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Research article
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Evaluation of Sweet Sorghum (*Sorghum Bicolor* (L.) Moench) Genotypes for Bioethanol Yield Potential

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Abstract: Bioethanol is a raw material for pharmaceutical and food production and a renewable and sustainable energy source. Sweet sorghum has great potential for producing bioethanol because of its characteristics, such as high sugar accumulation in the stems, fast plant growth, wide adaptability, and low input requirements. The objective of this research is to observe the morphological characteristics and bioethanol yield potential of promising lines of sweet sorghum, including the lines 15020B, 1115-C, 4-183ABEOTO, AB-6-1-1, EA-13-1-1, KL2, 10(1-1), 23(1-1), Buleleng Abang, and WR2, and as checks, the Super-1 and Super-2 varieties. The observed variables include the plant growth variables, crop yield components, and ethanol yield of the stem juice and bagasse. The results showed that the lines WR2, KL2, and Buleleng Abang had the most potential for bioethanol production with volumes of 1090.82 l ha^{-1}, 1116.50 l ha^{-1}, and 961.08 l ha^{-1}, respectively. The juice of sweet sorghum can yield higher ethanol than bagasse. Several morphological characteristics exhibit differences that can aid the selection process for high bioethanol potential lines. These results indicate that sweet sorghum can be developed as a raw material for bioethanol, but further multi-location trials are needed to ensure genotypic stability and sustainability.
Keywords: bioethanol; genotype; sweet sorghum

甜高粱（高粱(L.)莫恩奇）基因型生物乙醇产量潜力的评估

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摘要:

生物乙醇是药品和食品生产的原材料，也是可再生和可持续的能源。甜高粱具有茎秆糖分积累高、植株生长快、适应性广、投入要求低等特点，具有生产生物乙醇的巨大潜力。本研究的目的是观察甜高粱有前景的品系的形态特征和生物乙醇产量潜力，包括品系15020乙、1115-C、4-183阿贝奥托、AB-6-1-1、EA-13-1-1、吉隆坡2、10(1-1)、23(1-1)、布勒冷阿邦和WR2，以及作为检查的超级1和超级2品种。观察到的变量包括植物生长变量、作物产量成分以及茎汁和甘蔗渣的乙醇产量。结果表明，品系WR2、吉隆坡2和布勒冷阿邦的生物乙醇生产潜力最大，产量分别为1090.82 l ha-1、1116.50 l ha-1和961.08 l ha-1。甜高粱汁比甘蔗渣可产生更高的乙醇。一些形态特征表现出的差异可以帮助高生物乙醇潜力品系的选择过程。这些结果表明，甜高粱可以作为生物乙醇的原料开发，但需要进一步的多地点试验以确保基因型稳定性和可持续性。

关键词：生物乙醇； 基因型; 甜高粱

1 Introduction

Bioethanol is currently used as a raw material in the pharmaceutical industry, as a solvent in the cosmetics industry, and as a biofuel to reduce the use of fossil fuels[1]. The United States, Mexico, and European countries are the world's largest bioethanol producers and continue to promote bioethanol as a fuel oil substitute.

Bioethanol (C_2H_5OH) is a liquid biochemical derived from sugar fermentation and is sourced using microorganisms. The raw material for bioethanol is derived from agricultural products containing sugar and starch and processing agricultural waste and other biomass sources. Three types of bioethanol feedstock, namely sugar extracts, starchy plants, and lignocellulosic materials, are used for this purpose[2,3]. Bioethanol is generally produced from sugarcane molasses and corn kernels. However, ethanol raw materials have recently been produced from cheaper raw materials or non-food sources[4].

Sweet sorghum (*Sorghum bicolor* (L) Moench) is a drought-tolerant C4 plant of the grass family. It is considered a potential bioenergy crop in most tropical and temperate zones of the world[5]. It can be adapted to a variety of temperate and tropical climates as a perennial plant with a short growth cycle, high nitrogen and water use efficiency, high tolerance to environmental stress, and adaptability to marginal soils[6,7].

The use of sweet sorghum (*Sorghum bicolor* L. Moench) as a raw material for mass-produced bioethanol provides a promising opportunity. The characteristics of sweet sorghum, which has high sugar accumulation in stems and seeds, fast plant growth, wide adaptability, and low input requirements, are believed to have great potential as ethanol feedstock. In addition, sweet sorghum in its production process has lower production costs and does not compete with food sources[8,9].

The raw material for ethanol from sweet sorghum comes from biomass, which can be divided into three categories, namely the raw material for brix sugar, which comes from stems (juice), bagasse (cellulose), and grains (starch)[1,3]. Sweet sorghum juice is obtained by squeezing sorghum stems with the main sugar sucrose[9]. On the other hand, the remaining solid after juice extraction (sweet sorghum bagasse) is a lignocellulosic residue whose use as a raw material for bioethanol production is profitable due to the lack of competition with food applications and its relatively high sugar content (such as cellulose and hemicellulose)[9,10].

Indonesia is a tropical area with various natural characteristics. The development of sweet sorghum is generally carried out on land with low fertility levels. Therefore, the agronomic and biochemical characteristics of sweet sorghum make it promising for cultivation in marginal environments. When sweet sorghum grains reach physiological maturity, the total biomass consists of approximately 75% stems, 10% leaves, 5% grains, and 10% roots[11]. The composition of the biomass can vary depending on the sweet sorghum genotype.

This study aims to evaluate promising sweet sorghum lines with bioethanol yield potential by comparing plant morphological characteristics and factors related to bioethanol production potential from the stem juice and bagasse.

The objective of the present study was to evaluate promising lines of sweet sorghum for bioethanol production by comparing them with their whole-crop (juice and bagasse) bioethanol production potential and morphological crop characteristics.

2 Materials and Methods

2.1 Study Area

Field experiments were conducted at the Indonesian Cereal Research Institute (ICERI) in Maros, South Sulawesi, Indonesia, from August to December 2019. Based on data from the Indonesian Meteorology, Climatology, and Geophysics Agency (BMKG) for the research location, the average temperature ranged from 22°C to 33°C, with an average humidity of 60% to 82%. The rainfall was classified as low to medium (51-300 mm) from May to November 2019 and high (300-500 mm) from December 2019 to April 2020.

2.2 Procedures

A month prior to sowing, herbicides were applied to control weeds. The soil was plowed to a depth of 30-40 cm. Each genotype was planted in plot sizes of 4 x 6 m with a planting distance of 70 x 25 cm. Sweet sorghum seeds were planted with two plants per hole, resulting in 36 plots.

Each genotype showed an average of 106.666 plant ha^{-1} each. The average number of stalks of sweet sorghum around 16 stalks m^{-2} was harvested. Base fertilization was performed using NPK fertilizers according to the recommended fertilization guidelines for sweet sorghum, which included 250 kg ha^{-1} (46% N), SP36 100 kg ha^{-1} (36% P2O5), and 100 kg KCl ha^{-1} (60% K2O). Urea was applied twice, and SP36 and KCl were applied once each.

2.2.1 Plant Materials and Experimental Design

The genetic material used in this study consisted of ten sweet sorghum lines, namely 15020B, 1115-C, 4-183ABEOTO, AB-6-1-1, EA-13-1-1, KL2, 10(1-1), 23(1-1), Buleleng Abang, and WR2. Super-1 and Super-2 varieties were included as checks. The genetic materials were obtained from ICERI in Maros, South Sulawesi. The experimental design was a randomized complete block design with 12 sweet sorghum genotypes and three replications, resulting in 36 experimental plots.

2.2.2 The Plant Height, Diameter, and Weight

The sample plants were taken from the two center rows of each genotype, randomly selected from each plot, and manually harvested. The plant height, leaf area, stem diameter, and number of stem nodes were measured 80 days after planting (DAP). The number of leaves and nodes was calculated for each stem. The stem diameter was measured at three locations (top, middle, and bottom) of the stem using a digital calliper. The dry weight of the stems was determined by weighing the dried leaves after oven-drying at 105°C for 48 h or until reaching a constant weight.

2.2.3 The Harvest and Juice Extraction

Harvesting is performed when the plants reach physiological maturity, indicated by a black layer on the sweet sorghum seeds. Six stalks were selected, and their leaves were stripped off. The juice was then extracted using a pressing machine. The volume of juice was measured for each individual stem.

2.2.4 The Stem Sugar Content

The total soluble solid (^{o}Brix) was measured using a hand-held refractometer. The concentrations of glucose, sucrose, fructose, and ethanol were determined using high-pressure liquid chromatography (HPLC). Total sugar as invert (TSAI) was determined using the Luff-Schoorl method by Icumsad $GS1_{/3/4/7/8}$-13.

2.3 Data Analysis

All measurements were collected from the two middle rows of each experimental unit. Data were subjected to analysis of variance (ANOVA) using the general linear model procedures in Excel. Comparison of the means was performed using Tukey's HSD. Means were considered significant at $p < 0.05$.

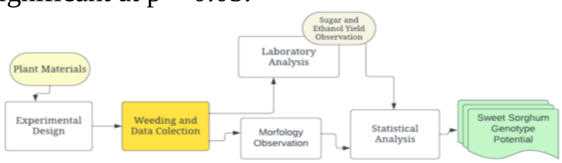

Fig. 1 Flowchart of the research methodology

3 Results and Discussion

3.1 Soil

The soil analysis results of the research site indicate the physicochemical properties of the soil. The soil pH is acidic, the organic carbon (C) content is high, the total nitrogen (N) content is low, and the C/N ratio is considered high. The soil phosphorus (P) content was classified as high, whereas the soil potassium (K) content was considered moderate (Tab. 1).

Tab. 1 Characteristics of the soil at the research site

	Analysis Result	Category
C-Organic (%)	3,55	High
N-total (%)	0,11	Low
C/N	32	High
P_2O_5 (HCl 25%) (mg/kg)	79	High
K_2O (HCl 25%) (cmol/kg)	36	Moderate
pH (H_2O)	4,19	Acidic
KTK (cmol/kg)	31,44	High

3.2 The Plant Height, Diameter, and Number of the Nodes

Genotypic factors showed significant differences in the plant height, leaf area, stem diameter, panicle length, and leaf number.

High plant performance of sweet sorghum is a criterion for selecting it as a raw material for ethanol production[12]. Plant height is in the range of 226.9–367.7 cm (Tab. 2). The Buleleng Abang line has a higher plant height than the Super 1 and Super 2 varieties (check) in this study. This is because of environmental and genetic differences in plants. Plant height is a growth parameter commonly used to determine environmental or genetic influences. Plant height correlates with the ability of plants to accumulate photosynthesis and produce more stem biomass for sugar accumulation[13]. Environmental influences can cause genotypes to express their characteristics. Unsuitable environmental conditions can affect the expression of genetic traits and result in suboptimal performance. Stem diameter varies among different genotypes. Statistical analysis results indicate a significant influence among the lines from early growth to reproductive phases ($p > 0.05$). The stem diameter ranges from 1.79 to 2.44 (Tab. 2). The line Buleleng Abang has a higher stem diameter, approximately 20% and 13% higher than the check varieties, Super 1 and Super 2, respectively. Sweet sorghum's bioethanol production is most promising in stems that produce juice. Therefore, the agronomic characteristic that closely relates to this is the stem diameter. In addition, plants with a larger stem diameter are likely to have better growth. A larger stem diameter provides stronger support to the plant, thereby reducing the chances of lodging. Sweet sorghum produces lower grain yields but due to higher sugar content in its juice and more important yields of green biomass[14].

Tab. 2 Morphological characteristics of the sweet sorghum genotype

Genotype	Plant height (cm)	Leaf area	Number of the leaves	Stem diameter (cm)	Number of the internodes	1000 Seed Weight	Day of flowering (DAT)
15020B	274.1 cd	542.8 abc	13.94 abc	1.87 ab	12.78 abc	29.57	66.7 e
1115-C	226.9 a	560.8 abcd	12.33 a	1.79 a	9.56 a	28.31	63.3 c
4-183ABEOTO	251.2 bc	588.8 abcd	12.78 a	1.87 ab	10.94 ab	31.11	66.7 e
61(1-1)	308.6 ef	601.7 bcd	14.78 abc	2.13 d	12.72 ab	26.22	70.0 g
EA-13-1-1	317.8 f	630.7 d	16.28 bc	2.13 d	14.67 b	27.65	68.3 f
KL 2	299.5 ef	600.1 bcd	12.89 a	2.16 d	13.11 ab	28.69	61.7 b
10 (1-1)	288.8 de	581.7 abcd	14.94 abc	1.72 a	12.28 ab	31.91	65.0 d
23 (1-1)	245 .6 ab	522.8 a	16.61 c	1.8 1ab	12.00 ab	29.85	70.0 g
Buleleng Abang	367.7 g	614.0 cd	21.17 d	2.44 e	16.44 c	27.26	96.7 h
WR-2	294.3 def	538.0 ab	12.83 a	2.22 d	12.22 ab	26.55	60.0 a
Super-1 (check)	290.6 de	554.3 abc	13.56 ab	1.93 bc	12.11 ab	27.38	60.0 a
Super-2 (check)	294.3 def	600.3 bcd	13.28 a	2.11 d	12.28 ab	27.06	70.0 g
Mean	288.3	578.0	14.62	2.00	12,59	29.20	68.50

Note: The numbers followed by the same letter in the same column are not significantly different at the 5% level of Tukey's HSD.

Analysis of variance showed that the number of internodes in the Buleleng Abang line significantly differs from the Super 1 and Super 2 varieties ($p > 0.05$). The highest number of internodes was obtained in Buleleng Abang and the lowest in the 1115-C line. Nodes and internodes in sorghum plants play a role in determining the vertical distribution of leaf area, which is the most important photosynthetic organ that produces carbohydrates. The internode dimensions also play an important role in inducing resistance during the plant's photosynthesis and determining cereal crop biomass and grain yield[15].

3.3 Sweet Sorghum Leaf and Stem Biomass at Harvest

The ability of plants to perform photosynthesis is highly influenced by leaf area. Increasing the leaf area can enhance the interception of solar radiation for photosynthesis. The observation results indicate that the EA-13-1-1 line has a higher leaf area than the Super 1 variety but has the same leaf area as the Super 2 variety (Tab. 2). Leaf area partly determines the photosynthetic rate per unit of a plant, i.e., the information obtained about the plant photosynthesis[16].

Observations on the number of leaves in the plants indicate that the Buleleng Abang line has a higher leaf number than the Super 1 and Super 2 (check) varieties. An increasing leaf number indicates good plant growth. More leaves imply better photosynthesis, which leads to greater production and distribution of assimilates to plant organs. However, an excessively high number of leaves are not always beneficial for plants.

Too many leaves can result in increased transpiration and greater water loss, disrupting photosynthetic activity and leading to uneven distribution of assimilates, with a larger proportion allocated to leaves. Leaves, as the primary organ in photosynthesis, generate assimilates for plant growth and development. Observation of leaves is crucial as a growth indicator and supporting data to explain growth processes, such as plant biomass formation[16] .

Observations of the dry weight of the stems at harvest indicate a decrease in the stem dry weight during the advanced generative phase (hard dough), which is related to the seed-filling phase in sorghum plants. During the seed filling, assimilates are translocated to the generative part (inflorescence). According to Vanderlip[17], approximately 10% of the seed weight comes from reduced stem weight. The decrease in the proportion of leaves and stems during the hard dough phase is also caused by the movement of nutrient compounds from the leaves and stems to the seed[17,18]. The 15020B, 4-183ABEOTO, 61(1-1), EA-13-1-1, KL 2, 10 (1-1), 23 (1-1), and WR 2 lines can have the same stem dry weight as Super 1 and Super 2.

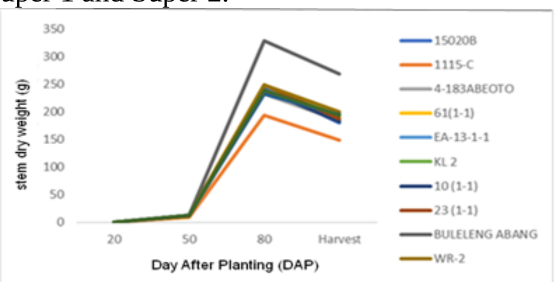

Fig. 2 Sweet sorghum stem dry weight

3.4 Sweet Sorghum Sugar Composition

The results of previous studies indicated that the chemical composition of sweet sorghum and its bioethanol potency varied greatly according to genotype[19–22]. The total soluble solid (TSS) is a reflection of the amount or content of dissolved sugar (solid substances) in a juice solution, which consists of sugar, aconitic acid, and starch, with sugar brix being the largest component[23]. The results of the study indicate that the WR2 line genotype has a higher level of TSS in the stems than the Super 1 and Super 2 varieties. The KL 2, 15020B, 10 (1-1), Buleleng Abang, 4-183ABEOTO, and 23 (1-1) line genotypes can have the same level of soluble sugar as the Super 1 and Super 2 varieties. Although the measurement of sugar brix does not solely represent the measurable sugar content, it can still indicate high ethanol yield potential because the sugar content in brix remains the largest component.

The TSS in the tested genotypes increases with the age of the plants. The soluble sugar content of sweet sorghum ranges from 10.00° Brix to 15.11° Brix. These findings are consistent with the research conducted by Wortmann and Regassa[24], where the sugar content in the stalk is still low during the flowering phase, ranging from 8.3° Brix to 14.0° Brix. However, it increases during the soft dough phase to approximately 12.8° Brix to 16.6° Brix. This is because photosynthates are translocated during the flowering phase for seed formation, leading to the use of some sugar in the sorghum stalk as energy to support plant physiological processes. The TSS in the stem reaches its maximum when sorghum plants have undergone physiological maturity[25].

The TSS reported in this study at harvest is consistent with those reported under various ecological conditions. Reddy et al.[23] reported TSS ranging from 11.2° Brix to 14.2° Brix during the post-rainy season in a semi-arid tropical region for Icsv 700 and Icsv 93046. In a humid subtropical region, Erickson et al.[26] reported values of 12.8° Brix to 16.5° Brix in Florida, USA; Kering et al.[27] reported values of 13.0° Brix to 16.8% in Virginia, USA, for Dale, M81-E, and Top76-6, respectively. The genetic capacity of each genotype and specific soil and climate conditions contribute to the variation in TSS among genotypes[28].

Similarly to sugarcane, sweet sorghum also contains sucrose and glucose/fructose; the proportions vary for each genotype. The observations indicate that the KL 2 and WR-2 line genotypes have the potential for sucrose and glucose/fructose content in the juice of the stem, similarly to the control varieties (Super 1 and Super 2). The sucrose content in the juice of the stem ranges from 9.49% to 16.49%, and the glucose/fructose content ranges from 1.70% to 2.65%. The sucrose content in the bagasse shows that the 4-183ABEOTO, EA-13-1-1, 1115-C, 1115-C10 (1-1), and WR-2 lines have the same sucrose level as the Super 1 variety. However, the Buleleng Abang genotype has higher glucose/fructose content than the control varieties. The sucrose content in the stem juice and bagasse, the 10 (1-1), KL2, EA-13-1-1, and 23 (1-1) lines have the same sucrose content as the control varieties. Stem juice is an important part of ethanol production because of its high sugar concentration and ease of fermentation[1].

Sucrose has the largest proportion among the sugars in sweet sorghum. This can be seen in the findings of this study, where the sucrose content in the juice of stem and bagasse of sweet sorghum is higher than the content of glucose/fructose. The same was reported by Khalil et al.[8], where sucrose was identified as the dominant sugar in sweet sorghum. Abazied[29] showed that varieties with high sucrose content tended to have high %TSS and lower reducing sugar content. The difference in composition is attributed to differences in the cultivar and soil types and environmental factors[1].

Tab. 3 Sugar composition of sweet sorghum

Ganotype	Total Soluble Solid (°Brix)	Sugar Content (%)							
		Stem Juice				Baggasse			
		Sucrosa		Glucosa/Fructosa		Sucrosa		Glucosa/Fructosa	
15020B	13.90 bc	8,64	ab	1,70	a	7,50	bcde	1,23	ab
1115-C	10.00 a	7,86	a	1,75	a	8,14	cde	1,32	ab
4-183ABEOTO	11.68 ab	11,18	abcd	2,21	ab	9,50	e	1,51	bcd
61(1-1)	10.50 a	9,49	abc	2,60	b	5,03	a	1,59	abcd
EA-13-1-1	12.06 abc	13,19	cd	2,20	ab	9,33	e	0,95	d
KL 2	15.02 cd	16,39	e	2,63	b	5,18	ab	1,35	abcd
10 (1-1)	12.24 abc	13,56	cd	2,16	ab	5,98	abc	1,74	bcd
23 (1-1)	13.03 abc	12,57	bcd	2,65	b	5,29	ab	1,41	bcd
Buleleng Abang	14.33 bcd	13,62	cd	2,53	b	5,83	ab	1,69	e
WR-2	15.11 d	15,97	e	2,63	b	8,52	de	1,42	a
SUPER 1	12.96 abc	15,64	e	2,58	b	8,25	cde	1,31	abc
SUPER 2	14.08 bcd	14,92	de	2,59	b	6,61	abcd	1,37	abcd
Mean	13.07	12,78		2.35		7,10		1.41	

Note: The numbers followed by the same letter in the same column are not significantly different at the 5% level of Tukey's HSD.

3.5 Total Sugar as Invert (TSAI) and Ethanol Yield

There are six promising lines that have the same TSAI value of sweet sorghum juice as Super 1 and Super 2 varieties, namely the KL2, EA-13-1-1, 10 (1-1), 23 (1-1), Buleleng Abang, and WR2 lines. The TSAI value of the juice of the stem ranges from 13.12% to 19.58%. For bagasse, with TSAI values ranging from 5.86% to 11.08%, the EA-13-1-1 line had the same TSAI potential as the Super 1 variety and higher than the Super 2 variety. In general, the results of this study indicate that the total sugar content derived from the stem juice has higher TSAI than that found in bagasse.

The results of observations of ethanol 70% yield from juice and bagasse found three promising lines that have the potential as raw material for bioethanol, namely lines KL2 (1090.82 l ha^{-1}), WL2 (1102.48 l ha^{-1}), and Buleleng Abang (961.08 l ha^{-1}) (Tab. 4). These quantities were relatively similar to those reported in Indonesia by previous studies for 70% ethanol production of the Numbu, Super 1, and Super 2 varieties (827–1116 l ha^{-1})[30]. The stem juice had greater potential to produce higher bioethanol than bagasse. After juice extraction, bagasse, which is a dry fibrous lignocellulosic material, can be used for cellulosic ethanol, butanol, biomass pellets, and second-generation power[3,31]. The bagasse from line EA-13-1-1 (255.67 l ha^{-1}) exhibits higher ethanol production than the Super 1 and Super 2 varieties. The production of bioethanol from sweet sorghum bagasse (lignocellulose) holds great potential for future development because of its high biomass yield per hectare and lower production costs than bioethanol production from other crops[3].

Tab. 4 Total sugar as invert and ethanol yield of sweet sorghum

Genotype	Total Sugar as Invert (%)		Ethanol yield 70% (l ha^{-1})	
	Stem Juice	Bagasse	Stem Juice	Bagasse
15020B	11.86 ab	8.50 ab	481.97 a	231.67 bc
1115-C	10.94 a	8.42 ab	408.41 a	183.00 abc
4-183ABEOTO	13.42 abcd	11.02 c	534.45 abc	235.33 bc
61(1-1)	13.12 abc	7.62 a	499.53 ab	215.00 abc
EA-13-1-1	16.14 cde	11.08 c	669.16 cd	255.67 d
KL 2	19.58 e	6.20 a	912.15 e	178.67 ab
10 (1-1)	15.78 bcde	7.93 a	485.72 a	201.00 abc

Continuation of Tab. 4

23 (1-1)	17.36 de	6.64 a	644.21 bc	168.00 ab
BULELENG ABANG	16.57 cde	5.86 a	818.75 e	142.33 a
WR-2	19.06 e	10.28 bc	879.48 e	223.00 bc
SUPER 1	18.82 e	10.70 c	837.35 e	215.00 abc
SUPER 2	17.73 e	8.07 a	789.43 de	185.00 abc
Mean	15.86	8.69	663.47	198.64

Note: The numbers followed by the same letter in the same column are not significantly different at the 5% level of Tukey's HSD.

4 Conclusions

The results of this study concluded that the WR2, KL2, and Buleleng Abang lines had the greatest potential for bioethanol production with volumes of 1090.82 l ha^{-1}, 1116.50 l ha^{-1}, and 961.08 l ha^{-1}, respectively. Morphological characteristics and bioethanol production are important indicators for selecting sweet sorghum genotypes with high bioethanol production potential. The juice of sweet sorghum can yield higher bioethanol than bagasse. The efficiency of using sweet sorghum as a raw material for bioethanol can be increased by making better use of bagasse in addition to sweet sorghum juice. Thus, sweet sorghum is profitable for the first- and second-generation bioethanol production. This research is a stage of selecting potential lines of sweet sorghum as raw materials for bioethanol. However, further research is needed in the form of multi-location trials to validate these results and use them in sorghum breeding programs to obtain and develop superior varieties of sweet sorghum with high bioethanol potential.

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