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Mining Sorghum Biodiversity—Potential of Dual-Purpose Hybrids for Bio-Economy

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Abstract: Sweet, grain, and dual-purpose sorghums differ in a number of important traits, including biomass production, total solutes in the stem juice, and sugar accumulation across the stem. Ten dual-purpose hybrids, two sweet genotypes, and two grain landraces of sorghums were characterized under temperate environmental conditions to determine their potential for bioethanol production. Five sorghum hybrids (Ganymed, Hannibal, Tarzan, Merlin, and Zerberus) performed better with respect to cane yield, juice yield, potential sugar, and ethanol yields compared to sweet and grain genotypes. While the sweet genotype KIT1 produced the highest sugar concentration in the stem, the lowest concentration was produced by the grain landrace Razinieh. The study showed that plant height, leaf number, leaf weight, cane yield, and juice yield were positively correlated with the sugar yield in fresh stalk. Sugar accumulation was higher in the central internodes of all genotypes. Clustering analysis showed that sweet genotypes are located more closely to dual-purpose hybrids than grain landraces. We discuss the results with respect to the potential of dual-purpose sorghum hybrids for bio-economy in Germany.

Keywords: sorghum (grain; sweet; dual-purpose); sugar accumulation; stem internode; bio-economy

1. Introduction

The global population growth consequently leads to an increase in food, fodder, and energy demand in form of fossil fuel, and therefore exacerbates the negative effects of global climate change further [1]. As one strategy to contain the excess of carbon dioxide, the use of natural carbon sinks (most efficient for the preferential use of plants with C_4 photosynthesis) allows absorbing ambient carbon dioxide for transformation into biomass [2]. Further, effects of greenhouse gas emissions can be mitigated by a moderate shift of fossil energy to biomass-derived renewable energy [3]. However, the usage of food crops for bioethanol production is progressively assessed critically. To circumvent this "no food for fuel" dilemma, the use of plants that do not compete with food crops, i.e., those able to grow on marginal lands, is a promising and strategic approach [4].

Sorghum (*Sorghum bicolor* L. Moench) is the world's fifth most important cereal crop after maize, rice, wheat, and barley due to its multipurpose economically important yields such as food, fodder, bioethanol, and further industrial uses [5,6]. The aspect that sorghum, as a C_4 photosynthesis plant, has a high efficiency in terms of absorbing ambient atmospheric carbon dioxide and its transformation into biomass, underlines its multipurpose versatility and potential for bio-economy [7]. Besides the high yield of biomass, several advantages, such as low input requirements, good adaptation to the constraints, characteristic for marginal lands, and rich genomic resources, led to the use of sorghum for fuel [8]. In contrast to many other sources, all parts of sorghum—its biomass, its grain, and its sugary juice from the stem—can be used for ethanol production [9].

Sorghum varieties can be divided into four major classes based on the major forms of use: sweet, grain, fodder, and high biomass types [10,11]. Grain sorghum is an important staple crop for its dry stem and gluten-free grains, most popular in Africa and China [12,13], as also observed in some ancient wheats of the Mediterranean Basin [14]. Sweet sorghum (*S. bicolor* ssp. *bicolor*) is well suited for plant-based bioethanol production, due to its sugary stalk [15]. However, its use has not yet been implemented in many developing countries, as these varieties are currently not available for small- and large-scale farmers [16]. "Ideal" varieties should feature and combine certain traits, such as high yield of grain and biomass and high stem sugar content, each in an acceptable amount, to allow use as dual-purpose crops [17,18].

Sweet and grain sorghum varieties differ in several important traits like plant height, grain, biomass production, and accumulation of stem sugar and juice [19]. Sweet sorghum is a natural variant of the common grain sorghum, featuring thicker stems acting as primary sink tissue for synthesized sugar during flowering and translocation into seeds during seed filling [20]. Due to its ability to produce high biomass under adverse conditions, sweet sorghum has been considered as a source of raw material for second-generation bioethanol and lignocellulosic feedstocks in temperate areas like Germany [21,22]. In contrast, grain sorghum stores carbohydrates as starch in seeds [12]. Unlike grain sorghum, all sweet sorghum genotypes are classified within *S. bicolor* ssp. *bicolor* [23].

Previous research investigating the accumulation of sucrose in sweet sorghum stems indicated that sucrose accumulation begins with the start of the reproductive phase [24]. Interestingly, however, it could be shown by genetic analysis that grain and sweet sorghum share striking genetic similarities, where genotypes of both sorghum types clustered together based on the geography of origin rather than with respect to sugar-related differences [23]. Nevertheless, key differences between sweet and grain sorghum were found in regulatory genes and sugar metabolism genes, which are likely to play major roles in stem sugar accumulation, despite the high genetic similarity at structural levels [25]. Both, sweet and grain sorghums accumulated sugar in higher concentrations in central internodes, while accumulating more weight in the basal internodes [26]. Compared to the grain types, sweet genotypes double or even triple the sugar concentration in all internodes, with the highest amounts of sugar in the central internodes. Juice volume can increase by six- to ninefold [27].

Therefore, the objectives of the study were: (1) to characterize dual-purpose sorghum hybrids and determining their potential for use as source of bioethanol production and (2) to investigate major differences between sweet and grain sorghum in terms of stem-sugar accumulation.

2. Materials and Methods

2.1. Plant Materials

For this study, 14 sorghum genotypes of *S. bicolor* L. Moench were chosen and grouped according to their use in agricultural practice (Table 1). These included sweet varieties (KIT1 and So-29), grain landraces (Razinieh and Ruzrok), and dual-purpose hybrids (Zerberus, Juno, Bulldozer, Ganymed, Phoenix, Hannibal, Freya, Merlin, Sole, and Tarzan). KIT1 is a sweet sorghum genotype developed by us using pedigree selection. Razinieh is an improved Syrian landrace whose grain productivity was enhanced by bulk breeding [19]. 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 [28]. The sweet variety So-29 was maintained by Crop Research Institute, Prague, Czech Republic, and released in 1971. All hybrids used in this study were bred by KWS company (KWS SAAT SE & Co. KGaA, Einbeck, Germany) with the targets of high yield and high disease tolerance for production of grain and biomass.

Table 1. List of genotypes used in this study, their agronomic group, type, origin, and resistant to lodging.

ID	Cultivars	Group	Lodging	Type	Origin
9472	Zerberus	Grain, biomass	Resistant	*S. bicolor x S. bicolor*	KWS, Germany
9473	Juno	Grain, biomass	Resistant	*S. bicolor x S. bicolor*	KWS, Germany
9474	Bulldozer	Grain, biomass	Resistant	*S. bicolor x S. bicolor*	KWS, Germany
9475	Ganymed	Grain, biomass	Resistant	*S. bicolor x S. bicolor*	KWS, Germany
9576	Phoenix	Grain, biomass	Resistant	*S. bicolor x S. bicolor*	KWS, Germany
9477	Hannibal	Grain, biomass	Resistant	*S. bicolor x S. bicolor*	KWS, Germany
9478	Freya	Grain, biomass	susceptible	*S. bicolor x S. sudanense*	KWS, Germany
9479	Merlin	Grain, biomass	Resistant	*S. bicolor x S. bicolor*	KWS, Germany
9480	Tarzan	Grain, biomass	Resistant	*S. bicolor x S. bicolor*	KWS, Germany
9481	Sole	Grain, biomass	susceptible	*S. bicolor x S. sudanense*	KWS, Germany
9145	Razinieh	Grain	Moderate	Landrace	Syria
9482	Ruzrok	Grain	Moderate	variety	Czech Republic
9483	So-29	Sweet, biofuel	susceptible	variety	Czech Republic
9484	KIT1	Sweet, biofuel	Resistant	Elite line	KIT, Germany

2.2. Plant Cultivation

Plants were grown at the Botanical Garden of Karlsruhe Institute of Technology (Karlsruhe, Germany) during the summer (May–August) of 2018. Karlsruhe is located in the Rhine Valley of Southeast of Germany (latitude: 49°0′24.8004″ N, longitude: 8°24′13.1508″ E), with an average elevation of 119 m above sea level (based on the World Geodetic System 1984 datum). Temperatures ranged from around −1 °C during winter to 26 °C during summer in a temperate oceanic climate. 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. The two outer rows of each plot served as buffers, while all measured parameters were taken from the plants in the two middle rows. A spacing of 60 cm between rows and 25 cm between individuals within rows was maintained. In total, 80 seeds were planted in each row and thinned manually to 20 individuals per row at the two-leaf stage. Environmental factors such as temperature and rainfall were monitored during the experiment and are presented as monthly average values in Supplementary Table S1. Based on soil analysis, 100 g/m^2 organic fertilizer (Hauert Hornoska®Special, Nürnberg, Germany) and 90:60:40 kg/ha of NPK were added.

2.3. Phenotyping of Plants and Internodes

As recommended by previous studies, sorghum plants were harvested at dough stage (the seeds are still soft and immature, and embryogenesis was completed), when optimal sugar concentration is mostly stored in stem sink tissue as reported [19,26,29] during the month of August. For analysis, from each replicate, five plants located in the center of a plot were sampled randomly for recording plant height (cm), leaf number, internode number, fresh leaf weight (g/plant), cane yield (t/ha), juice yield (kl/ha), bagasse yield (t/ha), and sugar concentration (°Brix). The theoretical ethanol yield was estimated as 40 liters per ton of cane yield [30]. Sugar yield (t/ha) and percentage was estimated using formulas of [31].

In addition to entire plants, the following measurements were conducted in each internode individually: internode length (cm), internode bagasse weight (g), internode juice volume (mL), internode weight (g), and sugar concentration (°Brix). As to quantify the juice yield, and to measure the sugar concentration of the juice directly after harvest, a conventional cane crusher (Sugarcane Juicer 110LBS/H, VEVOR, Shanghai, China) was used. Sugar concentration as °Brix was recorded with a manual refractometer (Model PAL, Atago Co. Ltd., Tokyo, Japan) for each individual cane.

2.4. Statistical Analysis

Data were subjected to individual ANOVA for different characters in order to assess the variability among the genotypes using PROC ANOVA in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Mean comparisons were separated with least significant difference (LSD) at 5% level of significance. Additionally, grand mean and coefficient of variation as percentage (C.V.%) were recorded for each measured trait individually. To estimate the degree of linear association between the studied traits, the simple correlation coefficient (*r*) was computed using the standard formula of [32]. The significance of correlation coefficients was tested at n-2 degrees of freedom on a "t" table from Fisher and Yates at 5% and 1% significance levels.

The multi-collinearity in path analysis was investigated to avoid bias in the decision-making process regarding the partial effect of independent variables on the dependent variable. For this purpose, the method of tolerance index (TOL) was employed. The procedure of computing TOL is first to make each independent variable as the criterion variable regressing against all other explanatory variables. Then TOL is calculated as following: $TOL = 1 - R^2$, where, R^2 = coefficient of multiple determination. TOL is simply the unexplained variance. A small TOL denotes high multi-collinearity [33]. As cut-off for TOL, we used a value of 0.2. Path coefficient analysis was carried out using the phenotypic correlation coefficients to determine the direct and indirect effects of morphological characters on sugar yield. No independent trait caused multi-collinearity. Morphological data were standardized using Minitab 17 (Minitab Ltd., Coventry, UK), and a Euclidean distance matrix was generated according to [34] with Minitab 17 (Minitab Ltd., Coventry, UK). General agglomerative hierarchical clustering was conducted with Average linkage method and subsequently used to plot a dendrogram. The principal component analysis (PCA) was applied to plot the relationship between distance matrix elements with respect to their first two principal components, using Minitab 17.

3. Results

3.1. Agro-Morphological Descriptive Analysis

The analysis of variance using a randomized complete block design showed significant variation ($p < 0.05$) for all 10 quantitative traits (Supplementary Table S2) with considerable ranges in plant height (277.67–442.50 cm), leaf number (7.67–14.0), internode number (7.67–13.0), green-leaf weight (5.97–80.75 g), cane yield (16.65–124.80 t/ha), juice yield (2.67–52.20 kl/ha), bagasse yield (12.74–49.80 t/ha), sugar concentration in °Brix (6.90–15.16), estimated sugar yield (0.19–5.43 t/ha), and estimated ethanol yield (0.67–4.99 kl/ha) (Table 2).

The coefficient of variation was between low and moderate, ranging from 3.56% to 15.84%. The range of parameters was considerable. For instance, the sorghum hybrid Ganymed produced the highest green leaf weight (80.75 g), cane yield (124.80 t/ha), juice yield (52.20 kl/ha), bagasse yield (49.80 t/ha), and predicted ethanol yield (4.99 kl/ha). In contrast, the grain variety Ruzrok produced the lowest plant height (277.67 cm), leaf number (7.67), internode number (7.67), green leaf weight (5.97 g), cane yield (16.65 t/ha), juice yield (2.67 kl/ha), bagasse yield (12.74 t/ha), and predicted sugar (0.19 t/ha) and ethanol (0.67 kl/ha) yields compared to the other genotypes (Table 2). While the sweet genotype KIT1 produced a significantly higher sugar concentration (15.16 °Brix), the grain landrace Razinieh showed the lowest sugar concentration (6.90 °Brix). However, all the hybrids registered sugar concentration higher than 10, except Sole (9.70 °Brix), while grain sorghum genotypes recorded sugar concentration less than 9.

Table 2. Mean values of agro-morphological traits recorded from 14 sweet, grain, and dual-purpose sorghum genotypes, their grouped overall mean values, least significant difference, and coefficient of variation as percentage (C.V%) at dough stage under temperate climatic conditions.

Cultivar	Plant Height (cm)	Leaf Number	Internode Number	Leaf Weight (g/Plant)	Cane Yield (t/ha)	Juice Yield (kl/ha)	Bagasse yield (t/ha)	Sugar Content (°Brix)	Sugar Yield (t/ha)	Ethanol Yield (kl/ha)
Zerberus	361.66	12.00	12.00	46.87	93.87	41.87	33.53	11.27	4.20	3.77
Juno	382.50	13.00	13.00	44.30	82.80	35.00	38.20	12.80	3.97	3.31
Bulldozer	315.50	14.00	12.00	65.00	93.70	38.50	33.80	11.80	4.03	3.75
Ganymed	426.50	14.00	12.00	80.75	124.80	52.20	49.80	11.10	5.14	4.99
Phoenix	305.50	10.00	10.00	39.40	54.50	21.80	26.30	13.60	2.63	2.18
Hannibal	397.50	12.00	12.00	54.40	106.50	46.10	47.60	13.30	5.43	4.26
Freya	415.00	10.00	11.00	25.85	55.10	18.20	29.00	12.70	2.05	2.20
Merlin	403.00	11.00	11.00	36.65	84.20	41.40	35.20	13.70	5.02	3.37
Tarzan	442.50	11.00	12.00	47.50	109.40	38.70	45.80	13.60	4.66	4.38
Sole	396.00	10.00	10.00	15.05	38.11	9.10	21.50	9.70	0.78	1.52
Razinieh	284.00	10.00	10.00	30.50	42.39	11.46	18.95	6.90	0.71	1.69
Ruzrok	277.67	7.67	7.67	5.97	16.65	2.67	12.74	8.10	0.19	0.67
So-29	308.50	11.00	11.00	24.50	76.00	35.30	32.00	12.70	3.97	3.04
KIT1	284.00	9.33	9.33	63.43	78.57	35.40	23.45	15.16	4.75	3.14
Overall mean	357.13	11.07	10.92	41.44	75.47	30.55	31.99	11.88	3.39	3.01
F-test	****	****	****	****	****	****	****	****	****	****
LSD (0.05)	12.25	0.36	0.36	4.02	4.87	1.82	2.57	0.56	0.32	0.19
C.V (%)	9.06	3.78	3.56	13.78	15.84	11.55	9.79	6.82	8.62	11.85

Data were analyzed by F-test. LSD: least significant differences within the trait for all the varieties ($p < 0.05$). **** Significant at 0.0001 probability level.

3.2. Phenotypic Correlation Coefficients and Path Analysis

The results of correlations analysis carried out among the 10 quantitative traits are given in Table 3. Sugar concentration (in °Brix) showed significant positive correlations with number of internode ($r = 0.37$*), leaf weight ($r = 0.44$**), cane yield ($r = 0.54$***), juice yield ($r = 0.60$***), and Bagasse yield ($r = 0.49$***). However, sugar concentration did not correlate with plant height ($r = 0.28^{ns}$), nor with leaf number ($r = 0.21^{ns}$). Sugar yield was also significantly and positively correlated with plant height ($r = 0.40$**), leaf number ($r = 0.64$****), internode number ($r=0.66$****), leaf weight ($r = 0.77$****), cane yield ($r = 0.92$****), juice yield ($r = 0.97$****), and bagasse yield ($r = 0.82$****).

The direct and indirect effects of plant height, leaf number, internode number, leaf weight, cane yield, juice yield, bagasse yield, and sugar concentration (in °Brix) on sugar yield were estimated by path analysis. They are presented in Table 4. The path coefficient analysis based on sugar yield as a dependent variable was consistent with a model where juice yield (0.894), °Brix (0.211), bagasse yield (0.062), cane yield (0.011), and internode number (0.002) exhibited positive direct effects, while plant height (−0.039), leaf number (−0.123), and leaf weight (−0.005) showed negative direct effects on sugar yield. Highly positive indirect effects on sugar yield were exerted by juice yield via plant height (0.386), leaf number (0.681), internode number (0.662), leaf weight (0.732), cane yield (0.864), bagasse yield (0.777), and sugar content as °Brix (0.544). In contrast, leaf weight contributed negatively and very little on sugar yield via all the studied traits. Internode number had positive and very low indirect effect on sugar yield. Bagasse yield and °Brix had moderate indirect effect on sugar yield via other traits under study.

Table 3. Phenotypic correlation coefficient of different agro-morphological traits obtained from 14 sorghum genotypes.

Traits	Leaf Number	Internode Number	Leaf Weight	Cane Yield	Juice Yield	Bagasse Yield	°Brix	Sugar Yield
Plant height	0.43 **	0.62 ****	0.23ns	0.54 ***	0.43 **	0.71 ***	0.28ns	0.40 **
Leaf number	1.00	0.88 ****	0.72 ****	0.79 ****	0.76 ****	0.76 ****	0.21ns	0.64 ****
Internode number		1.00	0.56 ****	0.79 ****	0.74 ****	0.83 ****	0.37 *	0.66 ****
Leaf weight			1.00	0.84 ****	0.81 ****	0.68 ****	0.44 **	0.77 ****
Cane yield				1.00	0.96 ****	0.92 ****	0.54 ***	0.92 ****
Juice yield					1.00	0.86 ****	0.60 ***	0.97 ****
Bagasse yield						1.00	0.49 ***	0.82 ****
°Brix							1.00	0.75 ****

ns = non-significant; *, **, *** and **** Significant at 0.05, 0.01, 0.001, and 0.0001 probability levels, respectively.

Table 4. Direct (bold diagonal) and indirect effect (off diagonal) of different quantitative characters on sugar yield of sorghum.

Characters	Plant Height	Leaf Number	Internode Number	Leaf Weight	Cane Yield	Juice Yield	Bagasse Yield	°Brix	Sugar Yield
Plant height	**−0.039**	−0.053	0.001	−0.001	0.006	0.386	0.044	0.061	0.405 **
Leaf number	−0.017	**−0.123**	0.001	−0.003	0.008	0.681	0.047	0.046	0.640 ****
Internode number	−0.025	−0.109	**0.002**	−0.003	0.009	0.662	0.052	0.079	0.666 ****
Leaf weight	−0.009	−0.089	0.001	**−0.005**	0.010	0.732	0.042	0.095	0.776 ****
Cane yield	−0.021	−0.098	0.001	−0.004	**0.011**	0.864	0.057	0.115	0.925 ****
Juice yield	−0.017	−0.094	0.001	−0.004	0.011	**0.894**	0.053	0.128	0.972 ****
Bagasse yield	−0.028	−0.095	0.001	−0.004	0.010	0.777	**0.062**	0.104	0.828 ****
°Brix	−0.011	−0.027	0.001	−0.002	0.006	0.544	0.031	**0.211**	0.752 ****

Residual effect = 0.0899. ** and **** Significant at 0.01 and 0.0001 probability levels, respectively.

3.3. Sugar Accumulation Across Internodes of the Stem

For each internode individually (from base to top), sugar concentration (in °Brix), internode weight, internode bagasse weight, internode juice volume, and internode length were analyzed for all the 14 genotypes (Figure 1). Similar patterns were observed between all the genotypes in sugar accumulation across the stem. The central internodes accumulated higher sugar concentration, especially from the fourth to the seventh internode (Figure 1A), while the basal internodes accumulated more weight, especially from the second to the fourth or fifth internode (Figure 1B). This heavier weight for basal internodes was correlated with a higher juice content (Figure 1C). As a result, the central internodes although storing less juice, nevertheless produced the highest sugar concentration (measured as °Brix). In KIT1 and So-29 (sweet types), and in Buldozer, Hannibal, and Merlin (dual-purpose types), sugar concentration was significantly higher compared to the other genotypes studied (Figure 1A). Hannibal, Ganymed, and So-29 displayed heavier internode weight and higher juice volume for basal internodes, which increased significantly after the internode number 3, while the sweet genotype KIT1 had a stable internode weight and juice volume up to the internode number 8 (Figure 1B,C).

Sugar concentration (in °Brix) showed a positive and significant correlation with internode weight ($r = 0.34$, $p < 0.0001$), internode juice volume ($r = 0.35$, $p < 0.0001$), and internode bagasse weight ($r = 0.25$, $p < 0.01$) (Figure 2A–C). No significant relation was observed between sugar content (in °Brix) and internode length ($r = −0.03$, $p < 0.06$) (Figure 2D). The internode juice volume exhibited a significant positive correlation with internode weight ($r = 0.91$, $p < 0.0001$), but no significant correlation with internode length ($r = −0.15$, $p < 0.06$) (Figure 2E,F, respectively).
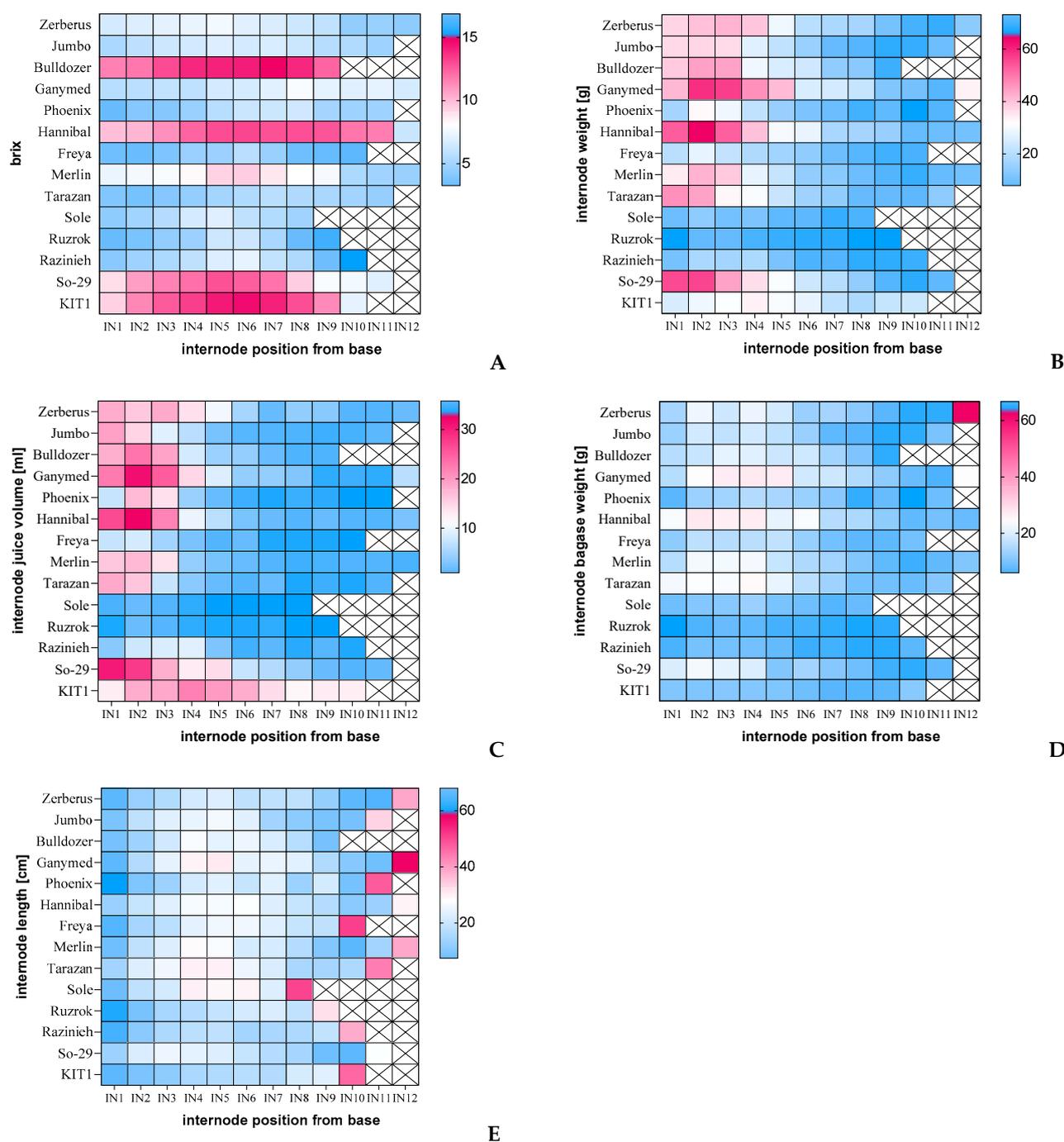
Figure 1. Differences in stem biomass and sugar-related parameters in the individual internodes of 14 sorghum genotypes grown in 2018 season in South-West Germany: (**A**) sugar concentration as °Brix, (**B**) internode weight, (**C**) internode juice volume, (**D**) internode bagasse weight, and (**E**) internodes length. Values are means of three replicates (5 samples in each replicate). Boxes with x indicate the absence of the internode. The blue color represents the trend of decrease and the purple color represents a rising trend. Heat map was generated using GraphPad Prism 8.4.3 software.
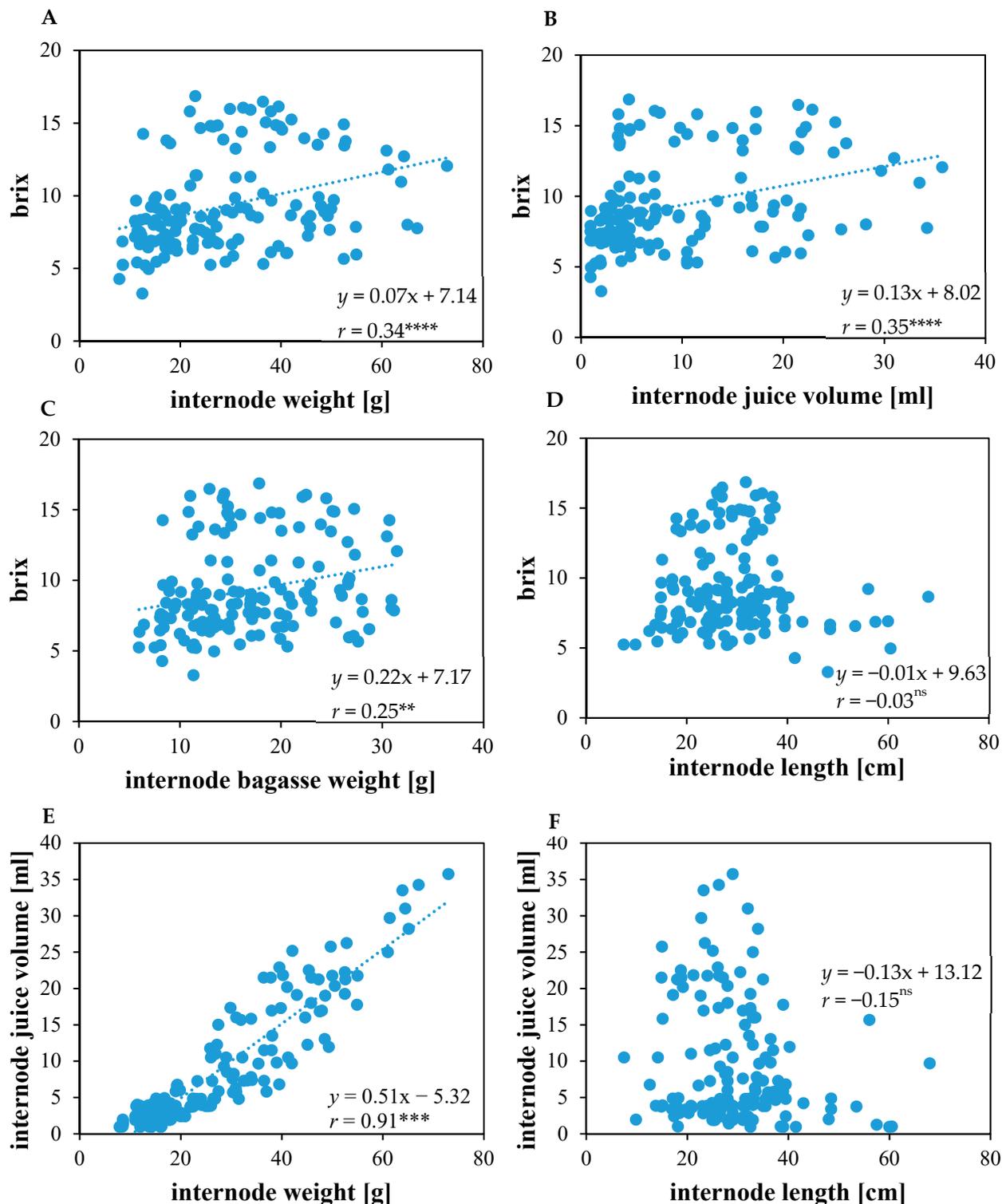
Figure 2. Relationship between sugar-related stem parameters of 14 sorghum genotypes grown in 2018 season in South-West Germany: (**A**) °Brix over weight in individual internodes, (**B**) °Brix over juice volume in individual internodes, (**C**) °Brix over bagasse weight in individual internodes, (**D**) °Brix over length in individual internodes, (**E**) juice volume over weight in individual internodes, and (**F**) juice volume over length in individual internodes. ns = non-significant; *, **, ***, and **** Significant at 0.05, 0.01, 0.001, and 0.0001 probability levels, respectively.

3.4. Diversity Analysis of Agro-Morphological Traits

Data recorded on 8 quantitative traits were used for clustering by the Average linkage method after the data had been standardized. In the resulting dendrogram, the investigated sorghum genotypes are separated into two main clusters (Figure 3A). Cluster I comprises two grain sorghum genotypes (Razinieh and Ruzrok), which recorded low in sugar content, plant height, internode number, cane yield, juice yield, bagasse yield, sugar yield, and ethanol yield. Cluster II is composed of all hybrids and sweet sorghum genotypes. Additionally, cluster II can be subdivided into two groups. Although group A contains seven hybrids (Zerberus, Juno, Bulldozer, Hannibal, Tarzan, Merlin, and Ganymed), with generally higher plant height, leaf number, internode number, fresh leaf weight, cane yield, bagasse yield, and sugar yield, group B contains two sweet genotypes (KIT1 and So-29) and three dual-purpose hybrids (Phoenix, Freya, and Sole). Group B is defined by its superior sugar concentration (in °Brix) with exception of the hybrid Sole.
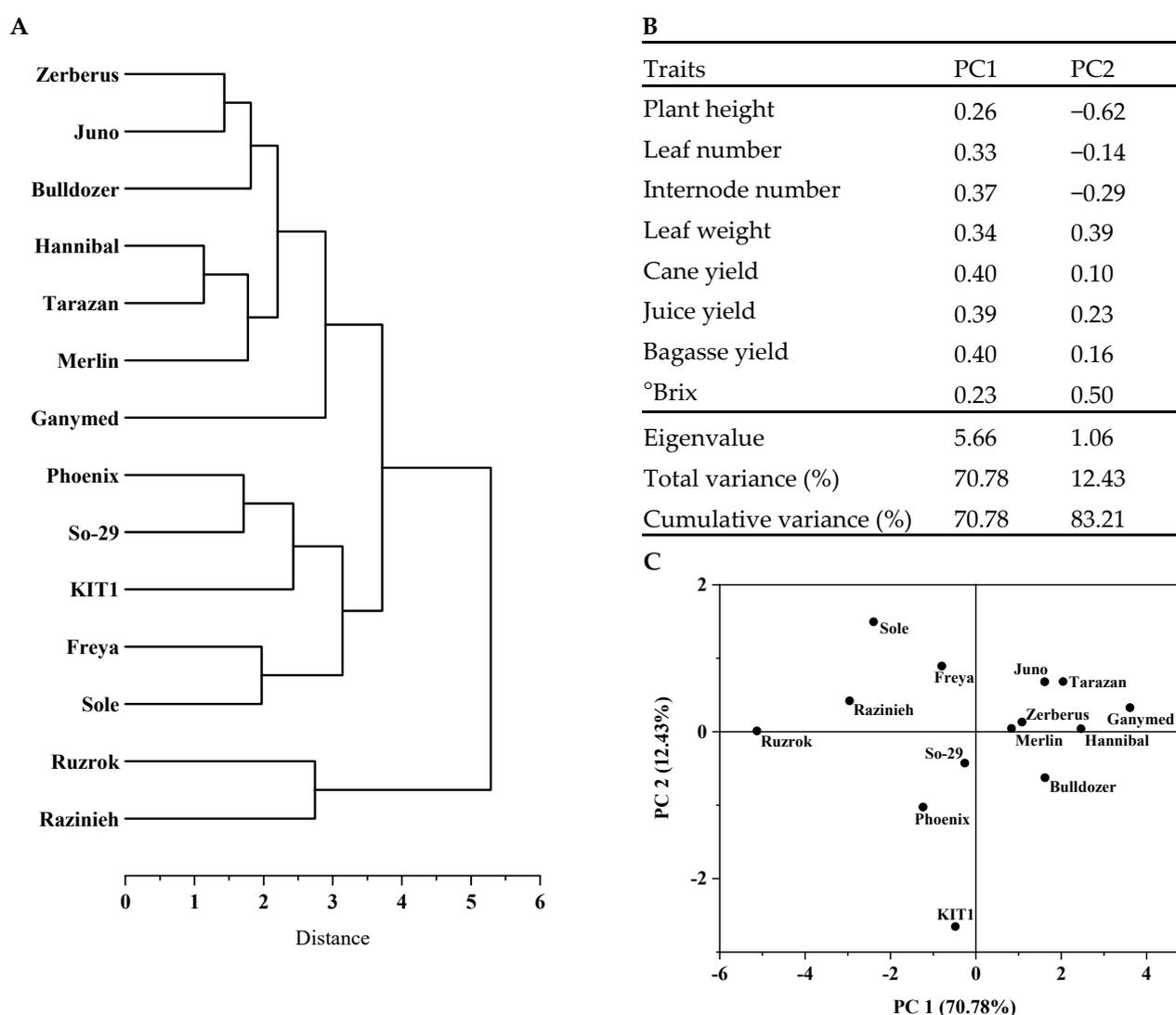
A

B

Traits	PC1	PC2
Plant height	0.26	−0.62
Leaf number	0.33	−0.14
Internode number	0.37	−0.29
Leaf weight	0.34	0.39
Cane yield	0.40	0.10
Juice yield	0.39	0.23
Bagasse yield	0.40	0.16
°Brix	0.23	0.50
Eigenvalue	5.66	1.06
Total variance (%)	70.78	12.43
Cumulative variance (%)	70.78	83.21

C

Figure 3. Clustering and principal component analysis based on 8 agro-morphological and sugar-related traits in 14 sorghum genotypes. (**A**) Dendrogram based on Euclidean distance and Average linkage method. (**B**) Eigenvectors, total variation, eigenvalue, and cumulative variance. (**C**) PCA plot showing scores for PC1 and PC2.

PCA results show that 83.21% of total variation among genotypes is contained within the first two principal components, having an eigenvalue (1.06) greater than one (Figure 3B). The score plot of 14 genotypes based on the first two principal components is presented in

Figure 3C. The first principal component is strongly correlated with four of the original variables. The first principal component increases with increasing cane yield, bagasse yield, juice yield, and internode number and explains 71% of the variation. The second principal component increases with only two of the values, decreasing plant height and increasing sugar concentration (in °Brix), and contributes only with 12% of the variation.

4. Discussion

Biofuels are a sustainable and renewable energy source deriving from biomass as organic matter [35]. As suggested by several studies, plants grown for the purpose of biofuel production have the potential to reduce net greenhouse gas emissions [36]. About 2.5% of the world's transportation fuels are currently produced from crop plants like sweet sorghum [37]. The multiple-purpose crop sorghum is used for food and fodder, as well as for bioethanol production due to its easily fermentable sugar [37,38]. The current study provides details of major differences between sweet, grain, and dual-purpose sorghums in terms of stem-sugar accumulation.

4.1. More Cane and Sugar Yields in Dual-Purpose Hybrid Sorghum but Higher Sugar Content in Sweet Sorghum

In the present investigation, morphological and sugar-related traits like plant height, leaf and internode numbers, leaf weight, cane yield, juice yield, bagasse yield, sugar concentration (as °Brix), as well as predicted ethanol yield significantly differed among the 14 tested sorghum genotypes (Table 2). Genotypic differences with respect to morphological characters and ethanol production have also been reported by previous studies [19,39–42]. In the set of the current work, the hybrid sorghum Ganymed, Tarzan, Hannibal, Zerberus, Bulldozer, and Merlin produced the highest cane yield, juice yield and bagasse yield compared with the two sweet genotypes, KIT1 and So-29, and the two grain sorghum landraces, Razinieh and Ruzrok. These six hybrids are expected to produce the highest ethanol yield, which depends on cane yield and juice yield [43,44].

Due to the wide variability in juice yield and extractability between sweet and grain sorghum genotypes, an assessment of genotypes for potential ethanol and sugar yields is of great importance [9,10,13,16,23,27]. By comparison of the total average of grain and sweet sorghum genotypes with dual-purpose hybrids, phenotypic differences exhibiting a taller, sugar-rich juicy stem, and a higher fresh biomass production were observed in hybrid plants (Table 2). Because of their frequently tall habitus, hybrid sorghum accumulated large biomass. For a range of morphological and agronomical traits, similar variation has been reported, indicating the prospect of an improvement of crops as biofuel feedstock sources [31,39,45]. Even though grain and sweet sorghum are highly similar with respect to structural genes, in regulatory genes as well as in sugar metabolism genes potential deletions and loss-of-function mutations were identified, that are likely crucial for the accumulation of stem sugar [25]. Regarding these discrepancies, further factors affecting physiology, morphology and molecular characteristics are needed to be included to understand the difference between sorghum types in their stem storage capacity.

The sweet sorghum KIT1 was superior to other evaluated genotypes in terms of sugar concentration (measured as °Brix). However, the superiority of dual-purpose hybrids, Hannibal, Ganymed, and Merlin in juice yield compared to KIT1 consequently leads to a higher potential of sugar and ethanol yield than that of KIT1 (Table 2). Although sorghum is naturally self-pollinating, heterosis is well-described for grain yield and biomass production [46]. With the discovery of cytoplasmic male sterility in the 1950s, the commercial production of grain sorghum was completely replaced by hybrids [47]. At present, a significant proportion of grain, biomass, and forage production is based on hybrids in Germany [48]. To enhance the value of this crop for ethanol production, the same genetic mechanism can be utilized to improve the concentration of soluble sugars in grain sorghum varieties. As already described by [49], the reduction and fluctuations of the photoperiod were observed to negatively affect the quantity and quality of stem and juice yields in KIT1. The parents of KIT1 originated from South India, which are adopted to semi-arid

environmental conditions. However, all the hybrids were developed by the company KWS in Germany, and they are suitable to grow in the temperate environmental conditions.

The grain sorghum genotypes Ruzrok and Razinieh were bred and improved from landraces. Ruzrok recorded the lowest values for all the studies parameters compared with other genotypes. The Syrian landrace Razinieh showed better performance than Ruzrok with respect to growth, biomass yield, sugar yield, and ethanol productivity under temperate environmental condition. This genotype has been cultivated in Syria for a long time to alleviate fodder and food shortages during dry seasons. Later, its grain yield was improved by a bulk breeding program under respect to maintain its drought tolerance capability [19,50].

4.2. Higher Cane and Juice Yields and More Sugar Accumulation in the Stem

To predict the consequences of selections for one trait on the performance of others, the examination of genetic relationships between different traits is of great importance. The statistically determined relationships visualized in a correlation matrix showed interesting positive correlations between sugar concentration (as °Brix) and internode number, leaf weight, cane yield, juice yield, Bagasse yield, and sugar yield (Table 3). Sugar concentration (as °Brix) was positively correlated with plant height, but not significantly. Prior research activities had identified a positive correlation between sugar concentration and plant height in sweet sorghum genotypes [51]. The observed positive relation of sugar concentration (as °Brix) and leaf weight may be a desirable trait for further sorghum breeding activities, since plants need leaf areas to synthesize carbohydrates through photosynthesis. For higher yields of biomass and storage of energy in its stalk through the growth period, plants need more photosynthetic active areas in form of leaf [23,52,53]. The positive relations between internode numbers and cane yield suggest that taller plants were accumulating more biomass in the stalk [19,54]. This study showed that plant height, leaf number, leaf weight, cane yield, and juice yield positively affected the sugar yield in fresh stalk. Similar results were obtained by [19,51,54]. Furthermore, the accumulation of stem sucrose was correlated with stem growth during anthesis [55].

Partitioning sugar-related traits and other morphological parameters into direct and indirect effects is of importance for breeding so that traits that directly contribute to sugar yield are selected. The results show that juice yield and concentration of soluble sugars (as °Brix) had significant direct and indirect effects on sugar yield (Table 4). The remaining traits had low negative indirect effect on sugar yield. Therefore, sugar yield can be improved by selecting for high juice volume and °Brix. Genotypes developing a juicy stem and high sugar concentrations (°Brix values) were described to lead to high sugar and ethanol yield [17,43]. Cane and juice yield in sorghum are mainly determined by stem properties that concern the capacity to act as a sink for soluble sugars [56]. In dual-purpose hybrids and the sweet KIT1 genotype, °Brix, fresh weight of internodes, juice content per internode, and leaf weight were significantly higher than in Razinieh and Ruzrok landraces. This result is consistent with previous observations, where sweet sorghum was shown to produce more weight and to exhibit higher sugar concentration reflected as higher refraction (in °Brix) compared with grain sorghum varieties [11,13,15,27,39,52,53,57]. Comparative studies between sorghum hybrids and open-pollinated sorghum varieties (OPV) described that hybrid sorghum produced higher yields in fresh biomass, juice, and sugar compared to OPV [46]. Because of the higher activity of photosynthesis and starch metabolism genes during daytime, hybrid plants of corn or sorghum tend to grow better and bigger than their stable varieties [58].

4.3. Internode Weight Rather Than Length Can Be Used as Predictor for Sugar Yield

In sorghum yields of cane and juice are mainly determined by those properties that concern the stem capacity to act as sink tissue for soluble sugars. All tested genotypes exhibited higher values for sugar concentration in terms of °Brix in the central internodes (Figure 1A), while accumulating more weight and juice volume in the basal internodes

(Figure 1B,C). Thus, the central internodes although storing comparatively less juice, nevertheless produced the highest sugar concentration (Figure 1C). This result is consistent with earlier observations showing that it was the central internodes in sorghum stems that displayed the highest °Brix [27,51,59]. The sweet sorghum cultivars Della and Rio showed similarities in the internode sugar concentration dynamics, whereas in both genotypes, the total sugar concentration was remarkably increased after anthesis and the upper and lower internodes had lower sugar concentrations compared to the middle internodes [60].

In KIT1, Bulldozer, Hannibal, Merlin, and So-29, the sugar concentration was significantly higher compared to other tested genotypes (Figure 1A). Thus, the superiority of these genotypes over other hybrids and landraces on the level of the entire stem is due to its heavier, juicier, and more sugary internodes. This result is consistent with previous observations, where sweet sorghum was shown to produce more weight and to exhibit higher sugar concentration compared to grain sorghum varieties [11,13,15,27,39,57,61–63]. A common feature of sweet sorghum cultivars observed in Keller, NK 405, and Tracy, was the significantly lower sucrose contents (in °Brix) in the upper and lower three internodes during anthesis [59]. In the last top internodes and peduncle of the landrace IS2848, lower sucrose concentrations were observed [64]. Hence, these data suggest that KIT1, So-29, Bulldozer, Merlin, and Hannibal accumulate higher stem solutes than landraces and other hybrids through a combination of increased internode number, higher juice volume per internode, and a greater sugar content per internode that occurs because of significant increases in sugar content during post-flowering stage.

Sugar concentration (in °Brix) showed non-significant but positive correlation with plant height (Table 3) and negative correlation with internode length (Figure 2D). However, especially when the correlation coefficients are low, there will likely be genotypes that do combine taller internode with high sugar concentration in the stem. In the case of dual-purpose hybrids, these genotypes tend to be taller and to contain a moderate concentration of soluble sugars in their stems compared to sweet genotypes, while grain landraces tend to be shorter and accumulate less sugar in the stem (Table 2; Figure 1A,E). [51] reported a weak relation between plant height and sugar concentration. However, there are several reports that describe a positive correlation between plant height and sugar accumulation in sorghum [13,23,61,62,65,66], suggesting that sugar accumulation in the stem may depend on increased plant height.

4.4. Sweet Sorghums Are More Closer to Dual-Purpose Hybrids Than Grain Landraces

As to assess genetic diversity, phenotypic traits are commonly used as simple indicators to quantify genetic variations that are of interest for plant breeding programs [67,68]. Trait clustering analysis revealed that the two grain landraces Razinieh and Ruzrok were clustered together far from 12 other dual-purpose hybrids and sweet sorghums (Figure 3A). These landraces were recorded low in sugar concentration (°Brix), plant height, internode number, cane yield, juice yield, bagasse yield, sugar yield, and ethanol yield compared with other genotypes (Table 2). All examined sweet and hybrid sorghums were grouped in one cluster. In general, the genotypes of this cluster were taller and had more leaf and internode numbers, higher cane yield, fresh leaf weight, and bagasse yield (Table 2). The broad trait diversity among and between sweet, grain, and dual-purpose sorghum genotypes provides ample opportunities for genotype enhancement through breeding programs. To select parents for crossing, grouping of accessions with agro-morphological and, most likely, genetic similarities may help [69]. Large morphological variations were found among different Indian sweet and grain sorghums [70]. While sweet sorghums are selected and optimized for increased size and thickness of their stem, grain sorghums are mainly bred to optimize grain yield, and dual-purpose hybrids are developed for high shoot biomass and grain yield by heterosis effects [71]. The variation in the magnitude of heterosis between the hybrids in sub-cluster one and two (Figure 3A) likely reflects the increased genetic distance between the parental lines of these hybrids in relation to adaptation to temperate environmental conditions. In addition, compared to the grain

landraces and sweet sorghums, all the hybrids (except Sole) showed superiority in their adaptation to the temperate environmental conditions, indicating the potential of using these hybrids as a source for biofuel production in Germany.

To find traits that contribute most to the total amount of variation in large data sets and to assess the magnitude of variation, PCA analyses proved a valuable tool [6]. PCA results show that 83.21% of total variation among genotypes is contained within the first two principal components (Figure 3B,C). With only four traits (cane yield, bagasse yield, juice yield, and internode number) explaining most of the variation, strong correlations between agro-morphological traits are evident. To achieve better separation of different genotypes, other traits need to be considered.

5. Conclusions

Ten dual-purpose hybrids (grain and biomass), two sweet genotypes, and two grain landraces of sorghum were phenotypically characterized to determine their potential for use as source of bioethanol production under temperate environmental conditions. Dual-purpose hybrids Ganymed, Hannibal, Tarzan, Merlin, and Zerberus showed internodes that were significantly heavier, were richer in sugar and juice, had taller height, and had higher sugar and ethanol potential yields compared with sweet and grain genotypes, indicating the potential of these five hybrids as a source for bioethanol production in Germany. All genotypes showed similar pattern of sugar and juice accumulation in the stem, where more sugar was accumulated in the central internodes and more weight and juice volume were found in the basal internodes. Significant and positive interrelationships were recorded between sugar-related parameters. Individual internode weight rather than internode length can be used as predictor for sugar yield in our sorghum materials. Phenotypic clustering analysis showed that sweet sorghums are closer to dual-purpose hybrids than to grain landraces. Further research is needed to estimate the genetic diversity among the genotypes at molecular level.

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