen et al., 2014; Simeone et al., 2017), which allows inferring sugar content refractometrically (Murray et al., 2009). The readout (°Brix) is widely accepted as a reflection of sucrose percentage in a solute, and the positive and significant correlation between °Brix and sucrose levels in sorghum syrup has been demonstrated across different genotypes of sweet sorghum (Morey et al., 2018). However, the readout in °Brix does not adequately reflect the contents of the monosaccharides glucose and fructose, and other techniques such as high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) are necessary to determine the monosaccharide composition in detail.

Plant cell walls represent the framework of the plant cell and serve to stabilize the cell, protect it from external influences, and separate the cells from each other. In addition, cell walls play an important role in signal transmission and in the transport of substances (Doblin et al., 2010). Plant cell walls consist of the middle lamella and primary cell walls. A secondary cell wall can additionally be deposited after the termination of cell growth. In general, plant cell walls are composed of structurally diverse nonstarch polysaccharides, structural proteins, and lignin. However, the composition of plant cell walls is complex and strongly depends on, among others, phylogeny, tissue type, and/or maturation stage of the plant. The composition of primary cell walls differs between the so-called type I and type II cell walls (Carpita & Gibeaut, 1993). Type II cell walls are typical for grasses, such as sorghum, and are mainly composed of cellulose and hemicelluloses, most importantly heteroxylans such as (glucurono-)arabinoxylans and, in particular grasses, mixed-linked β-glucans (Carpita & Gibeaut, 1993; Vogel, 2008). In grass heteroxylans, the backbone, which is made up of β-(1,4)-xylopyranose units, is commonly substituted with α-arabinofuranosyl residues that are attached at the *O*-2 and/or *O*-3 positions of the xylosyl residues (Vogel, 2008). In addition, arabinofuranosyl substituents can be esterified with hydroxycinnamic acids such as ferulic acid, which is responsible for cross-link formation between polysaccharides and polysaccharides, and lignin (Bunzel, 2010). Besides cell wall polysaccharides, lignin is often deposited with the beginning of secondary cell wall formation, resulting in higher stability of the cells

and decreased water permeability (Boerjan et al., 2003). Because the cell wall composition affects both forage digestibility and fermentation processes to produce bioethanol, knowledge about the cell wall composition of sorghum stems is important to improve both (Burton et al., 2010; Byrt et al., 2013; Paterson et al., 2009; Vancov & McIntosh, 2011, 2012).

Lignin negatively affects biomass digestibility and has been a long-standing barrier to efficient monosaccharide release from lignified plant materials (Weng et al., 2008). To reduce biomass recalcitrance, genetic factors that contribute to a reduction of lignin content have attracted considerable interest (Martin et al., 2013; Saballos et al., 2012). However, reduced lignin contents will also render the plants prone to lodging, because the bending momentum at breaking will decrease as well (Oda et al., 1966). A strategy where the bioeconomic value of sorghum does not derive from a single use (sugar extraction) but is the result of a multistep valorization, where the residues from sugar extraction can be processed further, including the use of lignocellulose for the extraction of interesting compounds for industrial purposes, would allow circumventing the antagonism of lodging resistance and sugar extractability. As a first step toward this goal, we mapped three types of sorghum (grain, sweet, and dual-purpose) raised under a temperate climate with respect to morphological and sugar-related parameters, and also with respect to the composition of the cell wall.

2 | MATERIALS AND METHODS

2.1 | Plant materials

For this study, 10 sorghum genotypes were chosen and grouped according to their use in agricultural practice (Table 1). These included sweet varieties (KIT1, Ramada, and So-29), grain landraces (Razinieh and Ruzrok), and hybrids with high grain yield, along with stem weight, which were bred for the dual purpose using the grains for food or feed and the stems for biogas production (Zerberus, Ganymed, Freya, Sole, and Tarzan). All hybrids used in this study were bred by the company KWS (KWS SAAT SE & Co. KGaA) for high grain and biomass yield, as well as disease resistance. The sweet sorghum genotype KIT1 was developed by us using pedigree selection (Kanbar, Flubacher, et al., 2021). The other sweet variety, So-29, was derived from the Crop Research Institute (Prague, Czech Republic) and was released in 1971 (Kanbar, Flubacher, et al., 2021). Ramada is also a sweet sorghum variety that was selected from the progeny of the cross ("MER 45–45" × "MN 1056") ×

TABLE 1 List of genotypes used in this study, their agronomic group, type, origin, and characteristics

ID	Cultivars	Type	Pedigree	Source	Characteristics
9475	Ganymed	Grain, biomass	*S. bicolor* × *S. bicolor*	KWS, Germany	Resistant to lodging, high grain and biomass yields, tall, juicy stem, and disease resistance
9478	Freya	Grain, biomass	*S. bicolor* × *S. sudanense*	KWS, Germany	Susceptible to lodging, high grain and biomass yields, tall, juicy stem, and disease resistance
9472	Zerberus	Grain, biomass	*S. bicolor* × *S. bicolor*	KWS, Germany	Resistant to lodging, high grain and biomass yields, tall, juicy stem, and disease resistance
9480	Tarzan	Grain, biomass	*S. bicolor* × *S. bicolor*	KWS, Germany	Resistant to lodging, high grain and biomass yields, tall, juicy stem, and disease resistance
9481	Sole	Grain, biomass	*S. bicolor* × *S. sudanense*	KWS, Germany	Susceptible to lodging, high grain and biomass yields, tall, semi-juicy stem, and disease resistance
9484	KIT1	Sweet, biofuel	Elite line	KIT, Germany	Resistant to lodging, high grain, biomass and sugar yields, tall, medium height, and juicy stem
9483	So-29	Sweet, biofuel	Variety	Czech Republic	Susceptible to lodging, medium height, juicy stem, moderate grain yield, and high sugar yield
9485	Ramada	Sweet, biofuel	Variety	USA	Resistant to lodging, juicy stem, medium height, high grain, biomass, and sugar yields
9482	Ruzrok	Grain	Variety	Czech Republic	Moderate to lodging, early maturity, medium height, pithy stem, high grain yield, black seeds.
9145	Razinieh	Grain	Landrace	Syria	Moderate to lodging, medium grain yield and low biomass, medium height, pithy stem, drought and salt resistance.

("MN 1054" × "MN1060") made at Meridian (Mississippi, USA) in 1974 (Inman-Bamber, 1980). The grain sorghum genotype Razinieh is a Syrian landrace, whose grain yield was improved by bulk breeding (Kanbar, Flubacher, et al., 2020; Kanbar, Flubacher, et al., 2021; Kanbar, Mirzai, et al., 2021). Ruzrok is a grain sorghum variety that was bred from a landrace collected by V. Holubec in the Bílé Karpaty region in Moravia, Czech Republic (Hermuth & Kosova, 2017).

2.2 | Field experiment

The seeds were planted in early May at the Botanical Garden of Karlsruhe Institute of Technology (Karlsruhe, Germany) during the summer of 2018. Karlsruhe city is located in the Rhine Valley, in SouthWest Germany (latitude: 49°0′24.8004″N, longitude: 8°24′13.1508″E), and has an average elevation of 119 m above sea level. Temperatures ranged from around −1°C in the winter to around 26°C in the summer in a temperate oceanic climate. The seeds of 10 genotypes were treated with a fungicide before planting to ensure better seedling emergence and stand establishment. Then, the seeds were planted in three randomized experimental blocks, each block containing one plot of six adjacent rows of 5.0 m length for each sorghum genotype. At the second leave stage, plots were thinned maintaining a spacing of 60 cm between rows and 25 cm between individuals within rows. A total of 20 individual plants was kept per row. The temperature and rainfall were monitored during the experiment and are shown as monthly average values in Table S1. Based on soil analysis, 100 g/m² organic fertilizer (Hauert Hornoska® Special, Nürnberg, Germany) and 90:60:40 kg/ha of NPK were added.

Sorghum plants were harvested at the dough seed stage as recommended by previous studies (Kanbar, Flubacher, et al., 2021). This stage provides the optimum concentration of stored sugar in the stem sink tissues. Five random plants located in the center of a plot were harvested from each replicate, recording plant height (cm), leaf number, internode number, green leaf weight (g/plant), green leaf area (cm^2/plant), cane weight (g/plant), juice volume (ml/plant), bagasse weight (g/plant), sugar content (°Brix), and sugar weight (g/plant). A cane crusher was used to extract the juice and to measure the sugar concentration as °Brix. Stem sugar percentage was estimated for each individual cane from the readout in °Brix, determined by a hand-held refractometer (Model PAL, Atago Co. Ltd.), by means of a regression equation developed in the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) as described in (Kanbar et al., 2020). Sugar weight (g/plant) and percentage were estimated according to Reddy et al. (2005).

2.3 | Preparation of plant materials for chemical analyses

Replicates were prepared by pooling 2–5 plants each. Leaves were removed directly after harvest. Sorghum stems were freeze-dried (estimation of dry weight) and ground to a size of ≤1 mm.

2.4 | Chemicals and enzymes

Heat stable α-amylase Termamyl 120 L (from *Bacillus licheniformis,* 120 KNU/g), the protease Alcalase 2.5 (from *B. licheniformis*, 2.5 AU/g), and the amyloglucosidase AMG 300 L (from *Aspergillus niger*, 300 AGU/g) were kindly donated by Novozymes (Bagsvaerd, Denmark). Chemicals used were purchased either from VWR, Part of Avantor, Sigma-Aldrich, Alfa Aesar, or Roth.

2.5 | Analysis of soluble carbohydrates

Freeze-dried and milled plant material as prepared in 2.3 (200 mg) was suspended in 10 ml of H_2O and treated in an ultrasonic bath for 10 min (temperature ≤ 30°C). Following mixing for 30 s, the suspension was centrifuged (4500 rpm, 10 min), and the supernatant was removed. The extraction procedure was repeated three times. Supernatants were combined, adjusted to a defined volume, and filtered (polytetrafluoroethylene, 45 μm). Soluble polysaccharides and larger oligosaccharides were precipitated by adding the fourfold volume of 99.5% ethanol. Samples were centrifuged, the supernatant was evaporated, and monoccharides and disaccharides were dissolved in H_2O and diluted. Fucose (0.6 μg) was added as a measurement standard, and samples were analyzed by HPAEC-PAD equipped with a CarboPac PA-20 column (150 mm × 3 mm; Thermo Fisher Scientific) using a flow rate of 0.4 ml/min and H_2O (A), 0.1 M NaOH (B), and 0.1 M NaOH + 0.2 M sodium acetate (C) as eluents. Before each run, the column was rinsed for 10 min with 100% B, and equilibrated for 10 min with 90% A and 10% B. After injection, the following gradient was applied: linear over 1.5 min to 96% A and 4% B, held for 20.5 min; linear over 10 min to 100% B; linear over 0.1 min to 100% C, held for 9.9 min.

2.6 | Preparation of cell wall material

Cell wall material was isolated by enzymatic degradation of starch and protein using the principle of AOAC method 985.291 with minor modifications (Prosky et al., 1985; Schäfer et al., 2016). In brief, dried plant material (15 g, see section 2.3) was suspended in 200 ml of sodium phosphate buffer (0.08 M, pH 6.2), α-amylase (1.5 ml) was added, and incubation was performed at 90°C for 20 min. The suspension was cooled to room temperature, pH was adjusted to 7.5, and incubation with protease (700 μl) was carried out at 60°C for 30 min. Incubation with amyloglucosidase (700 μl) was performed at pH 4.5 at 60°C for 30 min. Cell wall material was precipitated overnight by adding the fourfold volume of ethanol (99.5%). Following centrifugation, the residue was washed twice each with ethanol (80% and 99.5%) and acetone and dried at 60°C. After drying, aliquots of the residues were additionally washed four times with 80% ethanol (to minimize soluble carbohydrate contents), once with 80% ethanol, twice with ethyl acetate, and three times with hexane. Cell wall material was finally dried at 45°C in a vacuum oven and used for cell wall polymer characterization.

2.7 | Determination of lignin contents

Lignin contents were determined as Klason lignin (Adams, 1965; Theander & Westerlund, 1986) and acetyl bromide soluble lignin (ABSL) (Hatfield, Grabber, et al., 1999; Iiyama & Wallis, 1990). Determination of Klason lignin contents was performed according to Bunzel et al. (2011). Cell wall material (200 mg) was mixed with 5 ml of 12 M sulfuric acid, and the suspension was held for 30 min on ice and additionally for 2 h at room temperature. The suspension was diluted with 32.5 ml of H_2O and hydrolyzed for 3 h at 100°C. Samples were filtered through glass microfiber filters (1.6 μm; Whatman), and residues were washed acid-free and dried at 60°C. Filtrates were used for monosaccharide determination (see Section 2.8). Klason lignin samples were corrected for ash and residual protein contents. Ash contents were determined gravimetrically after incineration at 550°C. Protein contents were calculated from the nitrogen contents of the Klason lignin samples by using the general conversion factor of 6.25. Nitrogen contents were analyzed after Kjeldahl digestion using an ammonium selective electrode (Thermo Scientific) (Urbat et al., 2019).

ABSL contents were analyzed according to Bunzel et al. (2005). In brief, cell wall material (25 mg) was incubated with 4 ml of 25% acetyl bromide in glacial acetic acid at 50°C for 2 h. The solution was diluted with 12 ml of glacial acetic acid and 1 ml of the sample solution was mixed with 2.5 ml of glacial acetic acid, 1.5 ml of 0.3 M NaOH, and 0.5 ml of 0.5 M hydroxylamine hydrochloride solution, and the volume was adjusted to 10 ml with glacial acetic acid. Absorbance was read at 280 nm on a Jasco V-550 photospectrometer (Jasco). ABSL contents were calculated using an absorption coefficient of 20 ml/(cm × mg) (Iiyama & Wallis, 1990).

2.8 | Monomer composition of cell wall polysaccharides

The monomer composition of cell wall polysaccharides was determined after sulfuric acid hydrolysis (Saeman et al., 1945). For this purpose, Klason lignin filtrates were used (see Section 2.7). Following neutralization of Klason lignin filtrates, liberated monosaccharides were analyzed by HPAEC-PAD on an ICS5000 system (Thermo Scientific Dionex) equipped with a CarboPac PA20 column (150 mm × 3 mm i.d., 6.5 μm particle size; Thermo Scientific Dionex). The following gradient composed of (A) bidistilled water, (B) 0.1 M sodium hydroxide, (C) 0.1 M sodium hydroxide + 0.2 M sodium acetate, and a flow rate of 0.4 ml/min were used: Before every run, the column was rinsed with 100% B for 10 min and equilibrated for 10 min with 90% A and 10% B. After injection, the following gradient was applied: 0–1.5 min, linear to 96% A and 4% B; 1.5–22 min, isocratic, 96% A and 4% B; 22–32 min, linear to 100% B; 32–42 min, isocratic, 100% C. The temperature was held at 25°C and the injection volume was 25 μl.

2.9 | Determination of cell wall-bound phenolic components

Cell wall-bound phenolic components were determined according to Dobberstein and Bunzel (2010). Cell wall material (50 mg) was saponified in 2 M NaOH for 18 h. To determine ferulic acid dehydrodimers (DFA), 5-5(methylated)-DFA (10 μg) was added, and the solution was acidified to pH < 2. Extraction was performed three times using diethyl ether. Extracts were evaporated under nitrogen, and residues were dissolved in MeOH/H_2O (50/50, v/v). Phenolic components were analyzed by reversed-phase HPLC coupled to a photodiode array detector on a Luna phenylhexyl column (250 mm × 4.6 mm, 5 μm; Phenomenex) using 1 mM trifluoroacetic acid (A), methanol/1 mM trifluoroacetic acid (90/10, v/v) (B), and acetonitrile/1 mM trifluoroacetic acid (90/10, v/v) (C) as eluents. The following gradient was used to analyze phenolic monomers: initially, 87% A, 0% B, and 13% C held for 10 min; linear over 10 min to 77% A, 3% B, and 20% C; linear over 5 min to 70% A, 5% B, and 25% C; linear over

5 min to 25% A, 25% B, and 50% C; linear over 5 min to 0% A, 50% B, and 50% C, held for 5 min, following an equilibration for 5 min step at initial conditions. The following gradient was used to analyze DFAs: initially 85% A, 0% B, and 15% C; linear over 15 min to 82% A, 0% B, and 18% C; linear over 5 min to 80% A, 0% B, and 20% C; linear over 5 min to 72% A, 3% B, and 25% C; linear over 5 min to 70% A, 5% B, and 25% C, held for 25 min; linear over 5 min to 65% A, 5% B, and 30% C; linear over 5 min to 55% A, 5% B, and 40% C, held for 5 min; linear over 3 min to 0%A, 20% B, and 80% C, held for 5 min, following an equilibration for 5 min at initial conditions. Quantitation of ferulic acid and *p*-coumaric acid was performed at 308 and 321 nm using an external calibration. DFAs were quantitated at 280 nm using previously described correction factors (Dobberstein & Bunzel, 2010).

2.10 | Statistical analysis

Phenotypic data were subjected to individual analysis of variance (ANOVA) for different traits to assess the variability among the genotypes using PROC ANOVA in SAS version 9.4 (SAS Institute Inc.). Mean comparisons were separated with the least significant difference at a 5% level of significance. Additionally, grand mean and coefficient of variation as a percentage (C.V.%) were recorded for each measured trait individually. For chemical analyses, two to five plants were combined to form a biological replicate. Each biological replicate was analyzed twice. Data for each replicate are indicated as mean ± range/2.

3 | RESULTS AND DISCUSSION

Ideally, the stem of sorghum plants provides large amounts of sugar that can be used as high sugar syrup in the food industry as well as large amounts of lignocellulosic biomass that is comparably low in lignin and contains less ferulate-based cross-links between arabinoxylans as well as between arabinoxylans and lignin. Besides these compositional/chemical traits, knowledge about agro-morphological traits such as plant height, cane yield, internode number, and so on, is necessary to judge the genotypic resources with respect to their applicability as "multipurpose" resources.

3.1 | Agro-morphological descriptive analysis

The ANOVA using a randomized complete block design showed significant variation ($p < .05$) among the genotypes for all 10 quantitative traits (Table S2) with considerable ranges in plant height (277.7–442.5 cm), cane weight (83.3–624.0 g/plant), bagasse weight (63.7–249.0 g/plant), juice volume (13.3–261.0 ml/plant), sugar concentration as °Brix (6.9–15.2), and total sugar weight per plant (1.0–25.7 g/plant) (Figure 1 and Table S3). Similar genotypic differences with respect to morphological characters and sugar-related traits have also been reported in previous studies (Abdi et al., 2002; Deu et al., 2006; Kanbar et al., 2020; Kanbar, Flubacher, et al., 2021; Motlhaodi et al., 2017; Wang et al., 2008).

For instance, the dual-purpose sorghum hybrid Ganymed produced the highest leaf number (14.0), green leaf weight (80.8 g/plant), green leaf area (2831.7 cm^2/plant), cane weight (624.0 g/plant), juice content (261.0 ml/plant), bagasse weight (249.0 g/plant), and predicted sugar weight (25.7 g/plant) (Figure 1 and Table S3). Ganymed variety produced the highest sugar weight as this trait depends on juice content in the stem and °Brix (Reddy et al., 2005). Ramada, KIT1, Tarzan, and Zerberus ranged after Ganymed in producing higher sugar weights per plant as compared to other genotypes. In contrast, the grain variety Ruzrok showed the lowest values for all studied traits, except sugar content (8.1 °Brix), among all tested genotypes (Figure 1, Table S3). Regarding sugar content, the sweet genotype KIT1 produced the highest sugar concentrations (15.2 °Brix), whereas the grain landrace Razinieh showed the lowest sugar concentration (6.9 °Brix). However, all the hybrids displayed sugar concentrations higher than 10 °Brix, except Sole (9.7 °Brix), whereas grain sorghum genotypes exhibited sugar concentrations of less than 9 °Brix. Compared to the sweet sorghum genotype KIT1, the dual-purpose hybrid Ganymed showed higher juice content and cane weight, indicating a higher potential of sugar yield (Figure 1 and Table S3). When the studied sorghum genotypes are compared based on their uses, the mean performance of the dual-purpose sorghum group for plant height, leaf number, internode number, green leaf weight, green leaf area, cane weight, and bagasse weight was higher as compared to the sweet and grain genotypes (Figure 1 and Table S3). However, the group of sweet sorghums displayed a higher mean performance for °Brix and sugar weight over grain and dual-purpose sorghum groups (Figure 1 and Table S3). The group of sorghum hybrids (including all the dual-purpose genotypes) showed better performance with respect to all the morphological parameters over the group of homozygous cultivars (sweet and grain genotypes) (Figure 1 and Table S3).

Due to the wide variability in juice content and extractability between dual-purpose, sweet, and grain sorghum genotypes, an assessment of genotypes for

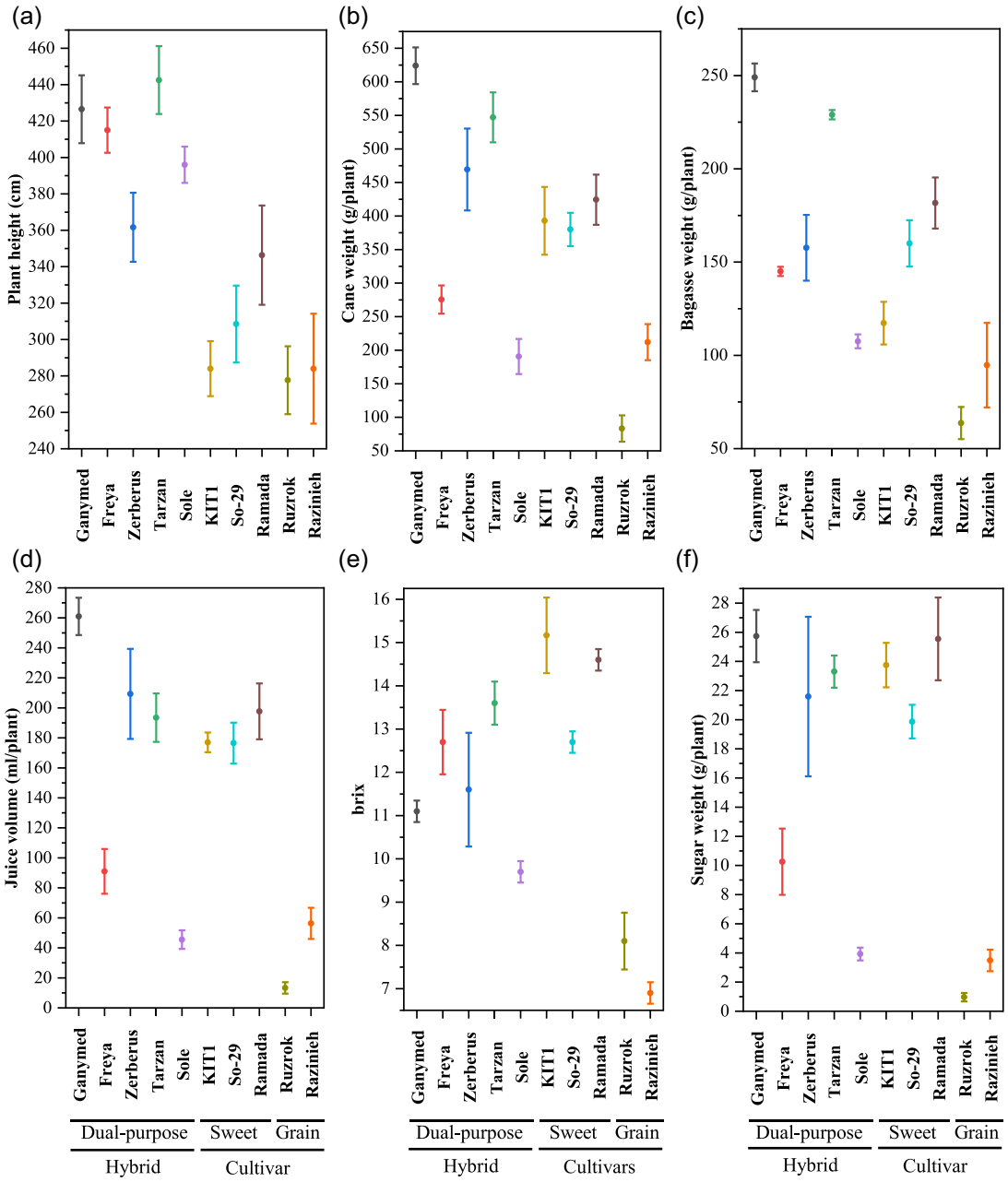

FIGURE 1 The means with 95% confidence intervals of (a) plant height (cm), (b) cane weight (g/plant), (c) bagasse weight (g/plant), (d) juice volume (ml/plant), (e) sugar content as °Brix, and (f) sugar weight (g/plant) for 10 sorghum genotypes. The genotypes were grown under temperate climatic conditions in the 2018 season in Southwest Germany and harvested at the dough seed stage. Values are means of three replicates (five samples in each replicate)

potential sugar yields is of great importance. Various investigators (Kawahigashi et al., 2013; Mak et al., 2011; Ritter et al., 2007; Shakoor et al., 2014; Wang et al., 2008) demonstrated that the sugar concentration measured as °Brix has limitations in reflecting fructose and glucose as monosaccharides, but showed better correlation with sucrose and total sugar contents. Therefore, and because the °Brix does not necessarily only reflect the sugar concentration (in the juice), but—as a density determination—also represents other water-soluble constituents, soluble sugars contents were analyzed in more detail.

For this purpose, two to five plants of each genotype were combined and served as starting material for all chemical analyses. Two of these bundled batches were analyzed (biological replicates a and b; Figure 2), and both biological replicates were analyzed twice. The temperature during the extraction of soluble sugars was kept below 30°C to reduce potential cleavage of sucrose into monomers and monosaccharide degradation.

Preliminary tests with an extraction temperature of 60°C resulted in lower total carbohydrate yields and poor reproducibility compared to the extraction procedure applied here. Extractable sugars were sucrose (8.0–299.6 mg/g dry weight) as the main sugar, followed by glucose (3.9–123.2 mg/g dry weight), and fructose (4.5–102.0 mg/g dry weight) (Figure 2). Sucrose, glucose, and fructose are described in the literature as the main soluble sugars of sorghum, too. However, their contents differ strongly, depending on several factors such as extraction and analytical procedures, genotype, and developmental stage (Billa et al., 1997; Chen et al., 2014; Goto et al., 1991; Li et al., 2014; Simeone et al., 2017). Analytical data for fructose, glucose and sucrose demonstrate partially large differences between the two biological replicates and also demonstrate genotypic differences in terms of sugar composition and concentrations. For example, Ramada (a sweet genotype) almost exclusively contained sucrose, whereas KIT1 (a sweet genotype as well) also contained large amounts of fructose and glucose (Figure 2). Although not as dominant as in KIT1, fructose and glucose were significant in So-29 (a sweet genotype), but also in Ganymed (a dual-purpose genotype). As expected, sweet genotypes contained the highest sugar concentrations compared to dual-purpose and grain genotypes. Also, slightly higher sugar concentrations were found for the homozygous sorghum cultivars (sweet and grain genotypes) compared to the hybrid group. By far the lowest sugar amounts per gram dry weight of stem material was found for the genotype Freya (a dual-purpose genotype). The Brix value for Freya was average; however, the juice volume per plant was at the lower end (Figure 1), such that the combination of the two factors can account for the low sugar value per gram stem dry weight.

The dual-purpose hybrids Ganymed and Tarzan exhibited a taller, sugar-rich juicy stem, and a higher cane production, and were more resistant to lodging (Table 1 and Figure 1). The large biomass of hybrid sorghum was linked with their tall habitus. A similar variation has been reported for a variety of morphological and agronomical traits, suggesting that crops as biofuel feedstock sources can be improved (Reddy et al., 2005; Rooney et al., 2007; Wang et al., 2008). Although sorghum is preferentially self-pollinating, heterosis has been well documented in terms of grain yield and biomass production (Rao et al., 2013). In Germany, sorghum hybrids account for a large portion of the grain, biomass, and forage production (Hermuth & Kosova, 2017). While all the hybrids in this study were developed by the company KWS in Germany, and they are suited for temperate environmental conditions, the parents of KIT1 derive from a context with semi-arid environmental conditions of South India.

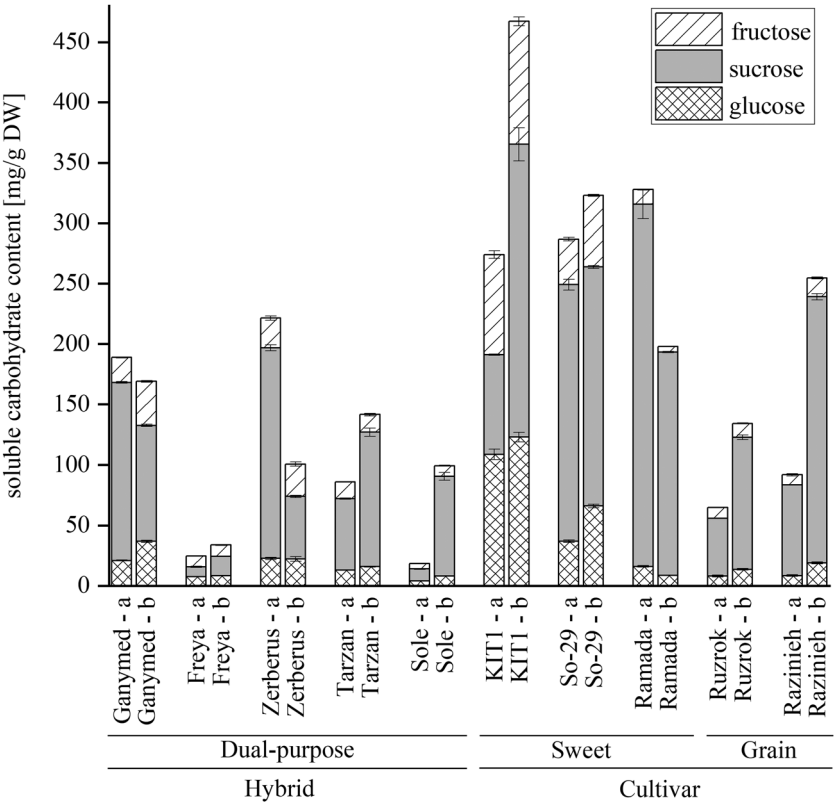

FIGURE 2 Contents of soluble carbohydrates extracted from stems of different sorghum genotypes. Results for each replicate (a, b) are shown ($n = 2$). DW, dry weight

3.2 | Cell-wall polysaccharides

The monomer composition of cell wall polysaccharides was determined after H_2SO_4 hydrolysis. Glucose was analyzed as the main monosaccharide (50.0–53.9 mol%), followed by xylose (43.2–46.3 mol%), and comparably low portions of arabinose (2.9–3.8 mol%) (Figure 3). In addition, traces of galactose, galacturonic acid, and glucuronic acid were detected.

The monosaccharide composition of the stem cell wall polysaccharides showed very little variability among replicates and genotypes, suggesting surprisingly similar polysaccharide structures within the samples. Because sorghum belongs to the family of *Poaceae*, cellulose and (glucurono-)arabinoxylans are expected to be the main cell wall polysaccharides. The predominance of mainly cellulose and (glucurono-)arabinoxylans in sorghum samples has already been described (Billa et al., 1997; Goto et al., 1991; Hatfield, Wilson, et al., 1999; Verbruggen et al., 1998). By using methylation analysis, Goto et al. (1991) confirmed that glucose is mainly 1,4-linked. Both cellulose and xyloglucans contain a backbone of 1,4-linked glucopyranose units. However, xyloglucans are not expected to significantly contribute to the cell walls of *Poaceae*. Therefore, glucose can mainly be attributed to cellulose contents. In addition, the authors showed that xylose is mainly 1,4-linked with substitution in position *O*-3 and to a lesser extent in position *O*-2 of certain xylopyranose units, indicating arabinoxylans as main hemicellulosic polysaccharides. The arabinose/xylose ratio of about 0.07 for all genotypes analyzed in this study indicates arabinoxylans of low substitution. Additional substitution of the xylan backbone with glucuronic acid is likely due to the detection of small amounts of glucuronic acid after H_2SO_4 hydrolysis.

To utilize sorghum as a feedstock, efficient hydrolysis of cell wall polysaccharides is required to yield monosaccharides, which can be further fermented to bioethanol. However, extensive hydrolysis depends on structural features and interactions of the polysaccharides in the cell wall network. Hydrolysis of cellulose into fermentable sugars is mainly limited by its crystallinity and by the presence of lignin (Bichot et al., 2018; Chang & Holtzapple, 2000; Puri, 1984; Yang et al., 2015). Hemicelluloses are often attributed to biomass recalcitrance, too, especially with regard to ester-linked ferulic acid that can form cross-links between polysaccharides or between polysaccharides and lignin (Bichot et al., 2018). Therefore, chemical, physical, and/or biological pretreatments (Sun & Cheng, 2002) are generally required to enhance the enzymatic degradability of cellulose. The efficiency of lignocellulosic biomass utilization can be improved by additionally converting pentoses (here mostly from arabinoxylans) into ethanol by using genetically engineered microorganisms (Kuhad et al., 2011; Lamichhane et al., 2021). Because all analyzed sorghum genotypes contain mainly cellulose and (glucurono-)arabinoxylans, they are, in principle, suitable bioethanol feedstocks.

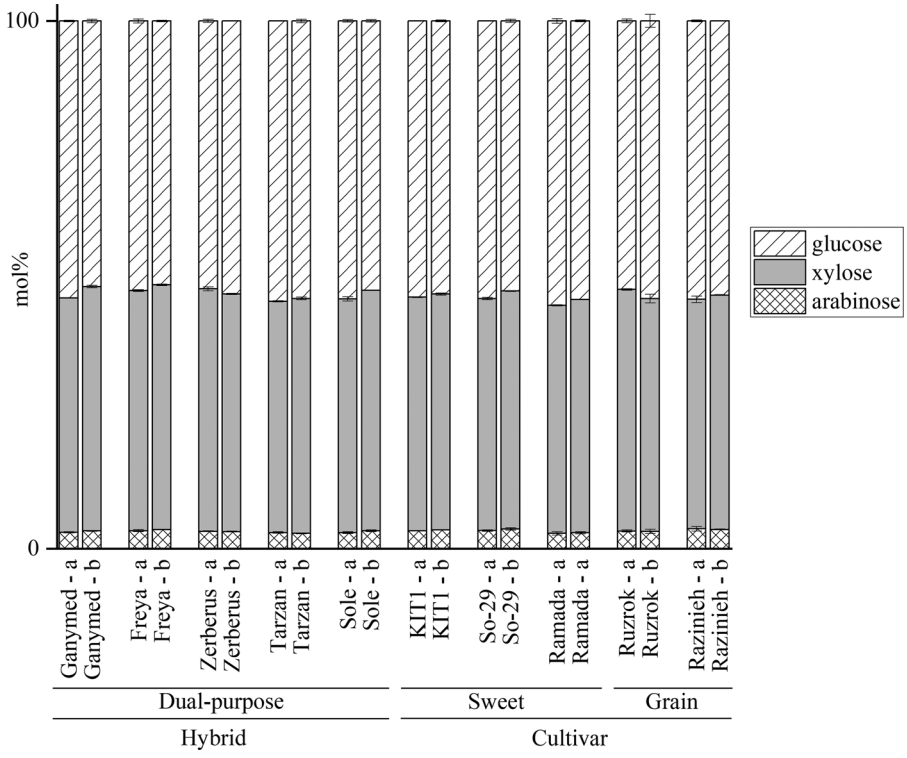

FIGURE 3 Monomer composition of cell wall polysaccharides from stems of different sorghum genotypes. Results for each replicate (a, b) are shown ($n = 2$). Traces of galactose, galacturonic acid, and glucuronic acid were identified for each genotype

However, other cell wall components (e.g., lignin) and interactions within the whole cell wall network (e.g., cross-links) may be limiting factors for specific genotypes.

3.3 | Lignin contents

Lignin contents were determined as both Klason lignin and ABSL to get a more reliable estimate of lignin contents. Because both methods are not specific and because both methods rely on either solubility (ABSL) or insolubility (Klason) of (derivatized, ABSL) lignin polymers, both methods may under-/overestimate lignin contents. Klason lignin is calculated gravimetrically after acidic hydrolysis of cell wall polysaccharides. However, Klason lignin contents can be overestimated due to capturing nonlignin components such as ash, proteins, waxes, and other polyaromatic/polyaliphatic plant constituents (Bunzel et al., 2011). To reduce the influence of nonlignin components, cell wall material was washed with ethanol, ethyl acetate, and hexane, and residual ash and protein contents were determined in the Klason lignin residues. However, also an underestimation of lignin contents may occur because acid-soluble lignin may be lost during sample preparation. ABSL is determined spectrophotometrically after dissolving lignin in acetyl bromide/acetic acid. However, lignin contents may be underestimated due to incomplete lignin solubility. On the other hand, nonlignin components such as degradation products of xylans or polysaccharide-bound ferulic acid that also absorb ultraviolet light at 280 nm can interfere resulting in an overestimation of lignin contents (Hatfield & Fukushima, 2005).

Klason lignin and ABSL contents of sorghum stem cell wall materials are shown in Figure 4. Analysis of ash and protein contents of Klason lignin preparations demonstrated low amounts of ash (<1.6%) and protein (<5%, Nx6.25). Thus, Klason lignin samples were not corrected for ash and protein contents.

Klason lignin (16.0 ± 0.2–20.1 ± 0.2 g/100 g cell wall material) and ABSL (17.6 ± 0.5–20.6 ± 0.3 g/100 g cell wall material) contents vary only moderately between genotypes. When these differences are linked to their use, dual-purpose genotypes show slightly higher lignin contents compared to sweet and grain genotypes. As all dual-purpose genotypes belong to the hybrid group, this group also contains slightly higher lignin contents compared to the homozygous cultivar group (Figure 4). It stands out that, in contrast to the abundance of soluble sugars and in accordance with the polysaccharide monomer data, lignin contents of the two replicates of each genotype were comparable. Taking into account that lignin contents depend on several factors, for example, the method used, the part of the plants extracted, cultivation conditions, and/or the harvest date, lignin contents determined in this study are roughly comparable to those reported in the literature for sorghum stems or sorghum biomass (Hatfield et al., 2009; Herrera et al., 2004; Wahyuni et al., 2019).

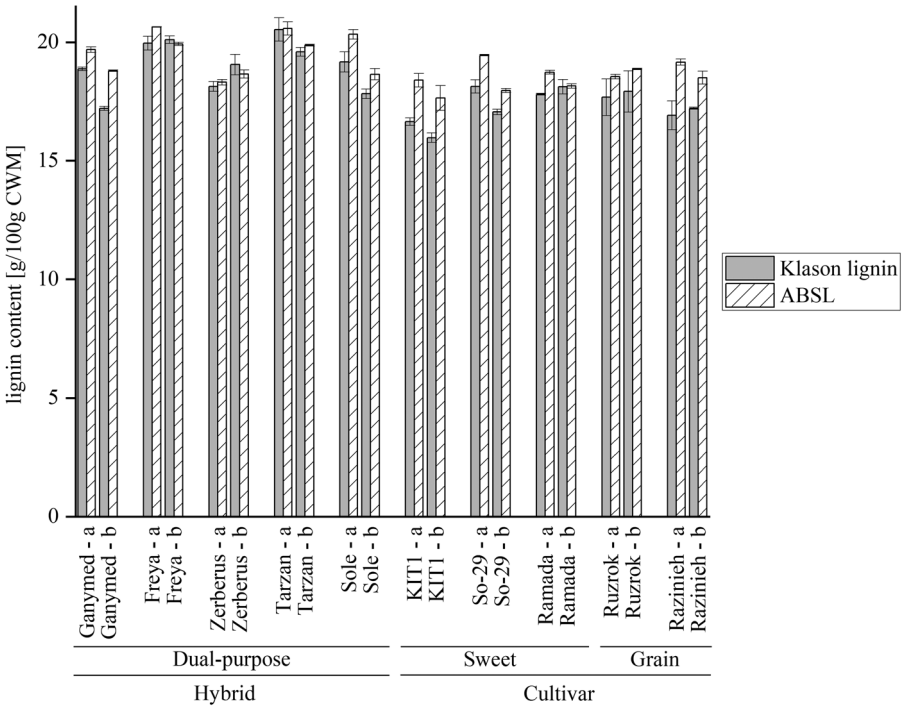

FIGURE 4 Klason lignin and acetyl bromide soluble lignin (ABSL) contents of cell wall material isolated from stems of different sorghum genotypes. Results for each replicate (a, b) are shown ($n = 2$)

Although the measured lignin contents depend on the respective method used for quantification, it appears that the sweet genotype KIT1 is among those genotypes with lower lignin contents, although the differences are minor. This is certainly remarkable as this genotype was also among the genotypes that showed the highest sugar contents. Given the fact that lignin largely contributes to the recalcitrance of the biomass against fermentation processes, lower lignin contents may favor this genotype for the production of second-generation biofuels following sugar extraction from the stems.

3.4 | Cell wall-bound hydroxycinnamates

Trans-p-coumaric acid was the dominant ester-linked hydroxycinnamic acid (15.7–24.7 mg/g cell wall material; 79%–87% of total cell wall-bound phenolic monomers), followed by *trans*-ferulic acid (3.6–5.3 mg/g cell wall material; 13%–21% of total cell wall-bound hydroxycinnamate monomers) for all sorghum genotypes (Figure 5). Although there was some variation between the biological replicates, this effect was generally smaller than observed for soluble sugars and comparable to data from cell wall polymers (see above).

Variability in the concentrations of total cell wall-bound hydroxycinnamate monomers appears to be moderate; however, genotypes such as KIT1 (a sweet genotype) and Razinieh (a grain genotype) contain roughly 20% more hydroxycinnamate monomers than Ruzrok (a grain genotype). Comparison of cell wall-bound hydroxycinnamate monomer contents between genotypes based on their use (dual-purpose vs. sweet vs. grain) and based on breeding type (hybrid vs. cultivar) do not reveal significant differences. Although the absolute contents described in the literature vary slightly (due to different protocols), the ratio of both phenolic acids is comparable to this study (Eraso & Hartley, 1990; Hatfield et al., 2009; Lam et al., 1996). Even though specific enzymes such as some arabinofuranosidases may be hindered by the arabinoxylan substitution with ferulic acid (Schendel et al., 2016), the overall impact of polysaccharide feruloylation (and *p*-coumaroylation) is deemed to be minor. Although arabinoxylan *p*-coumaroylation has been demonstrated for corn (Allerdings et al., 2006), this has not specifically been demonstrated for sorghum. Generally, *p*-coumaric acid in grasses is mostly attached to lignin and does not specifically (other than for lignin in general) affect enzymatic cell wall decomposition (Grabber et al., 2004; Ralph et al., 1994). Different from monomeric hydroxycinnamates, ferulate-based cross-links such as ferulate dimers are

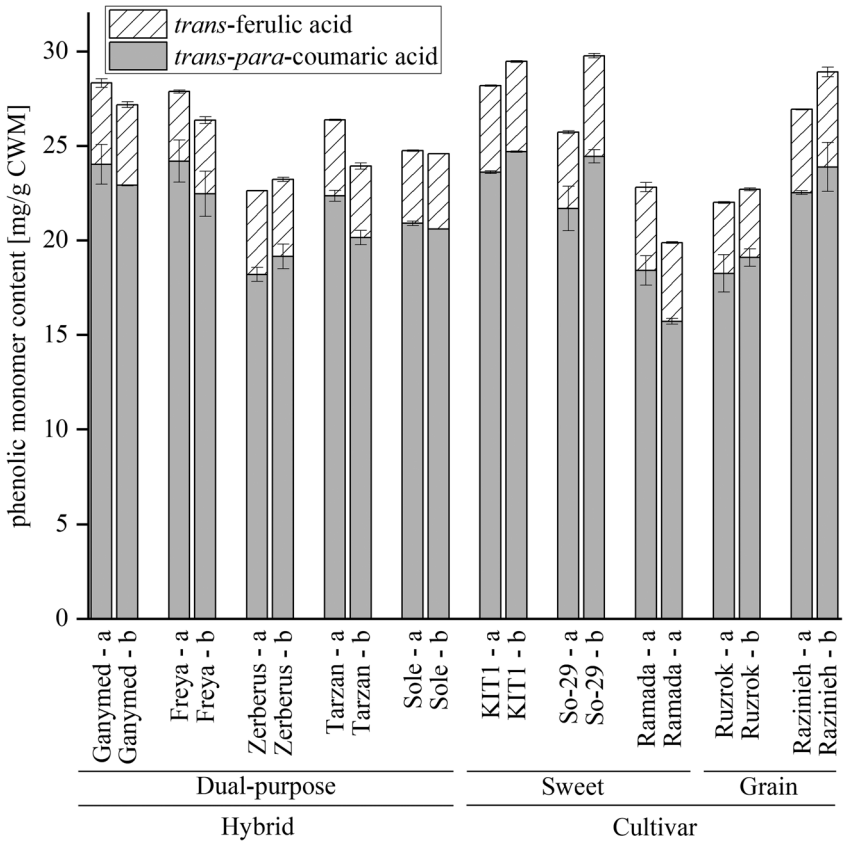

FIGURE 5 Phenolic monomer contents of cell wall material isolated from stems of different sorghum genotypes. Results for each replicate (a, b) are shown ($n = 2$)

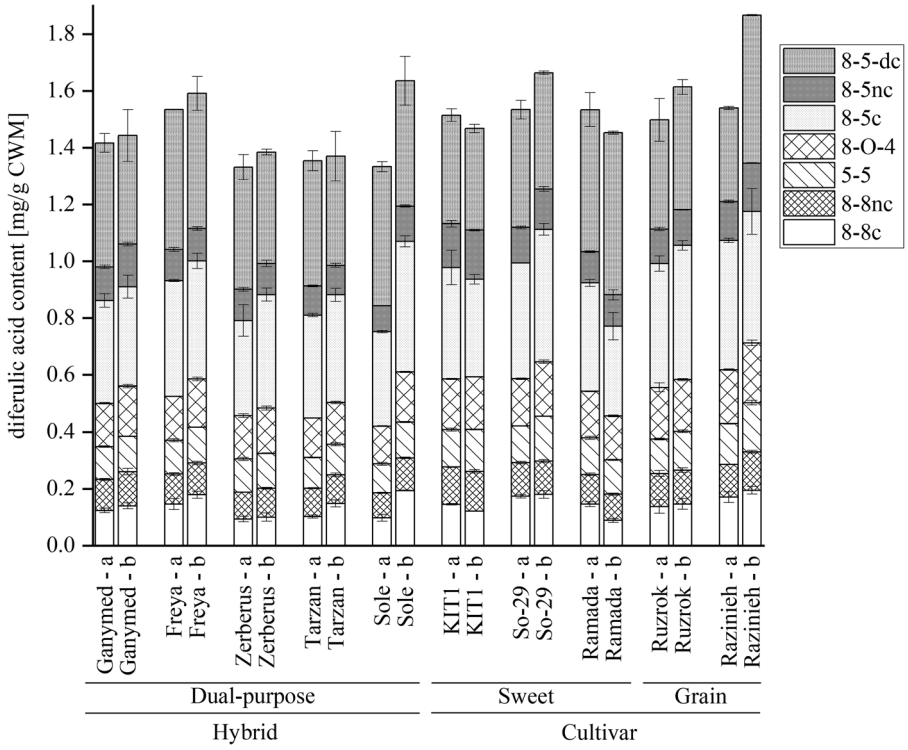

FIGURE 6 Contents of ferulic acid dehydrodimers of cell wall material isolated from stems of different sorghum genotypes. Results for each replicate (a, b) are shown ($n = 2$). c, cyclic; nc, noncyclic; dc, decarboxylated

supposed to reduce cell wall fermentability (Grabber et al., 1998, 2009).

For all sorghum genotypes, 8-8 cyclic (c)-, 8-8 noncyclic (nc)-, and 5-5-, 8-O-4-, and 8-5-coupled-DFA were determined (Figure 6). Contents of 8-5-DFA are composed of 8-5 c-, 8-5 nc-, and 8-5 decarboxylated (dc)-DFA. They are normally given as a sum because 8-5-c-DFA is the only native DFA, whereas 8-5 nc-DFA and 8-5 dc-DFA are formed during sample preparation (Bunzel, 2010). Additionally, 8-5 dc-DFA was suggested to originate as a degradation product of lignin (from dimers composed of ferulate and coniferyl alcohol) (Grabber et al., 2002). 8-8 c-, 8-8 nc-, 5-5-, and 8-O-4-DFA made up about 10% of total DFA contents each (8-8 c: 6%–12%; 8-8 nc: 6%–10%; 5-5: 8%–10%; 8-O-4: 10%–13%), whereas the sum of 8-5-coupled DFAs accounted for 60 %–69% of total DFA contents. As contents of 8-5 dc-DFA are noticeably high (Figure 6), 8–5 dc-DFA may (partially) arise from degradation of lignin, which results in an overestimation of total 8-5-DFA contents.

Again, some variation between replicates can be observed, especially for Sole and Razinieh. Also, there is some, but only moderate, variability in total DFA contents among the analyzed genotypes (between 1.3 mg/g cell wall material and 1.9 mg/g cell wall material). However, no distinct differences were observed between the hybrid and cultivar group nor between genotypes used for different purposes (dual-purpose, sweet, grain) (Figure 6). Literature data about DFA contents in sorghum stems are rarely available. Eraso and Hartley (1990) found 1.3 mg diferulic acids/g cell wall material, too. However, back in 1990, the spectrum of diferulic acids to be analyzed was different from now. 5-5-DFA was the only known DFA. Instead, cyclobutane dimers were considered. However, photochemically produced cyclobutane dimers may significantly contribute to the diferulic acid contents in leaves but are supposed to be less important than dehydrodiferulic acids in other plant organs.

4 | CONCLUSION

The study explored the high variability between sweet, grain, and dual-purpose sorghum genotypes for sugar content and related morphological traits, which provide valuable resources for sorghum improvement by breeding programs in the temperate zone. Despite the limited time frame (one season) and small sample size, first conclusions can be drawn that have to be, of course, validated in future studies. The sweet genotype KIT1 produced the highest sugar concentration in the stem. However, the dual-purpose sorghum hybrid group showed better performance with respect to all other morphological parameters over the cultivar group (sweet and grain genotypes). In general, cell wall composition varies only a little or is moderate between the genotypes analyzed in this study. Although the monosaccharide

composition of the stem cell wall polysaccharides showed very little variability between the genotypes, moderate variations were observed between the genotypes for Klason lignin and ABSL contents, with the dual-purpose group having slightly higher lignin contents compared to sweet and grain genotypes. Analysis of diferulate cell wall polymer cross-links demonstrated some but overall moderate differences between the ten genotypes, too. In sum, the sweet genotype KIT1 had slightly lower lignin concentrations and moderate contents of diferulate cross-links, which may be beneficial for biomass utilization of this genotype. However, as cell wall composition of all genotypes varies only moderately, the biomass of all genotypes appears to be suitable for bioeconomy use, which, however, needs to be further studied. Overall, the cell wall chemistry is mostly buffered against the more pronounced variations in sugar content. This finding is important for the potential of sorghum in a multistep bioeconomy strategy, where the residues from sugar extraction are further valorized by collecting the monolignol fraction.

ACKNOWLEDGMENTS

We thank KWS SAAT SE & Co. KGaA Company for providing the seed materials of sorghum hybrids. Special thanks go to Klára Kosová from Crop Research Institute, Czech Republic, and Eman Abuslima from Suez Canal University, Egypt for providing the seed materials of Ruzrok and Ramada genotypes, respectively. Open Access funding enabled and organized by Projekt DEAL.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, validation, formal analysis, investigation, resources (seeds), data curation, writing—original draft: Adnan Kanbar and Daniela S. Schäfer. *Conceptualization, methodology, formal analysis, writing—review*: Judith Keller. *Supervision, funding acquisition, resources, project administration, writing—review and editing*: Peter Nick. *Supervision, conceptualization, funding acquisition, resources, project administration, writing—review and editing*: Mirko Bunzel.

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How to cite this article: Kanbar, A., Schäfer, D. S., Keller, J., Nick, P., & Bunzel, M. (2022). Toward bioeconomy of a multipurpose cereal: Cell wall chemistry of sorghum is largely buffered against stem sugar content. *Cereal Chemistry*, 99, 786–801. https://doi.org/10.1002/cche.10536