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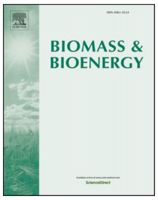

A comprehensive method to select energy sorghum hybrids for bioethanol production

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ABSTRACT

Breeding dedicated energy sorghum [*Sorghum bicolor* (L.) Moench] hybrids is an effective way to provide high-quality biomass feedstock for bioethanol production. However, there exist very limited energy sorghum cultivars/hybrids suitable for bioethanol production with different bioconversions, which require a comprehensive evaluation method that can select elite sorghum germplasms effectively. Here, an integrated approach (combing cluster analysis, principal component analysis, and grey relational analysis) was applied to evaluate the bioethanol potential of 96 sorghum hybrids at two locations with distinct environmental conditions in North China based on 10 energy-related traits. Results showed that 96 hybrids present extensive genetic diversity as reflected by a high coefficient of variation of theoretical ethanol yield and chemical components including soluble sugars, cellulose, hemicellulose, lignin, and ash content at two locations. The 96 sorghum hybrids can be consistently sorted into 4 groups targeted for different bioconversions for both locations, namely Cluster I (unsuitable sorghum type for bioethanol production), Cluster II (ideal sorghum type for bioethanol production), Cluster III (sweet sorghum type) and Cluster IV (biomass sorghum type). Hybrids No. 28 and 30, hybrids No. 64 and 33, and hybrid No. 10 were identified as optimal candidates for ideal sorghum type, sweet sorghum type, and biomass sorghum type for bioethanol production, respectively. Those results highlight that our comprehensive evaluation method can be effective to select elite sorghum hybrids targeted for different bioethanol bioconversions, which can facilitate the breeding process of high-quality energy sorghum hybrids.

1. Introduction

Industrial-scale cultivation of non-food crops for bioethanol production is one of the most promising ways for sustainable bioenergy development and climate change mitigation [1,2]. Moreover, China was projected to promote forecast biofuel production growth since 2019 [3]. Bioethanol, as a better alternative to replace fossil fuels, is expected to play a vital role in achieving this goal [4,5].

Energy sorghum [*Sorghum bicolor* (L.) Moench] has been considered as one of the promising crops for bioethanol production due to its high biomass yield, high water and nutrients use efficiency Bhattarai et al., 2020, short growth cycle, and wide adaptability [6–8]. It has been recommended to be cultivated on an industrial scale to improve the competitiveness of the bioethanol industry and contribute to the development of the bioeconomy [9,10]. However, sorghum is conventionally cultivated and genetic improved for grains and forage production [11,12]. The attention on biomass sorghum type, which is the most promising raw material for second-generation ethanol production, has

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been lacked. As a result, few breeding materials can be used in a bioenergy-oriented breeding program and the existing energy sorghum (including sweet sorghum and biomass sorghum) hybrids cannot meet the requirements for bioethanol production.

Hybrids were long ago demonstrated to present high productivity and better-quality traits of interest [13]. The energy-dedicated sorghum hybrids that have high biomass yield coupled with ideal chemical compositions can be gained by hybrids cultivation. Evaluation of the energy potential of sorghum hybrids is a prerequisite for its genetic improvement. Currently, methods for screening sorghum germplasms are primarily based on sugar and/or fiber production-associated and agronomic traits, far less attention was paid to bioenergy production and does not mention bioenergy-related quality traits. In addition, those methods were aimed to select elite sorghum germplasms for bioethanol production mainly focusing on only specific production technology (e.g., sugar-based bioconversion and lignocellulose-based bioconversion). The sugar-based bioconversion mainly ferments simple carbohydrates to produce bioethanol, which requires feedstocks with high soluble sugars content [14]. The lignocellulose-based bioconversion prefer feedstocks rich in cellulose and hemicellulose, while high lignin and ash content reduce the efficiency of biomass to bioethanol [15–17]. Different bioconversions require biomass with specific quality characteristics, and therefore the hybrid evaluation methods that aim at one specific bioconversion are not efficient for selecting cultivars/hybrids for different bioethanol production technologies. To enhance the energy sorghum breeding efficiency, it is necessary to establish an integrated evaluation framework with uniform and holistic indices to screen elite sorghum hybrids for different production technologies for bioethanol production simultaneously.

Evaluating the energy potential of sorghum hybrids involves agronomic-related and chemical composition-related traits, while those associated with each production technology are generally different. The development of an integrated evaluation method is normally a multi-criteria decision-making process. Grey relational analysis (GRA) could be efficiently used under complex multi-criteria circumstances as the complicated interrelationships between multiple variables can be determined by grey relational coefficients [18]. GRA has been successfully used to screen elite genotypes for fiber, biodiesel and bioenergy production [19,20]. Principal component analysis (PCA), which could effectively compress multiple traits into a few principal components, has been widely used to improve crops breeding efficiency. A comprehensive evaluation method based on GRA and PCA was successfully used to screen elite sorghum sterile lines and sweet sorghum varieties [21]. Cluster analysis can sort a given series of energy crop cultivars/hybrids into certain groups with a distinct characteristic that are suitable for different bioconversions.

Therefore, the objective of present study was to develop an integrated evaluation method (combining GRA, PCA, and cluster analysis) for effectively screening elite sorghum hybrids for bioethanol production with different production technologies andd also identify some optimal sorghum hybrids for cultivation at specific locations simultaneously.

2. Materials and methods

2.1. Germplasm and experimental sites

Ninety-six (96) energy sorghum hybrids were generated by using 12 cytoplasmic male-fertile (i.e., restore) lines as males and 8 cytoplasmic male-sterile (CMS) lines as females (Table 1). The parents of the 96 hybrids are listed in Table S1. The diverse male parents included types of sweet, biomass, and grain sorghum, most of which have been planted by farmers for several years in China. Particularly, 5 accessions of bloom type with very low grain yield and tall stems were collected from the countryside for this study (Table 1). Six female parents were A1 CMS lines which have been mostly used to generate hybrids for diverse purposes, whereas two other lines were A3 CMS lines which could generate hybrids with a very low level of grain yield due to a low seed-setting rate. One of the female parents (AMP450) was brown mid-rib type with a low level of lignin content. The parents with low levels of grain yield and lignin were selected in this study assuming these parents could be more suitable to generate biomass-type hybrids for using as bioethanol feedstock. Overall, these 20 parents should have extensive genetic diversity, which can generate dedicated energy sorghum hybrids with high biomass yield coupled with better energy-related chemical compositions.

Table 1
Origin and characteristics of parental sorghum lines used to generate hybrids.

Accession	Origin	Sorghum type	Role in crosses	Supplier
Awanlek	USA	Sweet type	Male	CI-CAAS
X097	China	Sweet type	Male	NECB-CAU
X098	China	Sweet type	Male	NECB-CAU
Tiansi-1	China	Sweet type	Male	BLCCI
NW2008-1	China	Bloom type	Male	NECB-CAU
NW2008-2	China	Bloom type	Male	NECB-CAU
NW2008-3	China	Bloom type	Male	NECB-CAU
NW2008-6	China	Bloom type	Male	NECB-CAU
NW2008-21	China	Bloom type	Male	NECB-CAU
HN2013	China	Grain type	Male	NECB-CAU
J7645zao	China	Grain type	Male	SI-SAAS
Katemu	Kenya	Grain type	Male	PGRCU-ARS-USDA
AMP439	USA	A1 cytoplasm	Female	PGRCU-ARS-USDA
AMP450	USA	A1 cytoplasm, brown mid-rib	Female	PGRCU-ARS-USDA
ATx622	USA	A1 cytoplasm	Female	CI-CAAS
ATx623	USA	A1 cytoplasm	Female	CI-CAAS
ATx624	USA	A1 cytoplasm	Female	CI-CAAS
ATx2924	USA	A1 cytoplasm	Female	PGRCU-ARS-USDA
A3SC103-12E	USA	A3 cytoplasm	Female	PGRCU-ARS-USDA
A3SM100	USA	A3 cytoplasm	Female	PGRCU-ARS-USDA

BLCCI: Beijing Lvneng Cash Crop Institute; CI-CAAS: Crop Institute, Chinese Academy of Agricultural Sciences; NECB-CAU: National Energy R&D Center for Non-food Biomass, China Agricultural University; PGRCU-ARS-USDA: Plant Genetic Resources Conservation Unit, Agricultural Research Service, United States Department of Agriculture. SI-SAAS: Sorghum Institute, Shanxi Academy of Agricultural Sciences.

Field experiments were conducted at Jiexiu (37°08′N, 112°06′E) in Shanxi and Zhuozhou (39°37′N, 115°51′E) in Hebei, China in 2017. Both locations are characterized by a continental monsoon climate with cold and dry winters and hot and humid summers. The annual temperature of the Jiexiu site is 10.4 °C and the mean annual frost-free period is 175 days according to the multi-year recordings. The mean annual precipitation is 450.0 mm, which occurred during July to September. The multi-year annual average temperature of the Zhuozhou site is 11.6 °C and the annual frost-free period is 178 days. The mean annual precipitation is 550.0 mm, which occurred during July to August. Soil samples for physical and chemical tests were collected one day before the sowing date. The main physical and chemical property data of the soils (0–30 cm depth) and the mean monthly precipitation and temperature during the growing seasons at two sites can be obtained from our previous study of He et al. [12]. In general, the Jiexiu site exhibited higher initial soil nutrients compared to the Zhuozhou site. Cumulative rainfall and daily mean temperatures were higher at the Zhuozhou site (559.3 mm and 22.7 °C) than at the Jiexiu site (461.0 mm and 21.1 °C) during the sorghum growing seasons in 2017.

2.2. Experimental design and crop management

Ninety-six hybrids were planted in a randomized complete block design with three replicates at Jiexiu and Zhuozhou in 2017, respectively. Basal fertilizers of 120 N kg ha^{-1} as urea, 60 P_2O_5 kg ha^{-1} as diammonium phosphate, and 60 K_2O kg ha^{-1} as potassium were applied to the soil just before sowing. Two to three seeds were sown for each hole at 0.6 × 0.2 m intervals oriented in a north-south direction using a manual hill-drop method. At the three-leaf growth stage, the seedling was manually thinned to leave one vigorous plant per hole resulting in a final population of 83,333 plants ha^{-1}, and concurrently weeds were manually removed. Besides the surface irrigation implemented one week before seeding, no supplementary surface irrigation was conducted during the growing period. A solution mixture of cypermethrin, acetamiprid, chlorpyrifos, and imidacloprid was sprayed onto the plots to control insect pests. Each sorghum panicle was covered by an opaque parchment bag for self-fertilization at the anthesis stages. After 10 days, these bags were removed and changed to fine mesh bags to prevent bird damage. Standard agronomic management practices were followed for the cultivation of good sorghum hybrids.

2.3. Sampling and measurements

On the harvest dates, aboveground sorghum plants (main and tiller) of 10 hills were randomly taken from each plot. A total of 10 plants were selected for each plot according to the equal spacing method of the plant height. Each sample plant was divided into leaves, stems, and panicles, and the fresh weights were separately measured. A machine (PSL sampler, Beijing, China) was used to cut leaves of 1.6 cm pieces as samples from every 12 cm leaf section and cut stems of 1.0 cm pieces as samples from every 15 cm section. The stem and leaf samples in each plot were weighed and oven-dried at 75 °C until a constant weight was achieved to gravimetrically determine moisture content. Dry stem and leaf biomass yields were calculated with the fresh weight and the moisture, respectively. The over-dried stem and leaf samples were ground to the particle sizes which could pass the 10–100 mm mesh using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA). These ground samples were stored in a plastic bag at 4 °C for subsequent chemical analyses.

2.4. Chemical composition determination, near-infrared spectroscopy, and TEY calculation

The near-infrared spectroscopy (NIR) models developed by Ref. [13] were used to determine the concentrations of soluble sugars, cellulose, hemicellulose, lignin, and ash in sorghum samples. The NIRS models were built by Chem Data Solution (2.0, Dalian Chem Data Solution Technology Co. Ltd., Dalian, China) based on 143 stem samples and 145 leaf samples of sorghum. Samples with a large number from different regions, years, and types make better goodness-of-fit of the quantitative models for chemical composition determination, and the detail model parameters can be found in Ref. [13]. These NIR models proved suitable to predict the above-mentioned chemical composition of sorghum biomass and grain, based on our previous studies [12,13].

Theoretical ethanol yield (TEY) was composed of that soluble sugar (TEY_{SS}) and insoluble sugar (TEY_{IS}), which were calculated using the following formulas, respectively:

$$TEY_{SS} = Soluble\ sugar\ content \times 0.51 \times 0.85 \times 1000 \div 0.79 \qquad \text{(Eq. 1)}$$

$$TEY_{IS} = (Cellulose\ content + hemicellulose\ content) \times 0.51 \times 0.85 \times 1.11 \times 0.85 \times 1000 \div 0.79 \qquad \text{(Eq.2)}$$

$$TE = TEY_{SS} + TEY_{IS} \qquad \text{(Eq. 3)}$$

Where *0.51* is the coefficient of the conversion factor of ethanol from sugar; *0.85* is the process efficiency of ethanol from sugar; *1.11* is the coefficient for the conversion factor of sugar from cellulose and hemicellulose [22]; *0.85* is the process efficiency of sugar from cellulose and hemicellulose; *0.79* (g mL^{-1}) is the specific gravity of ethanol.

2.5. Integrated method development

The integrated method framework for the bioethanol potential of sorghum hybrids evaluation was a three-step process to assess the comprehensive bioenergy performance of sorghum hybrids (Fig. 1). Firstly, cluster analysis was used to divide 96 sorghum hybrids into different groups according to a distinct characteristic target for different bioconversions. Secondly, PCA was applied to clearly visualize the distinct characteristicscs of each group. Finally, GRA was used to identify elite sorghum hybrids by rank sorghum hybrids based on grey relational grade. The detailed procedure of each step was presented as follows:

Step 1: Clustering and principal component analysis. Ninety-six sorghum hybrids can be grouped into 4 clusters based on the 10 energy-related traits at the Jiexiu and Zhuozhou sites, respectively, since the recommended optimal cluster numbers of the 96 sorghum hybrids were 4 in both sites (Fig. S1). We assumed that the 4 clusters were: (I) sorghum hybrids with low biomass yield and quality that are unsuitable for bioethanol production, (II) comprehensive ideal sorghum type that has better agronomic performance coupled with good chemical composition for bioethanol production, (III) sweet sorghum type with high soluble sugar for bioethanol production with sugar-based bioconversion route and, (IV) biomass sorghum type with high lignocellulose suitable for bioethanol production with lignocellulose-based bioconversion according to the present bioethanol production technology. After removing the hybrid group that is unsuitable for bioethanol production, the remaining three hybrid groups were used to further assess their energy potential. Additionally, a PCA biplot was used to visualize the characteristics of each group and the correlation between the investigated ten energy-related traits.

Step 2: Construct the grey relation matrix. Constructing the grey relation matrix of each hybrid and the best ideal species based on ten indicators including growth period, plant height, stem diameter, biomass yield, soluble sugar, cellulose, hemicellulose, lignin, ash, and TEY. These ten indicators were divided into two types of criteria, namely the higher the better criteria and the lower the better criteria. The indicators division is based on the results of cluster analysis and the correlation between ten indicators in each cluster. Growth period, plant height, stem diameter, biomass yield, soluble sugar, cellulose, hemicellulose, and TEY were listed as the higher the better criteria, whereas lignin and ash were considered as the lower-the-better criteria in the comprehensive ideal sorghum group as comprehensive ideal feedstock for bioethanol production requires materialss with higher biomass yield (longer growth period, higher plant height and higher stem diameter) coupled with ideal compositions (higher soluble sugars, cellulose, hemicellulose and lower lignin and ash). Growth period, plant height, stem diameter, biomass yield, soluble sugars, and TEY were listed as the larger the better criteria, and cellulose, hemicellulose, lignin, and ash were considered as the lower-the-better criteria in sweet sorghum type suitable for bioethanol production group with sugar-based bioconversion (Fig. S2). Growth period, plant height, stem diameter, biomass yield, TEY, cellulose, and hemicellulose were listed as the higher the better criteria, and soluble sugars, lignin, and ash were considered as the lower-the-better criteria in biomass sorghum type suitable for bioethanol production group with lignocellulose-based bioconversion (Fig. S2).

Step 3: Rank hybrids. Grey relational analysis was used to screen optimal sorghum hybrids that were suitable for bioethanol production. Herein, each group of sorghum hybrids at both sites was defined as the grey system respectively. By measuring the grey relational

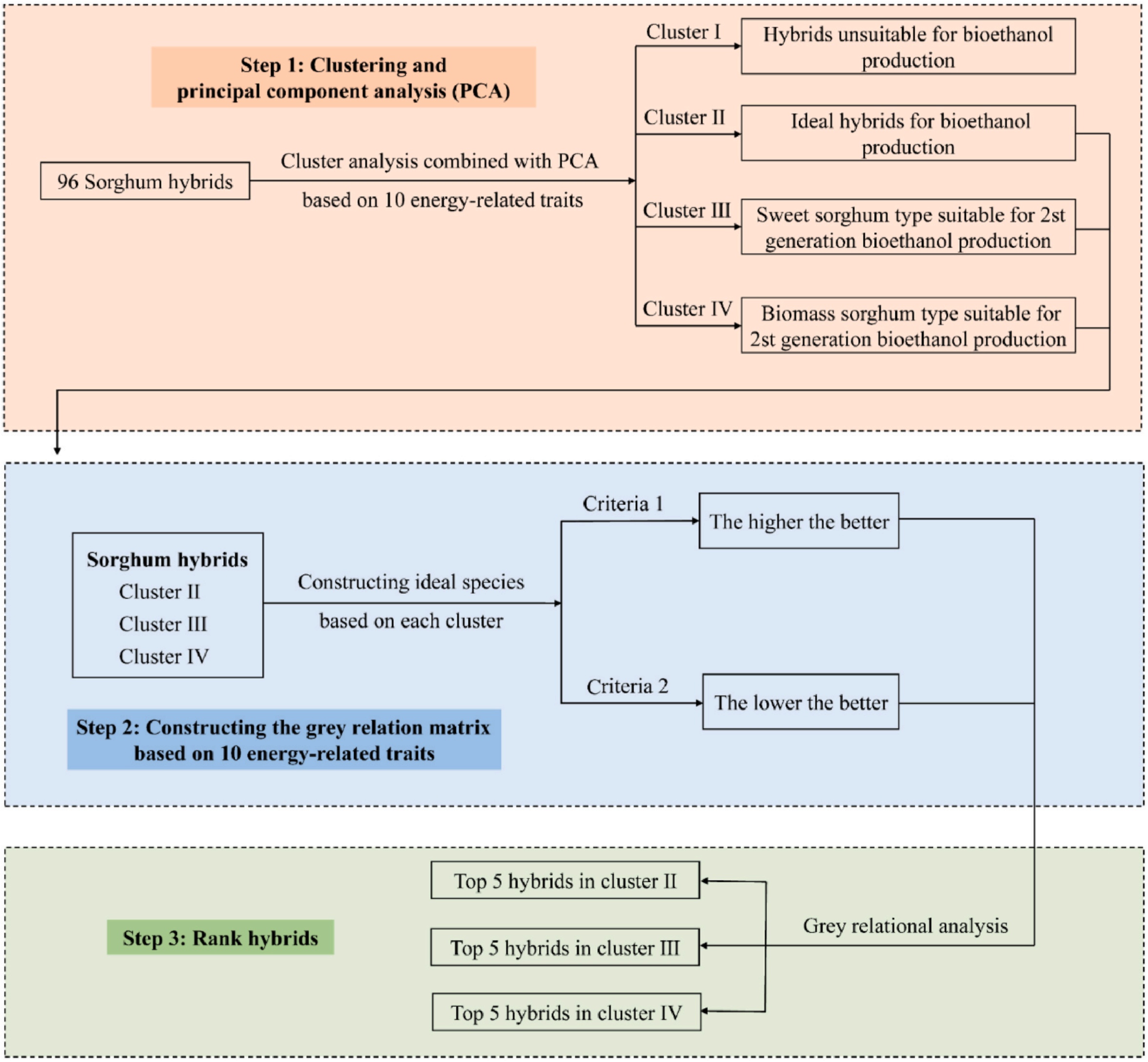

Fig. 1. The process to construct an integrated method for energy sorghum hybrids selection.

grade between each hybrid and the artificially designed ideal species (the best hybrids for the bioethanol production in each group), hybrids were ranked and the optimum hybrids with the higher grade of relation were selected in each group. The entire calculation procedures at both sites consist of three steps including dataset normalization, grey relational coefficient calculation, and grey relational grade determination are as follows [20]: The reference sequence was constructed based on each group of sorghum hybrids with the above ten indicators and ideal specials were constructed based on the characteristics of two types of criteria. Dataset normalization and grey relational coefficient calculations were the same as the method described by Ref. [20]. The grey relational grade, which is a weighted sum of the grey relational coefficients, is determined as follows:

$$GRDi = \sum_{k=1}^{n} W_k \times \varepsilon i(\mathrm{k}) \qquad \text{(Eq. 4)}$$

Where GRD_i represents the GRD of *i*th of hybrids and n is the number of the used traits; εi(k) is the grey relation coefficient. W_k is the weight of each indicator, which equals 1/multiple correlation coefficients [20].

2.6. *Data analysis*

SPSS 25.0 analytical software package (IBM SPSS Inc., Chicago, IL, USA) was used for the variance analysis and multiple comparison analysis at $p < 0.05$ level. Pearson correlation coefficient analysis, cluster analysis, and PCA were performed by R (version 3.2.0, R Development Core Team 2016).

3. Results

3.1. *Agronomic performance, chemical composition, and theoretical ethanol yield*

The effects of genotypes were significant ($p < 0.01$) for all the measured parameters, showing a wide genetic diversity among 96 sorghum hybrids (Table 2). Meanwhile, the significant ($p < 0.01$) effects of variables of the environment on all measured parameters of sorghum hybrids were observed in this study. By contrast, their interaction exerted a partly significant ($p < 0.05$) difference in plant height, cellulose, hemicellulose, lignin, and ash. From Table 3, the growth period, plant height, and stem diameter showed significant differences ($p < 0.05$) between the two experimental sites. Averaged across all the sorghum hybrids, the Zhuozhou site produced hybrids with longer growth periods and higher plant height than the Jiexiu site (Table 3), whereas

Table 2
Mean squares from combined analysis of variance (ANOVA) for the traits measured across environments.

Trait	Genotype	Environment	Genotype × Environment
	(*df* = 95)	(*df* = 1)	(*df* = 95)
Growth period (GP)	1377.5**	16121.1**	150.9
Plant height (PH)	46120.7**	820994.8**	2821.0**
Stem diameter (SD)	0.2**	1.5**	0.1
Biomass yield (BY)	1555.9**	26710.8**	290.5
Soluble sugar (SS)	18646.1**	103905.2**	2568.0
Cellulose (C)	2303.9**	37182.6**	615.7**
Hemicellulose (H)	1920.0**	4515.7**	406.8**
Lignin (L)	927.1**	21205.9**	218.1**
Ash (A)	719.9**	6260.6**	129.0**
TEY	169964005.4**	3969815310.3**	29787696.3

* and **: significantly different at 0.05 and 0.01 levels, respectively; *df:* degree of freedom; TEY: theoretical ethanol yield.

Table 3
Sorghum hybrids characteristics for performance at Jiexiu and Zhuozhou sites.

Trait	Jiexiu		Zhuozhou	
	Mean	CV (%)	Mean	CV (%)
Growth period (d)	139.6 b	12.1	150.4 a	14.0
Plant height (cm)	311.3 b	27.1	387.8 a	27.7
Stem diameter (cm)	2.1 a	14.3	2.0 b	10.0
Biomass yield (t ha^{-1})	36.9 b	43.4	50.6 a	52.2
Soluble sugar (g kg^{-1})	173.0 b	38.0	202.0 a	36.9
Cellulose (g kg^{-1})	249.6 b	9.3	266.4 a	12.0
Hemicellulose (g kg^{-1})	188.6 a	12.7	182.4 b	13.0
Lignin (g kg^{-1})	124.2 b	11.9	136.6 a	14.3
Ash (g kg^{-1})	59.5 a	21.8	52.9 b	26.7
TEY (L ha^{-1})	9613.8 b	54.7	14928.0 a	56.1

Different letters after mean values within the same row have a significant statistical difference at the $p < 0.05$ level. TEY: theoretical ethanol yield; CV: coefficient of variation.

stem diameter was higher for hybrids at the Jiexiu site. Biomass yield and TEY averaged among all sorghum hybrids grown at the Zhuozhou site (50.6 t ha^{-1}) were statistically ($p < 0.05$) higher than ones grown at the Jiexiu site (36.9 t ha^{-1}). In general, sorghum hybrids grown at the Zhuozhou site exhibited much higher soluble sugars, cellulose, and lignin than sorghum hybrids grown at the Jiexiu site (6.7–16.8 %) (Table 3). Conversely, hybrids grown at the Jiexiu site had a relatively higher content in hemicellulose (3.4 %) and ash (12.5 %), respectively. High TEY yield was mirrored by biomass yield in this study; averaged among all the hybrids, a mean TEY of 14,928.0 L ha^{-1} was observed at the Zhuozhou site, which produced a 55.3 % ($p < 0.05$) higher ethanol yield than that observed at the Jiexiu site (9613.8 L ha^{-1}; Table 3 and Fig. 2).

Among all hybrids, the highest coefficient of variation (CV) was observed for biomass yield and TEY with CV values of 43.4 % and 54.7 % at Jiexiu and 52.2 % and 56.1 % at Zhuozhou, respectively (Table 3). In terms of chemical composition, a relatively higher CV was observed for soluble sugars and ash, whereas cellulose, hemicellulose, and lignin exhibited relatively lower variability. Hybrid diversity was reflected by the changing CV levels of biomass yield, soluble sugars, cellulose, hemicellulose, lignin, ash, and TEY shown in Table 3.

3.2. *Cluster analysis and principal component analysis of 96 sorghum hybrids at the two sites*

As expected, ninety-six sorghum hybrids were sorted into 4 clusters based on 10 energy-related traits at both Jiexiu and Zhuozhou sites (Fig. 3), which is coincident with our assumption that the given 96 sorghum hybrids can be sorted into 4 groups according to bioethanol production technology. In general, the sorghum hybrids group division was highly stable as the same group shares most of the sorghum hybrids at two sites. The cluster I to cluster IV shared sixteen, twelve, twenty-eight, and nineteen sorghum hybrids at two sites, respectively. Cluster I with 19 hybrids in Jiexiu and 19 hybrids in Zhouzhou were characterized by lower TEY and high ash content, which can be listed as a sorghum group unsuitable for bioethanol production (Table 4). Cluster II (14 hybrids in Jiexiu and 19 hybrids in Zhouzhou) was considered a comprehensive ideal sorghum group for bioethanol production owing to its highest biomass yield and TEY and longest growth period as well as the biggest plant size (with the highest plant height and stem diameter). Cluster III in both sites (39 hybrids in Jiexiu and 29 hybrids in Zhuozhou) exhibited the highest soluble sugars and relatively high biomass yield and TEY and relatively low lignin and ash, which can be listed as sweet sorghum type suitable for bioethanol production with sugar-based bioconversion. Cluster IV in both sites (25 hybrids in Jiexiu and 29 hybrids in Zhouzhou) showed the highest content of cellulose, hemicellulose, and lignin as well as relatively high biomass yield and TEY, which can be considered as biomass sorghum type suitable for bioethanol production with lignocellulose-based bioconversion. Generally, the group division obtained from cluster analysis greatly matched the requirement of the current production technology of bioethanol.

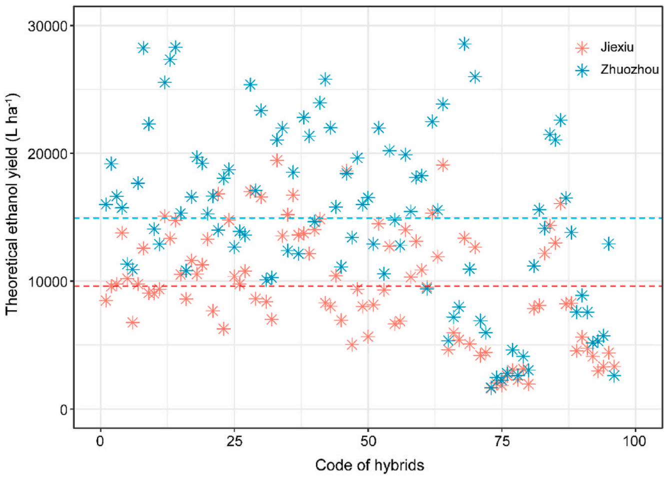

Fig. 2. Distribution of theoretical ethanol yield of 96 energy sorghum hybrids at Jiexiu and Zhuozhou. The red and cyan lines present the mean value of the theoretical ethanol yield of 96 sorghum hybrids at Jiexiu and Zhuozhou, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

PCA biplot confirmed the results of group division of cluster analysis and presented its characteristic of each cluster at the Jiexiu and Zhuozhou sites (Fig. 4). The sorghum hybrids form clusters II, III, and IV that can be clearly separated at both sites. In the PCA biplot, an individual that is on the same side of a given variable has a high value for this variable, while an individual that is on the opposite side of a given variable has a low value for this variable. Sorghum hybrids from cluster II present high biomass yield, TEY, plant height, and long growth period at both sites, indicating that those hybrids have high energy potential which can be considered as comprehensive ideal sorghum for bioethanol production. Most sorghum hybrids from cluster III are on the same side of soluble sugars, making those hybrids serve as sweet sorghum type suitable for bioethanol production with a sugar-based bioconversion. Sorghum hybrids from cluster IV were close to the arrows of cellulose, hemicellulose, and lignin, indicating those hybrids are suitable for bioethanol production with a lignocellulose-based bioconversion.

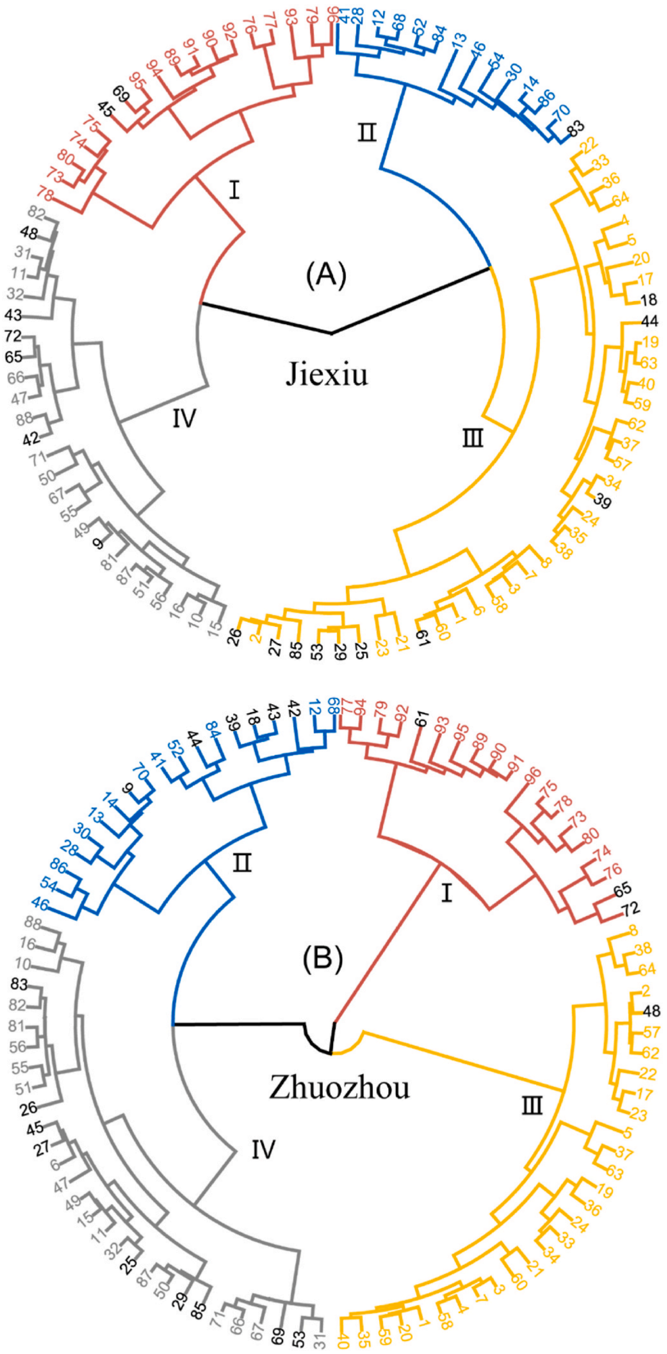

Fig. 3. Circular dendrogram obtained by cluster analysis of 10 energy-related traits in 96 energy sorghum hybrids at Jiexiu (A) and Zhuozhou (B). Sorghum hybrids in common between two locations for each cluster were indicated by same color, otherwise it present black. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

4.1. Diversity of selected energy sorghum hybrids

Exotic germplasm plays a fundamental role in crops genetic improvement and the USA-ARS National Plant Germplasm System has been a major source for global plant genetic resources [23]. Sterile lines Tx622 A/B, Tx623 A/B, Tx624 A/B, and TX625 A/B were induced from the USA to China in 1960s and used to make a few high-yielding grains sorghum hybrids for large commercial production till 1970s. A diverse of sweet sorghum germplasms were induced from the USA in the 1980s to improve cultivars feed and energy purposes in China [24]. This manuscript is a succeeding reports of sorghum germplasm evaluation and utilization based on the germplasm that was induced by the USA-ARS National Plant Germplasm System in 2012.

The multi-index analysis of sorghum hybrid diversity stands as a distinctive strength of this study. This approach facilitates the identification of sorghum hybrids optimally suited for widespread cultivation, thereby enhancing the production efficiency and sustainability of energy sorghum products [25]. Accordingly, this study conducted a comprehensive evaluation of 96 sorghum hybrids, aiming to elucidate the unique and shared attributes of different hybrids across multiple dimensions. This offers a refined and extensive theoretical basis for future bioethanol production from sorghum hybrids. Our findings revealed that the genetic makeup of different energy sorghum hybrids, the genotype-environment interaction, and the correlations among various traits exhibit significant diversity. Moreover, the multi-dimensional diversity among energy sorghum hybrids is not solely manifested in the number of varieties but is also evident in the disparities in growth habits, stress resilience, and biomass composition among hybrids [26,27]. Such diversity necessitates a more flexible variety selection tailored to specific application scenarios and objectives in the actual industrial layout, thereby elevating the overall ecological and economic benefits in the bioenergy industry. Our study's outcomes indicate that sorghum hybrids grown in Zhuozhou manifest superior biomass yield, enhanced chemical composition, elongated growth phases, and larger plant dimensions, theoretically presenting higher TEY, than those grown in Jiexiu. The index evaluation results reveal that energy sorghums from the two regions each possess distinct advantages across ten energy-related traits, with significant disparities observed. Moreover, TEY assessments among different energy sorghum hybrids within the same location demonstrate notable dispersion, indicating clear heterogeneity (Fig. 2). Considering the environmental conditions of both locations, certain hybrids still maintain substantial TEY under drought-prone conditions where water resources are limited. Ultimately, based on the integrated evaluation results, hybrids No. 28 (A3SC103-12E × NW-2008-21) and No. 30 (AMP439 × NW-2008-21) were identified as optimal raw materials for bioethanol production for various conversion routes due to their higher biomass yield, ideal chemical composition and wide adaptability (Table 5).

4.2. Importance of the selected traits for evaluating the energy potential

Distinct sorghum varieties exhibit significant heterogeneity in their growth characteristics and energy conversion efficiency. The bioethanol production potential of energy sorghum can be influenced by several factors Nasidi et al., 2010, and evaluations based solely on a limited set of indices may present constraints. To address the limitations encountered when relying on a singular metric for potential evaluation, it is imperative to consider a combination of pivotal agronomic and chemical composition associated traits.

The quality and growth parameters considered in this study hold significant implications for the bioethanol production potential of energy sorghum hybrids. Specifically, these indices collectively determine the suitability of sorghum as a feedstock for bioethanol production. There exists a positive correlation between plant height and biomass accumulation; the length of the growth cycle influences a hybrid's adaptability under specific environmental conditions. Pertaining to quality-associated attributes, lignin, cellulose, and hemicellulose stand out as decisive factors governing ethanol conversion efficiency. A high lignin content might impede the degradation of cellulose and hemicellulose, subsequently diminishing the ethanol conversion rate [28]. Conversely, high cellulose and hemicellulose content typically result in an elevated ethanol yield [29]. Additionally, ash content, soluble sugars, and dry matter also play pivotal roles in evaluating ethanol production potential. Generally, a high ash content might adversely impact the

Table 4
Average values of 10 energy-related traits of sorghum hybrids evaluated in four classifications at Jiexiu and Zhuozhou sites.

Parameter	Growth period (d)	Plant height (cm)	Stem diameter (cm)	Biomass yield (t ha^{-1})	Soluble sugar (g kg^{-1})	Cellulose (g kg^{-1})	Hemi-cellulose (g kg^{-1})	Lignin (g kg^{-1})	Ash (g kg^{-1})	TEY L ha^{-1}
Jiexiu										
I ($n = 18$)	117.6 c	178.2 c	2.0 b	17.2 d	131.8 c	250.1 c	202.3 a	123.4 c	73.6 a	3527.6 d
II ($n = 14$)	151.4 a	381.7 a	2.3 a	52.5 a	168.3 b	258.6 b	187.6 b	130.2 b	52.6 c	14710.1 a
III ($n = 39$)	149.2 a	331.3 b	2.1 b	41.8 b	232.0 a	232.2 d	168.6 c	113.2 d	51.2 c	11993.6 b
IV($n = 25$)	133.8 b	337.3 b	2.2 a	34.6 c	115.2 c	270.7 a	209.8 a	138.0 a	66.1 b	7402.8 c
Zhuozhou										
I ($n = 19$)	120.8 c	224.5 c	1.9 c	20.8 d	158.9 b	254.5 b	188.8 a	131.9 b	72.8 a	5262.9 d
II ($n = 19$)	165.2 a	500.7 a	2.1 a	76.3 a	176.4 b	290.9 a	194.6 a	150.3 a	43.8 c	23152.9 a
III ($n = 29$)	157.8 b	401.6 b	2.0 b	58.0 b	283.3 a	240.7 c	158.4 b	119.4 c	47.6 bc	17924.7 b
IV ($n = 29$)	153.6 b	407.9 b	2.0 ab	46.0 c	167.1 b	283.3 a	193.8 a	147.6 a	51.8 b	12924.7 c

Different lower-case letters: statistical significance among clusters at $p < 0.05$ level for the investigated trait within the same location; TEY: theoretical ethanol yield.

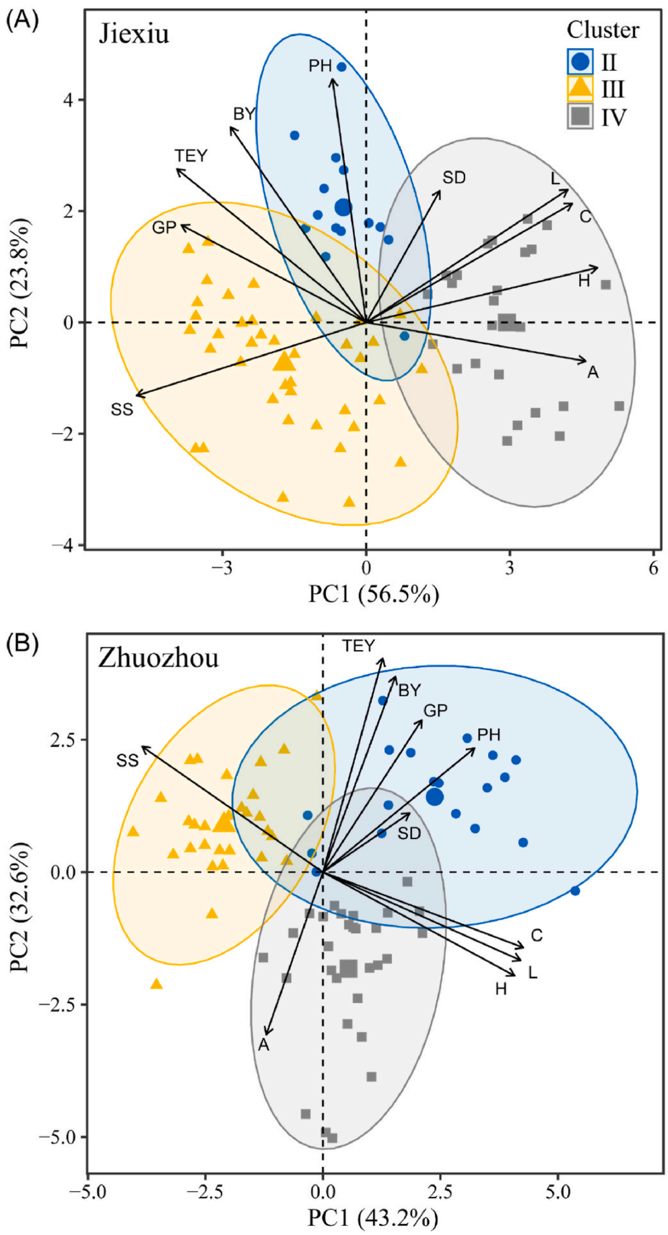

Fig. 4. Principal component analysis of the energy performance of three energy sorghum hybrid clusters at the Jiexiu (a) and Zhuozhou sites (b). GP: growth period; PH: plant height; SD: stem diameter; BY: biomass yield; S: soluble sugars; C: cellulose; H: hemicellulose; L: lignin; A: ash; TEY: theoretical ethanol yield.

efficiency of biomass pyrolysis and gasification, further reducing the ethanol conversion rate [30]. Soluble sugars, fundamental yet crucial in the ethanol fermentation process, directly influence the ultimate ethanol yield [31,32]. Hybrids with high dry matter content typically exhibit superior biomass accumulation potential, leading to an enhanced overall ethanol yield [33,34]. In aggregate, ethanol yield serving as the quintessential metric for evaluating ethanol production efficiency is intricately influenced by a confluence of agronomic and chemical composition quality-associated attributes. The TEY ultimately delineates the sustainability of industrializing such a variety for resource utilization [35]. In summary, this study not only comprehensively deciphers the potential of different sorghum hybrids for bioethanol production but also furnishes a scientific foundation for the meticulous selection of hybrids under specific requirements and environmental conditions.

4.3. Accuracy and applicability of the integrated method

The proposed integrative evaluation method in this study elevated precision and applicability advantages. Merging techniques such as grey relational analysis, principal component analysis, and cluster analysis, this method fosters a more diversified and comprehensive set of evaluation metrics. The scientific rigor and objectivity of the methodological application have been augmented, and the corroborative nature of the outcomes further ensures the reliability and accuracy of the conclusions drawn.

Compared to traditional singular evaluation methods such as grey relational analysis, principal component analysis, and cluster analysis, the integrative approach exhibited superior performance in terms of applicability and precision, thereby mitigating potential biases introduced by human factors. Through a comprehensive assessment of the ethanol-processing quality related characteristics of 96 energy sorghum hybrids, we successfully identified two hybrids (No. 28 and No. 30) with optimal potential for bioethanol production. Initially, using cluster analysis, samples from each region were effectively segregated into four distinct characteristic groups, facilitating preliminary differentiation based on quality excellence. Subsequently, based on clustering, principal component analysis enabled the simplification of multiple correlated variables into two independent principal components, streamlining the evaluation process and offering a more intuitive representation of the attributes of each cluster, further refining the selection criteria. Ultimately, grey relational degree analysis afforded data support through grey relational analysis, allowing us to evaluate the comprehensive performance of diverse hybrids [20] both intuitively and scientifically, thereby reinforcing the credibility of preceding clustering and principal component analysis findings. Furthermore, the synergy between principal component analysis and grey relational degree assigned varying weights to different indicators within each cluster, facilitating the quantitative analysis and ranking of the top five hybrids within each cluster based on their composite effects. In summary, when

Table 5

Characteristics of sorghum hybrids in the top fifth of grey relational grade from three clusters each at Jiexu and Zhuozhou sites.

Hybrid code	Group	GRD	Rank	GP (d)	PH (cm)	SD (cm)	BY (t ha^{-1})	S (g kg^{-1})	C (g kg^{-1})	H (g kg^{-1})	L (g kg^{-1})	A (g kg^{-1})	TEY (L ha^{-1})
Jiexiu													
46	II	0.786	1	160	440.3	2.2	65.4	137.1	278.1	202.1	143.7	50.0	18627.4
28	II	0.731	2	154	423.3	2.5	57.8	189.0	249.1	178.9	128.3	47.4	16998.6
30	II	0.665	3	162	309.2	2.3	53.9	189.3	255.5	190.3	129.2	46.3	16561.5
86	II	0.598	4	158	395.3	2.3	55.9	165.6	265.1	194.4	133.2	50.5	16053.7
12	II	0.592	5	147	422.8	2.3	51.0	180.7	254.0	177.8	129.5	49.5	15078.8
64	III	0.767	1	160	336.5	2.3	64.0	248.1	216.5	159.2	110.9	50.3	19081.6
33	III	0.738	2	155	359.3	2.1	62.6	245.9	231.7	163.1	112.9	50.7	19435.6
24	III	0.693	3	153	370.3	2.1	46.5	292.3	226.4	150.4	101.2	43.8	14767.4
22	III	0.683	4	150	365.2	2.1	56.5	275.8	230.8	162.7	107.9	46.1	16811.2
36	III	0.677	5	153	344.7	2.3	53.0	264.9	215.1	151.1	104.7	48.9	16720.6
15	IV	0.709	1	137	344.8	2.0	46.6	85.9	290.9	222.5	145.9	70.8	10513.3
10	IV	0.662	2	140	399.0	2.1	39.3	84.2	296.7	224.1	148.4	62.9	9033.7
16	IV	0.646	3	132	371.3	2.2	42.3	77.2	293.1	223.7	152.7	62.9	8607.6
49	IV	0.645	4	144	377.0	2.2	40.1	87.0	269.4	204.4	152.0	60.9	8016.7
43	IV	0.640	5	140	341.3	2.6	38.0	116.4	251.7	195.0	136.9	57.3	8027.3
Mean		0.682		150	373.4	2.2	51.5	176.0	254.9	186.6	129.2	53.2	14289.0
Zhuozhou													
68	II	0.746	1	169	495.5	2.2	88.9	229.9	269.4	179.2	131.8	44.1	28570.2
14	II	0.673	2	173	537.7	2.1	93.1	178.7	291.3	198.4	151.3	48.2	28295.1
28	II	0.649	3	173	587.7	2.3	74.0	173.9	315.8	209.0	153.2	42.3	25369.2
30	II	0.627	4	170	528.7	2.4	73.6	174.0	302.6	201.8	154.2	35.7	23343.5
12	II	0.619	5	167	557.3	2.2	80.3	208.4	267.0	176.9	137.5	52.3	25532.0
8	III	0.786	1	167	419.8	2.1	94.9	257.3	256.4	169.0	123.7	43.2	28243.1
19	III	0.669	2	152	408.2	2.3	63.6	324.3	222.5	143.6	107.7	43.5	19217.9
33	III	0.644	3	151	420.3	2.1	72.2	322.0	228.8	144.5	114.2	44.3	21045.3
64	III	0.607	4	167	442.5	2.1	69.7	275.2	245.8	163.5	123.5	47.4	23838.0
34	III	0.598	5	153	437.8	2.0	73.5	303.5	234.9	156.3	118.9	47.4	21975.2
85	IV	0.659	1	154	359.2	2.0	68.5	180.4	279.2	189.5	145.3	52.3	21051.4
50	IV	0.601	2	148	435.7	2.0	59.0	186.3	274.8	186.4	147.9	47.5	16516.2
87	IV	0.598	3	153	417.3	2.0	60.2	167.9	278.7	190.8	150.2	50.4	16495.7
82	IV	0.585	4	168	438.3	2.2	54.1	181.1	299.3	193.5	151.1	45.5	15573.6
29	IV	0.568	5	154	410.8	2.0	52.6	172.7	284.4	202.0	142.5	47.1	17089.2
Mean		0.642		161	459.8	2.1	71.9	222.4	270.1	180.3	136.9	46.1	22143.7

GRD: grey relational grade; GP: growth period; PH: plant height; SD: BY: biomass yield; S: soluble sugars; C: cellulose; H: hemicellulose; L: lignin; A: ash; TEY: theoretical ethanol yield.

juxtaposed with previous conventional assessment methodologies, this holistic analytical method effectively precludes potential biases stemming from human intervention, endorsing comprehensive evaluation metrics, objectives, and scientific methodologies, and corroborating reliable outcomes.

The evaluation methodology utilized in this study demonstrates significant applicability, as evidenced by the consistent classification of the 96 energy sorghum hybrids into four distinct groups targeting various conversion routes. This robust classification was further supported by the high stability of the sorghum hybrid group division, as the majority of sorghum hybrids within the same group were shared across the two sites. Specifically, cluster I, cluster II, cluster III, and cluster IV encompassed sixteen, twelve, twenty-eight, and nineteen sorghum hybrids at both sites, respectively (Fig. 3).

The integrated assessment approach excels when dealing with extensive datasets; however, its efficacy might be constrained in scenarios with limited data availability. Broadly speaking, this comprehensive evaluation method is widely applicable in the assessment of energy crop hybrids, especially in complex scenarios influenced by multiple factors such as genotypes, regional environmental conditions, and growth cycles. This method can proficiently facilitate systematic categorization and selection when most indicators exhibit significant heterogeneity. Nonetheless, prior to implementing this methodology, researchers must meticulously assess its relevance and suitability in the context of their specific research objectives and requirements.

4.4. Implications, limitations, and prospective

To the best of our knowledge, the present study for the first time established a comprehensive method for elite energy sorghum hybrids selection tailored to various conversion routes. In particular, the reliability and accuracy of this comprehensive method were verified using 96 sorghum hybrids in two sites with distinct climatic and soil properties. Thus, this comprehensive method can promote the progress of energy sorghum breeding when it is utilized by breeders. In addition, the selected elite energy sorghum in this study can be promoted to cultivation in arid or semiarid regions of China to boost bioethanol production. The present study has some limitations and prospects. Data on parents of energy sorghums were limited in our dataset, especially for maintainer lines. Thus, the heterosis of the energy sorghum hybrids was not reported, which is a drawback of the present study. In future research, we suggest that heterosis should be considered in comprehensive assessment method establishment.

5. Conclusions

This study compares sorghum hybrids performance under the two distinct environments based on 10 energy-related traits by integrated evaluation method, aiming to (1) classify them into different sorghum energy types; (2) identify optimal sorghum hybrids for cultivation under specific locations; and (3) use optimal conversion methods for bioethanol production. Sorghum hybrids grown at the Zhuozhou site exhibited greater biomass yield, better chemical components, longer growth periods, and bigger plant size and subsequently with higher ethanol potential compared to the Jiexiu site. We divided the hybrids into four groups based on TEY, soluble sugar, cellulose, and ash, which provides the research foundation for further classification screening of 96 hybrids and provides practical experience for similar research. In addition, the hybrid No. 28 (A3SC103-12E × NW-2008-21) and No. 30 (AMP439 × NW-2008-21) can be considered as the optimal feedstock for bioethanol production by the grey relational degree evaluation method. Overall, this study provides an integrated and efficient method for the evaluation of sorghum hybrids as feedstock for bioethanol production.

CRediT authorship contribution statement

Yi Xu: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ming Li Wang:** Writing – review & editing, Visualization, Validation, Resources, Conceptualization. **Abdulgani Devlet:** Writing – review & editing, Visualization, Validation. **Lipu Han:** Writing – review & editing, Validation. **Chaochen Tang:** Writing – review & editing, Software. **Tiansu Tan:** Writing – original draft, Data curation. **Guang Hui Xie:** Writing – review & editing, Validation, Project administration, Funding acquisition, Conceptualization.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

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Data availability

Data will be made available on request.

References

[1] R.K. Rathour, M. Behl, K. Dhashmana, et al., Non-food crops derived lignocellulose biorefinery for sustainable production of biomaterials, biochemicals and bioenergy: a review on trends and techniques, Ind. Crop. Prod. 204 (2023) 117220.

[2] Y. Wu, H. Huang, F. Chen, et al., Soil organic carbon sequestration rate and spatiotemporal dynamics under perennial energy crops cultivation: a global meta-analysis, Soil Tillage Res. 240 (2024) 106064.

[3] REN21Secretariat, Renewables 2023 Global Status Report, 2023. ISBN978–3–948393–03–8.

[4] P.S. Deora, Y. Verma, R.A. Muhal, et al., Biofuels: an alternative to conventional fuel and energy source, Mater. Today: Proc. 48 (2022) 1178–1184.

[5] L. Cherwoo, I. Gupta, G. Flora, et al., Biofuels an alternative to traditional fossil fuels: a comprehensive review, Sustain. Energy Technol. Assessments 60 (2023) 103503.

[6] L.G. Almeida, R.C. Parrella, M.L. Simeone, et al., Composition and growth of sorghum biomass genotypes for ethanol production, Biomass Bioenergy 122 (2019) 343–348.

[7] F.K. Kazungu, E.M. Muindi, J.M. Mulinge, Overview of sorghum (Sorghum bicolor. L), its economic importance, ecological requirements and production constraints in Kenya, Int. J. Phys. Soc. Sci. 35 (1) (2023) 62–71.

[8] H. Bakari, Djomdi, Z.F. Ruben, et al., Sorghum (Sorghum bicolor L. Moench) and its main parts (by-products) as promising sustainable sources of value-added ingredients, Waste and Biomass Valorization 14 (4) (2023) 1023–1044.

[9] B. Diallo, M. Li, C. Tang, et al., Biomass yield, chemical composition and theoretical ethanol yield for different genotypes of energy sorghum cultivated on marginal land in China, Ind. Crop. Prod. 137 (2019) 221–230.

[10] I. Lewandowski, E. Bahrs, N. Dahmen, et al., Biobased value chains for a growing bioeconomy, GCB Bioenergy 11 (1) (2019) 4–8.

[11] S. Qiu, M.P. Yadav, L. Yin, Characterization and functionalities study of hemicellulose and cellulose components isolated from sorghum bran, bagasse and biomass, Food Chem. 230 (2017) 225–233.

[12] S. He, C. Tang, M.L. Wang, et al., Combining ability of cytoplasmic male sterility on yield and agronomic traits of sorghum for grain and biomass dual-purpose use, Ind. Crop. Prod. 157 (2020) 112894.

[13] F. Zhou, S. He, M.L. Wang, et al., Correlation and combining ability of main chemical components in sorghum stems and leaves using cytoplasmic male sterile lines for improving biomass feedstocks, Ind. Crop. Prod. 167 (2021) 113552.

[14] C.E.C. Guimarães, F.S. Neto, Bizerra de Castro, et al., Sustainable bioethanol production from first-and second-generation sugar-based feedstocks: advanced bibliometric analysis, Bioresour. Technol. Rep. 23 (2023) 101543.

[15] K. Wu, Q. Lu, Q. Cao, et al., Hydrolytic and pyrolytic technologies of pretreatment lignocellulose for production of ethanol fuels–A comparative review, Ind. Crop. Prod. 218 (2024) 118840.

[16] A. Velidandi, P.K. Gandam, M.L. Chinta, et al., State-of-the-art and future directions of machine learning for biomass characterization and for sustainable biorefinery, J. Energy Chem. 81 (2023) 42–63.

[17] J. Xue, Z. Yang, L. Han, et al., On-line measurement of proximates and lignocellulose components of corn stover using NIRS, Appl. Energy 137 (2015) 18–25.

[18] S. Chakraborty, H.N. Datta, S. Chakraborty, Grey relational analysis-based optimization of machining processes: a comprehensive review, Process Integration and Optimization for Sustainability 7 (4) (2023) 609–639.

[19] N.A. Maidin, S. Mohd Sapuan, M.M. Taha, et al., Material selection of natural fibre using a grey relational analysis (GRA) approach, Bioresources 17 (1) (2022) 109.

[20] S. Xue, Y. Steinberger, J.S. Wang, et al., Biodiesel potential of nonfood plant resources from tsinling and zhongtiao mountains of China, Bioenergy Research 6 (3) (2013) 1104–1117.

[21] X. Liu, Z. Liu, J. Niyazi, et al., Comprehensive evaluation of sweet sorghum varieties based on principal component analysis and gray relational grade analysis, Xinjiang Agric. Sci. 53 (1) (2016) 99.

[22] Y.L. Zhao, A. Dolat, Y. Steinberger, et al., Biomass yield and changes in chemical composition of sweet sorghum cultivars grown for biofuel, Field Crops Res. 111 (1–2) (2009) 55–64.

[23] P.F. Byrne, G.M. Volk, C. Gardner, et al., Sustaining the future of plant breeding: the critical role of the USDA-ARS national plant germplasm system, Crop Sci. 58 (2018) 451–468.

[24] D.J. Li, F.S. Liao, Sweet Sorghum and its Utilization, Science Press, Beijing, 1992, pp. 56–76 (in Chinese).

[25] R.D. Huang, Research progress on plant tolerance to soil salinity and alkalinity in sorghum, J. Integr. Agric. 17 (4) (2018) 739–746.

[26] T.R. Stefaniak, J.A. Dahlberg, B.W. Bean, et al., Variation in biomass composition components among forage, biomass, sorghum-sudangrass, and sweet sorghum types, Crop Sci. 52 (4) (2012) 1949–1954.

[27] Y. Wang, J. Sun, S.S. Ali, et al., Identification and expression analysis of Sorghum bicolor gibberellin oxidase genes with varied gibberellin levels involved in regulation of stem biomass, Ind. Crop. Prod. 145 (2020) 111951.

[28] M.H. Thomsen, A. Thygesen, A.B. Thomsen, Hydrothermal treatment of wheat straw at pilot plant scale using a three-step reactor system aiming at high hemicellulose recovery, high cellulose digestibility and low lignin hydrolysis, Bioresour. Technol. 99 (10) (2008) 4221–4228.

[29] B. Godin, F. Ghysel, R. Agneessens, et al., Cellulose, hemicelluloses, lignin, and ash contents in various lignocellulosic crops for second generation bioethanol production, Biotechnol. Agron. Soc. Environ. 14 (S2) (2010) 549–560.

[30] M.A. de Almeida, R. Colombo, Production chain of first-generation sugarcane bioethanol: characterization and value-added application of wastes, BioEnergy Research 16 (2) (2023) 924–939.

[31] M. Madadi, K. Zhao, Y. Wang, et al., Modified lignocellulose and rich starch for complete saccharification to maximize bioethanol in distinct polyploidy potato straw, Carbohydr. Polym. 265 (2021) 118070.

[32] A. Montefusco, M. Durante, S. Grassi, et al., Assessment of sweet potato [*Ipomoea batatas* (L.) lam] for bioethanol production in southern Italy, Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology 148 (6) (2014) 1117–1126.

[33] R. Gurram, M. Al-Shannag, S. Knapp, et al., Technical possibilities of bioethanol production from coffee pulp: a renewable feedstock, Clean Technol. Environ. Policy 18 (2016) 269–278.

[34] C. Rachid-Casnati, F. Resquin, L. Carrasco-Letelier, Availability and environmental performance of wood for a second-generation biorefinery, Forests 12 (11) (2021) 1609.

[35] R. Suryaningsih, Irhas, Bioenergy Plants in Indonesia: sorghum for producing bioethanol as an alternative energy substitute of fossil fuels, Energy Proc. 47 (2014) 211–216.