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Agronomic and Bioconversion Assessment of Sweet Sorghum as a Sustainable Bioethanol Feedstock in Northern Kazakhstan

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ABSTRACT

Sugar sorghum is considered one of the promising energy crops due to its high biomass productivity, ability to accumulate soluble sugars, and suitability for bioethanol production under the conditions of Northern Kazakhstan. Field studies were conducted in 2024–2025 using four cultivars of sugar sorghum (Volonter, Kapital, Volzhskoye 51, and Sevilya). Green biomass yield, yield components, soluble sugar content (°Brix), sugar yield, the dynamics of sugar losses during juice storage, and laboratory-scale bioethanol yield were assessed. Two-way analysis of variance (ANOVA) was used to assess the effects of year, cultivar, and their interaction. Green biomass yield varied from 13.03 to 36.77 t/ha, while soluble sugar content ranged from 10.1 to 17.9 °Brix. Green biomass yield was mainly determined by growing-season conditions, whereas sugar accumulation depended to a greater extent on cultivar characteristics. Based on the combination of the studied characteristics, the Sevilya cultivar was identified as the most promising, with a soluble sugar content of 15.2–17.9 °Brix and a sugar yield of 1.04–2.44 t/ha. During storage, sugar losses ranged from 21.8 to 30.7%, with the most intensive reduction occurring within the first 24 hours. Under laboratory conditions, bioethanol yield reached 74.3% of the theoretical yield. These findings provide preliminary evidence for the suitability of sweet sorghum as a bioethanol feedstock under the conditions of Northern Kazakhstan and highlight the importance of selecting high-sugar cultivars and minimizing the time between harvesting and processing.

1. INTRODUCTION

In recent decades, the production of various types of liquid biofuels (biodiesel, biobutanol, bioethanol) has been considered one of the most promising areas for decarbonization of the transport sector and reduction of dependence on fossil energy sources [1, 2]. The development of technologies for processing plant raw materials into bioethanol corresponds to the concept of a circular bioeconomy, which provides for the integrated use of renewable biological resources with the minimization of waste generation and the reduction of greenhouse gas emissions [3]. Despite the steady increase in global bioethanol production, the reliance on conventional feedstocks, particularly corn and sugarcane, remains a subject of considerable scientific debate. The main concerns include competition with the food sector, the need for additional agricultural land, and the associated increase in total CO_2 emissions [4]. For this reason, in recent years there has been a significant increase in interest in specialized energy crops capable of providing high biomass yields with lower resource costs and greater resistance to adverse environmental conditions [5-7].

One of the promising crops in this group is considered to be sweet sorghum (*Sorghum bicolor* (L.) Moench). Its attractiveness is determined by the combination of high productivity, efficient use of moisture, and resistance to drought and soil salinity [8-10]. Unlike most cereal crops, sweet sorghum is grown primarily for its stem biomass, which is rich in soluble sugars [11], making it a versatile energy crop for bioethanol production while reducing the bioenergy sector's dependence on conventional food-based feedstocks [12-14].

Depending on the cultivar and growing conditions, the soluble sugar content in stem juice can exceed 20 °Brix [15, 16]. After juice extraction, a substantial amount of lignocellulosic bagasse remains, which can be used for the production of second-generation bioethanol or other bioproducts, thereby enabling nearly complete utilization of the produced biomass [17, 18]. This type of complex processing scheme is considered one of the advantages of sweet sorghum compared to most alternative energy crops.

The efficiency of bioethanol production is determined not only by the sugar content. After harvest, the concentration of soluble sugars begins to rapidly decrease due to tissue respiration and the development of microorganisms, which can significantly reduce the yield of the final product [19]. Bioethanol production is also influenced by the efficiency of alcoholic fermentation, which depends on the yeast strains used and the fermentation conditions [20]. The use of sweet sorghum for bioethanol production is determined not only by the biological characteristics of the crop but also by a range of factors, including soil and climatic growing conditions, post-harvest sugar retention, and the efficiency of subsequent processing.

Despite the high ecological plasticity of the crop, its wide distribution in northern latitudes is limited by high heat requirements during the growing season [21]. For regions with a short frost-free season, the selection of cultivars that combine early maturity, intensive sugar accumulation, and high biomass productivity is of particular importance. Studies in other regions have demonstrated genotypic variation in biomass and ethanol yield [22]. This study extends existing knowledge by evaluating the adaptation of mid-early cultivars across two contrasting growing seasons in Northern Kazakhstan and linking their field performance with post-harvest sugar retention and laboratory-scale ethanol conversion.

The aim of this study was to evaluate sweet sorghum cultivars as potential bioethanol feedstocks under the conditions of Northern Kazakhstan based on their agronomic performance, soluble sugar content and retention during storage, and laboratory-scale ethanol conversion.

2. MATERIALS AND METHODS

2.1 Experimental conditions

The experiments were carried out over two growing seasons (2024–2025) at an experimental site in the Akmola region of Northern Kazakhstan (Figure 1). The experiment was arranged in a randomized block design with three replicates. Sweet sorghum was sown on June 5, 2024, and May 20, 2025. The area of each experimental plot was 28 m². The seeding rate was set at 15 seeds per 1 m² with a row spacing of 0.70 m and a sowing depth of 4–5 cm. The preceding crop was clean fallow. The experiment was conducted under rainfed conditions without irrigation or fertilizer application. No plant protection products were applied against pests or diseases, as the cultivars were evaluated under natural growing conditions. During the growing season, three inter-row cultivations were carried out to control weeds and maintain optimal soil conditions.

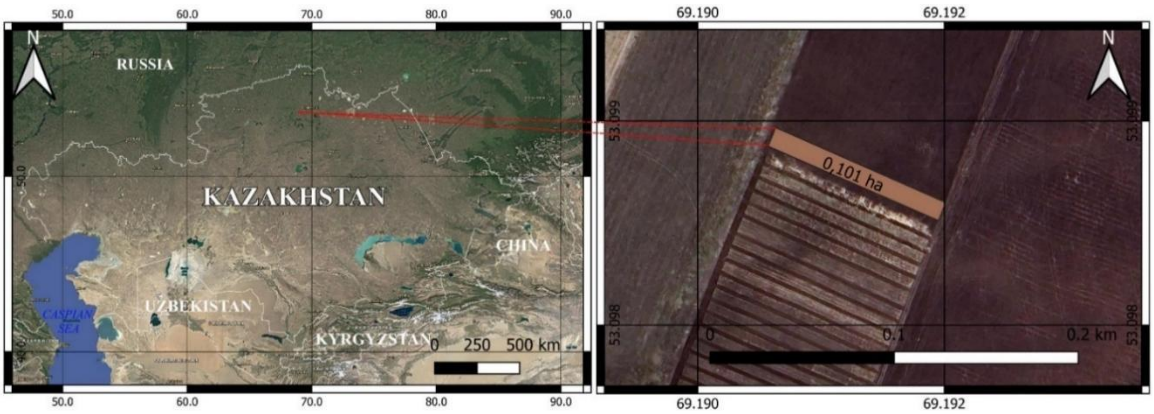

Figure 1. Experimental site location and coordinates (53°05'55.19"N, 69°11'31.44"E; 308 m above sea level)

Weather conditions differed considerably between the two growing seasons (Table 1). Rainfall from May to September totaled 275.4 mm in 2024 and 222.9 mm in 2025. In May 2025, rainfall was only 11.9 mm compared with 65.1 mm in 2024 and the long-term mean of 32.8 mm. Air temperatures from May to July were also higher in 2025. Reductions in rainfall of 53.2 and 21.8 mm in May and July 2025, respectively, coincided with crop establishment and intensive biomass accumulation.

Table 1. Meteorological conditions (2024–2025)

Parameter	May	June	July	August	September
2024					
Temperature (°C)	12.7	18.7	17.8	16.8	10.5
Rainfall (mm)	65.1	65	67.5	56	21.8
2025					
Temperature (°C)	15.1	19.1	19	16.7	11.8
Rainfall (mm)	11.9	73	45.7	51.5	40.8
Long-term mean					
Temperature (°C)	12.2	16.7	18	17.1	12
Rainfall (mm)	32.8	41	57	38.1	21.4

To assess the hydrothermal conditions during the growing season, Selyaninov's hydrothermal coefficient (HTC) was used (Figure 2) and calculated using the following formula:

$$HTC = \Sigma P_5 / (\Sigma T_5 / 10)$$

where, ΣP_5 is the total precipitation during the period with a mean daily air temperature above +5 °C, and ΣT_5 is the sum of mean daily temperatures over the same period [23].

The HTC values further characterized moisture availability during the growing seasons. In 2024, the HTC values varied only slightly during the growing season, indicating a relatively stable moisture regime and favorable conditions for plant growth.

In 2025, considerable fluctuations in moisture availability were observed: HTC was 0.3 in May, corresponding to very dry conditions, increased to 1.3 in June, and then decreased again in July. This pattern indicated uneven moisture availability during plant establishment and intensive biomass accumulation.

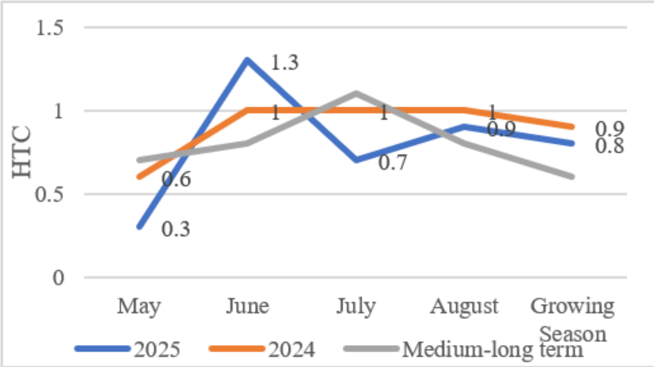

Figure 2. Hydrothermal coefficient (HTC) during the growing season (2024–2025)

Before starting the experiment, soil samples were taken from the 0–20 and 20–40 cm layers to assess the agrochemical properties of the root zone of sweet sorghum. Soil pH was determined in a KCl extract at a soil-to-solution ratio of 1:2.5 using a pH meter. The contents of available phosphorus (P_2O_5) and exchangeable potassium (K_2O) were determined by the Machigin method after extraction with a 1% $(NH_4)_2CO_3$ solution, using colorimetric determination for phosphorus and photometric determination for potassium. Organic matter content was determined by the Tyurin method as modified by Nikitina, based on the oxidation of organic carbon with potassium dichromate ($K_2Cr_2O_7$) in a sulfuric acid medium, followed by photometric determination of the reduced Cr^{3+}. Nitrate nitrogen (NO_3^-–N) content was determined by the Tyurin-Kononova method, based on alkaline hydrolysis followed by photometric determination of ammonium nitrogen.

Table 2. Agrochemical parameters of the experimental plot

Year	Soil Depth, cm	Content, mg/kg			Humus, %	pH
		NO_3^-–N	P_2O_5	K_2O		
2024	0–20	20.6	13.8	526.3	5.7	7.7
	20–40	17.7	13.7	444.0	5.2	7.8
2025	0–20	5.3	16.8	533.6	10.3	7.7
	20–40	5.0	16.8	410.0	8.6	7.9

The soil at the experimental site was a medium-humus chernozem with a 25–27 cm thick humus horizon. The results of the agrochemical analysis indicated low levels of nitrate nitrogen and available phosphorus in the 0–40 cm soil layer, whereas the level of exchangeable potassium was high. The soil reaction was close to neutral, with pH values ranging from 7.7 to 7.9 (Table 2). Humus content was lower in 2024 than in 2025 at both soil depths. Although the experiments were conducted within the same field, different adjacent plot areas were used each year. Therefore, this difference was attributed to spatial soil heterogeneity rather than to an annual increase in humus content.

2.2 Objects of study and analysis of plants

The study included four sweet sorghum (*Sorghum bicolor* (L.) Moench) cultivars: Volonter, Kapital, Volzhskoye 51, and Sevilya (Russia). The cultivars belong to the mid-early maturity group with a growing season of up to 120 days under the conditions of their region of origin.

Green biomass yield was determined by harvesting plants from 5 m² sampling plots, followed by weighing. To determine yield components, the plants were separated into stems, leaves, and inflorescences with seeds. Each fraction was then weighed separately, and its proportion of the total aboveground biomass was calculated. Harvesting was carried out at the milk–wax maturity stage. To determine the absolute dry matter content, plant biomass samples were oven-dried at 65 °C to constant weight. The concentration of soluble sugars in stem juice was determined using the refractometric method. For analysis, 1 mL of freshly extracted juice was applied to the refractometer prism, and the results were expressed in °Brix [24].

2.3 Evaluation of sugar retention and ethanol distillation in laboratory conditions

To assess post-harvest retention of soluble sugars, the stems were separated from the leaves and panicles, and the juice was subsequently extracted using a mechanical press. For each cultivar, one 500 mL bulked juice sample was stored in a single hermetically sealed container at a constant temperature of 26–27 °C. The initial soluble sugar content was determined immediately after juice extraction, and subsequent measurements were taken at 12-hour intervals over the following 10 days of storage. Sugar concentration was determined using the refractometric method and expressed in °Brix. At each storage time point, three technical measurements were performed, and their mean was used for analysis. Independent biological replicates were not included.

Laboratory-scale bioethanol production was carried out using the Sevilya cultivar, with plants harvested at the milk–wax maturity stage in a preliminary single-batch trial without independent fermentation replicates. Juice was extracted from the stems by mechanical pressing and then filtered to remove solid impurities. The initial pH value of the juice was 4.99. Alcoholic fermentation was carried out using dry yeast, *Saccharomyces cerevisiae*. The yeast dosage was 1 g/L of juice. Fermentation was carried out in sealed containers equipped with water seals at a temperature of 28–32 °C for 72 hours. The end of the process was determined by the cessation of carbon dioxide evolution and stabilization of the soluble sugar content. After fermentation was completed, the fermentation broth was filtered again to remove yeast biomass and suspended particles. The clarified fermented juice was subjected to simple distillation in a laboratory distillation apparatus with a condenser. Distillation was carried out at 78–85 °C (Figure 3), and the distillate was collected until ethanol content decreased noticeably. The concentration of ethanol was determined using an alcohol meter at room temperature. Bioethanol yield was calculated in milliliters per liter of fermented juice and was also expressed as a percentage of the initial volume of juice used for fermentation (Figure 3).

2.4 Calculation of theoretical and practical yield of bioethanol

The theoretical and practical bioethanol yields were calculated based on the content of soluble sugars in sweet sorghum juice and the results of laboratory fermentation followed by distillation.

The concentration of sugars in the juice was determined from the Brix value (%) using the conversion factor proposed by Nazli et al. [25]:

$$SC = Brix \times 0.75$$

where,

SC–sugar content, %;

0.75–conversion factor.

The total amount of sugars in the studied volume of juice was calculated using the formula:

$$\text{Sugar (g)} = \text{SC (\%)} \times \text{Juice volume (mL)} / 100$$

The theoretical ethanol yield was calculated based on the stoichiometric equation of alcoholic fermentation: $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$. The calculation was based on the assumption that 1 g of fermentable sugars theoretically yields 0.511 g of ethanol, equivalent to 0.581 mL of absolute ethanol.

$$\text{Theoretical ethanol (mL)} = \text{Sugar (g)} \times 0.581$$

The practical ethanol yield was determined based on the volume of the distillate obtained and the volumetric ethanol concentration:

$$\text{Practical ethanol (mL)} = \text{Distillate volume (mL)} \times \text{Ethanol strength (\%)} / 100$$

Fermentation efficiency was calculated as the ratio of the actual ethanol yield to the theoretical ethanol yield:

$$\text{Efficiency (\%)} = (\text{Practical ethanol} / \text{Theoretical ethanol}) \times 100$$

A B C

Figure 3. Laboratory bioethanol production process: (A) yeast inoculation; (B) fermentation under an airlock; (C) distillation

2.5 Statistical analysis

Statistical processing of experimental data was performed using STATISTICA 10 (StatSoft). Differences among cultivar means within each year were assessed by analysis of variance (ANOVA), followed by Fisher's LSD test at $p < 0.05$. To assess the effects of year, cultivar, and their interaction, two-way ANOVA with the block effect included in the model was used. For each source of variation, the sum of squares (SS), degrees of freedom (df), mean square (MS), and F-statistic (F) were calculated. Statistically significant effects were identified at $p < 0.05$ and marked with an asterisk (*) in the ANOVA tables.

3. RESULTS

3.1 Evaluation of sweet sorghum cultivars

Tables 3 and 4 present the yield indicators and morphobiological characteristics of sweet sorghum cultivars for two growing seasons. In 2024, green biomass yield ranged from 32.07 to 36.77 t/ha, with a mean value of 33.78 t/ha and a range of 4.70 t/ha, indicating relatively low variability in this trait. The highest green biomass yield was recorded for the Volzhskoye 51 cultivar, whereas the other cultivars showed similar values (32.07–34.03 t/ha), indicating a relatively uniform level of productivity. Stem yield ranged from 25.27 to 31.50 t/ha, with a mean value of 27.93 t/ha. The proportion of stems in the total aboveground biomass ranged from 78.8 to 85.7%, with a mean value of 82.6%, confirming the predominance of the stem fraction in yield formation. The Volzhskoye 51 cultivar was characterized by the highest values of these indicators. The high proportion of stems is of great technological importance, since it is in them that the bulk of the cell sap and fermentable sugars, which determine the potential yield of bioethanol, is concentrated.

The soluble sugar content (°Brix) ranged from 10.1 to 15.6% with an average value of 14.1%. The Kapital cultivar had the highest sugar content, while similar values were noted for the Volonter and Sevilya cultivars. Sugar yield varied within the range of 1.91–2.44 t/ha, with an average value of 2.23 t/ha. The Sevilya cultivar provided the highest sugar yield per unit area, which reflects the effective combination of high biomass and intensive sugar accumulation. The Kapital, Volonter, and Sevilya cultivars produced more than 2.0 t/ha of sugar, which allows them to be considered the most promising for bioethanol production under 2024 conditions. At the same time, the Volzhskoye 51 cultivar, with the highest green biomass yield, produced the lowest sugar yield per unit area, which reduces its value as a raw material for bioethanol production. This trade-off was reflected in the theoretical ethanol yield: Volzhskoye 51 had the lowest value (1111.2 L/ha), whereas Sevilya had the highest value (1417.5 L/ha).

In 2025, an overall decline in the productivity of the studied cultivars was observed compared with the previous growing season. The yield of green mass varied from 13.03 to 14.87 t/ha with an average value of 13.88 t/ha (the range of variability of the trait was 1.84 t/ha). The yield of stems was 9.83–11.33 t/ha (on average 10.68 t/ha), and their share in the structure of aboveground biomass was within 75.5–80.4% (on average 77.0%), which is 5.6% lower than the 2024 level on average for the studied samples. Despite the decrease in biomass, the average content of soluble sugars (°Brix) increased to 15.63%, varying from 13.6 to 17.9%. Sugar yield was 0.74–1.04 t/ha, with an average value of 0.89 t/ha. The theoretical ethanol yield ranged from 427.6 to 606.7 L/ha, with the highest value recorded for Sevilya. Considering biomass productivity, soluble sugar content, sugar yield, and theoretical ethanol yield per hectare across both growing seasons, Sevilya was selected for further laboratory evaluation. The results obtained in 2024–2025 indicate pronounced year-to-year variability in the studied traits. The LSD_{05} test revealed significant differences among cultivars for all evaluated traits in 2024. In 2025, significant cultivar differences were observed for stem yield, leaf proportion, soluble sugar content, sugar yield, and theoretical ethanol yield, whereas differences in green biomass yield, stem proportion, and juice yield were not significant.

Table 3. Mean traits of sweet sorghum in 2024

Cultivars	Green Biomass Yield, t/ha	Stem Yield, t/ha	Stem Proportion, %	Leaf Proportion, %	Brix (°Brix)	Juice Yield, t/ha	Sugar Yield, t/ha	Theoretical Ethanol, L/ha
Volonter	32.07	25.27	78.8	9.5	15.3	19.67	2.26	1314.0
Kapital	32.23	26.87	83.3	11.3	15.6	19.81	2.31	1343.7
Volzhskoye 51	36.77	31.50	85.7	8.4	10.1	25.25	1.91	1111.2
Sevilya	34.03	28.07	82.5	10.0	15.2	21.40	2.44	1417.5
LSD_{05}	2.56	2.09	0.12	0.26	2.30	1.60	0.18	106.01

Note: LSD_{05} = least significant difference at the 5% significance level.

Table 4. Mean traits of sweet sorghum in 2025

Cultivars	Green Biomass Yield, t/ha	Stem Yield, t/ha	Stem Proportion, %	Leaf Proportion, %	Brix (°Brix)	Juice Yield, t/ha	Sugar Yield, t/ha	Theoretical Ethanol, L/ha
Volonter	13.03	9.83	75.5	8.2	13.9	7.08	0.74	427.6
Kapital	13.67	10.97	80.4	6.8	17.1	7.85	1.00	583.6
Volzhskoye 51	13.93	10.57	75.9	11.6	13.6	7.49	0.77	445.0
Sevilya	14.87	11.33	76.3	6.4	17.9	7.78	1.04	606.7
LSD_{05}	2.32	1.28	6.75	4.01	1.54	0.90	0.11	65.87

Note: LSD_{05} = least significant difference at the 5% significance level.

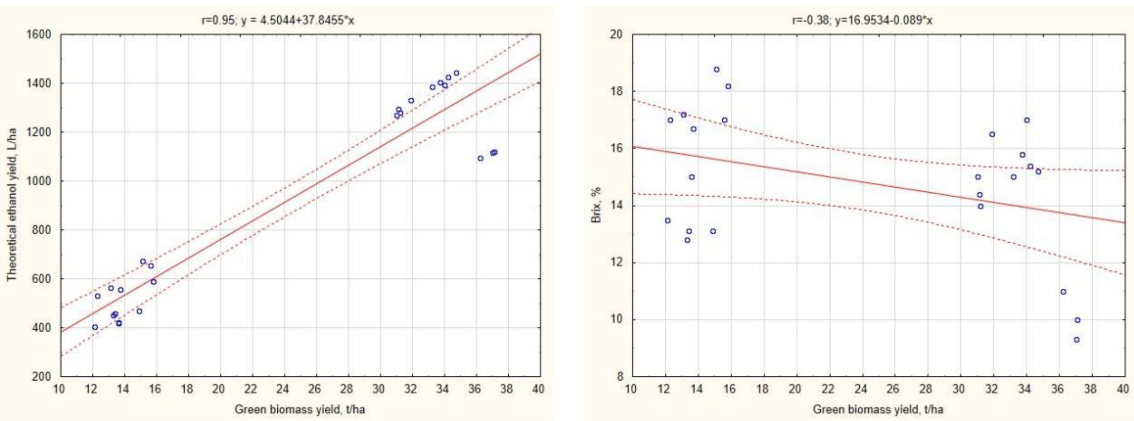

Figure 4. Relationships of green biomass yield with Brix and theoretical ethanol yield in sweet sorghum (2024–2025)

Green biomass yield was strongly correlated with sugar yield and theoretical ethanol yield ($r = 0.95$; $p < 0.001$). These coefficients were identical because theoretical ethanol yield was calculated directly from sugar yield. The relationship between green biomass yield and Brix was negative but not statistically significant ($r = -0.38$; $p = 0.069$) (Figure 4).

ANOVA showed that sweet sorghum green biomass yield was significantly affected by both growing-season conditions and genotype (Table 5; Figure 5). The Year factor had the strongest effect ($F = 1564.16$), indicating that differences between the two study years were the main source of variation in green biomass yield. The Genotype factor also had a statistically significant effect on green biomass yield ($F = 6.72$). A statistically significant Year × Genotype interaction was observed ($F = 3.84$), indicating different responses of the studied cultivars to growing conditions in different years. Figure 5 illustrates the predominant contribution of the Year factor to variation in green biomass yield. Thus, during the two study years, green biomass yield was influenced more strongly by the Year factor than by genotype. The observed reduction in 2025 may be associated with the recorded moisture deficit and temperature conditions; however, this interpretation is based on the available meteorological data and does not represent a validated climate-yield relationship.

In contrast to the yield of green mass, the content of soluble sugars in the juice of sweet sorghum stems was determined mainly by genotypic characteristics (Table 6; Figure 6). The results of two-way ANOVA showed that the Genotype factor had the most pronounced effect on the soluble sugar content (°Brix) (F = 29.15). The Year factor also had a statistically significant effect on this indicator (F = 15.33). A statistically significant Year × Genotype interaction was also observed (F = 7.47), indicating different cultivar responses to growing conditions in different years. Thus, under the conditions of the two study years, soluble sugar content was influenced more strongly by genotype than by growing-season conditions.

Table 5. Results of two-way analysis of variance (ANOVA) of green biomass yield of sweet sorghum (t/ha)

Source	SS	df	MS	F
Total	2446.02	23		
Year	2376.06	1	2376.06	1564.16*
Genotype	30.65	3	10.22	6.72*
Year × Genotype	17.51	3	5.84	3.84*
Error	21.26	14	1.52	

Note: SS = sum of squares; df = degrees of freedom; MS = mean square; F = F-statistic; * p ≤ 0.05.

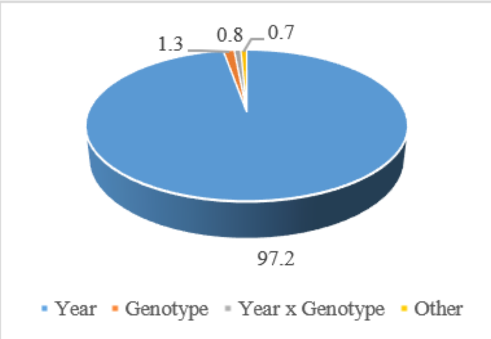

Figure 5. Contributions of environmental and genotypic factors to green biomass yield, averaged across 2024–2025

Table 6. Results of two-way analysis of variance (ANOVA) of soluble sugar content (°Brix) in the juice of sweet sorghum stems

Source	SS	df	MS	F
Total	135.99	23		
Year	14.73	1	14.73	15.33*
Genotype	84.02	3	28.01	29.15*
Year × Genotype	21.54	3	7.18	7.47*
Error	13.44	14	0.96	

Note: SS = sum of squares; df = degrees of freedom; MS = mean square; F = F-statistic.

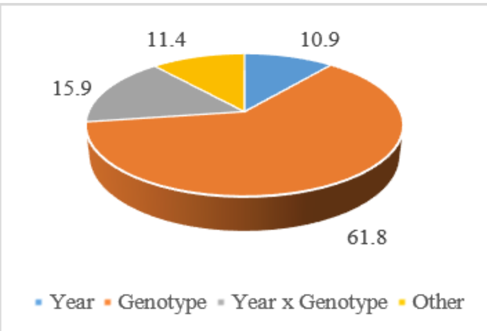

Figure 6. Contributions of environmental and genotypic factors to soluble sugar content (°Brix) in the juice of sweet sorghum stems, averaged across 2024–2025

3.2 Sugar retention in sweet sorghum stem juice during storage

The dynamics of the content of soluble sugars (°Brix) in the juice of sweet sorghum stems during storage are shown in Figure 7. Studies conducted in 2024 with measurement intervals of 12 hours over 10 days showed a gradual decrease in the content of soluble sugars in all samples. Moreover, the rate of change of the indicator differed between cultivars, which indicates a genotypic influence on the preservation of sugars. At the beginning of the experiment, the highest amount of soluble sugars was recorded in the cultivars Kapital (15.6%), Volonter (15.3%), and Sevilya (15.2%), while the Volzhskoye 51 was characterized by a lower sugar content (10.1%).

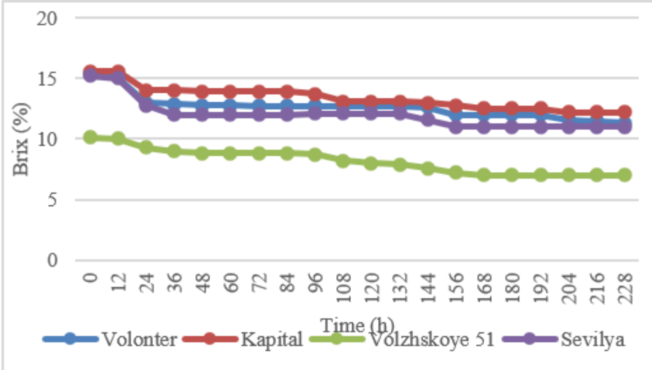

Figure 7. Dynamics of the content of soluble sugars in the juice of sweet sorghum stems during storage

Analysis of changes in sugar content made it possible to identify several stages of their degradation. The most intense reduction was observed in the first 24–36 hours of storage. During this period, the amount of soluble sugars in the Sevilya cultivar decreased from 15.2 to 12.0%, while in the Kapital cultivar the decrease was less pronounced, from 15.6 to 14.0%. Subsequently (48–132 hours), the rate of change of the indicator decreased significantly. The Kapital and Volonter cultivars showed the highest stability, with soluble sugar content remaining within the ranges of 13.1–13.9% and 12.7–12.8%, respectively. At the final stage of storage (144–228 h), a further increase in the rate of sugar loss was observed.

By the end of the experiment, the Kapital cultivar showed the highest retention of soluble sugars (12.2%), with a total decrease in sugar content of 21.8% relative to the initial value. In the Volonter and Sevilya cultivars, sugar losses reached 26.1% and 27.6%, respectively, whereas the highest losses were recorded for Volzhskoye 51 (30.7%). Thus, a gradual decline in soluble sugar content was observed in all cultivars, indicating the need to minimize the time between juice extraction and subsequent processing.

To assess the effects of cultivar and storage duration on the soluble sugar content of juice extracted from sweet sorghum stems, linear regression analysis was performed (Figure 8). The resulting linear relationship was described by the equation: $y = -0.0132x + 13.059$. The negative regression coefficient indicates a consistent decrease in soluble sugar content during storage. According to the obtained equation, after every 100 hours of storage, the Brix content (%) decreases by an average of 1.32%. The high coefficient of determination (R^2 = 0.8288) indicates a strong relationship between storage duration and changes in soluble sugar content, with a strong negative correlation (r = -0.91).

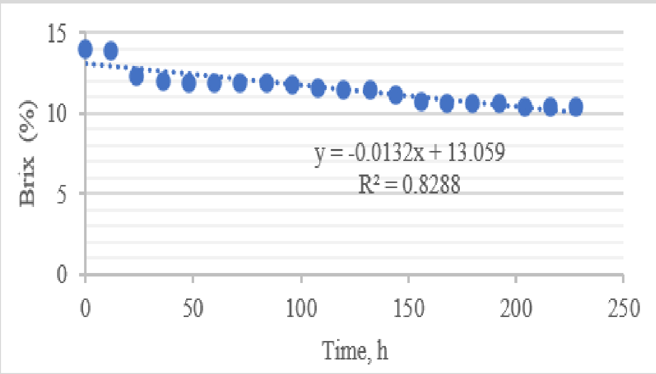

Figure 8. Scatter plot of Brix (%) vs. Time (h) (F_{05} = 43, 89*)

Note: * $p \leq 0.05$.

The results indicate that sugar degradation varied with storage duration and among the studied cultivars. However, the absence of independent biological replicates limits conclusions regarding genotypic differences in sugar degradation.

3.3 Distillation of ethanol in laboratory conditions

Table 7 presents the calculated and experimental indicators of bioethanol yield from the juice of sweet sorghum of the Sevilya cultivar. Sevilya was selected because it produced the highest sugar yield per unit area in both growing seasons. This preliminary single-batch laboratory trial was intended to demonstrate ethanol conversion using one selected cultivar rather than to compare conversion efficiency among cultivars. With a soluble solids content of 17.9 (°Brix) in 500 mL of wort, the amount of fermentable sugars was 67 g, which corresponded to a theoretical yield of 38.9 mL of absolute ethanol. Fermentation followed by distillation yielded 38 mL of distillate with an ethanol concentration of 76%, corresponding to 28.9 mL of absolute ethanol. Fermentation efficiency was 74.3% of the theoretical ethanol yield, while the difference between the theoretical and actual bioethanol yields amounted to 10.0 mL. The observed difference indicates losses during fermentation and distillation and suggests potential for further optimization of the laboratory process. These results represent a preliminary assessment of ethanol conversion for Sevilya and do not demonstrate its superiority in fermentation efficiency over the other cultivars.

Table 7. Theoretical and practical ethanol yield from Sevilya sorghum juice

°Brix	Juice Volume, mL	Sugar, g	Theoretical Ethanol, mL	Distillate, mL	Ethanol Strength, %	Practical Ethanol, mL	Efficiency, %
17.9	500	67	38.9	38	76	28.9	74.3

Applying the conversion efficiency of 74.3% to estimated ethanol yields of 1053.2 L/ha in 2024 and 450.8 L/ha in 2025. Estimated losses relative to the theoretical yield were 364.3 L/ha and 155.9 L/ha, respectively. These values are calculated estimates rather than directly measured field-scale yields.

4. DISCUSSION

The obtained results indicate genotypic variability of sweet sorghum for a complex of economically valuable traits, including biomass productivity, morphological characteristics, and the content of soluble sugars in stem juice. The soluble sugar content in the present study ranged from 10.1 to 17.9 °Brix, which is consistent with published data indicating significant variability in this parameter depending on the cultivar and growing conditions [11, 26]. In this study, the cultivars Kapital, Sevilya, and Volonter were characterized by the highest sugar content, which confirms their potential for use in bioenergy under similar soil and climatic conditions.

When assessing the suitability of sweet sorghum for bioethanol production, not only the total biomass yield but also its structure is important. Since the majority of soluble sugars are concentrated in the stems, increasing their proportion contributes to an increase in the potential yield of juice and bioethanol [27]. According to literature data, stems can form about 80% of the total above-ground biomass of sweet sorghum [28, 29]. In the present study, the proportion of stems ranged from 75.5 to 85.7%, which is consistent with previously published data. The strong positive relationship of green biomass yield with sugar yield and theoretical ethanol yield indicates that biomass productivity substantially influenced ethanol output per unit area. However, the non-significant negative relationship between green biomass yield and Brix shows that higher biomass did not necessarily result in greater sugar concentration. Shukla et al. [30] similarly found that greater plant height was not necessarily associated with increased sugar accumulation in sweet sorghum stems. Therefore, cultivar selection for bioethanol production should consider the balance between biomass productivity and Brix under specific growing conditions.

Analysis of variance showed that soluble sugar content was significantly affected by both genotype and growing-season conditions, with the Genotype factor having a stronger effect. A significant Year × Genotype interaction was established, which indicates different responses of the studied cultivars to the conditions of the growing season. In contrast, green biomass yield was predominantly affected by the Year factor, although the effects of Genotype and the Year × Genotype interaction were also statistically significant. The results obtained are consistent with previous studies indicating that sugar accumulation is largely determined by the genotypic characteristics of plants [31], whereas biomass yield is primarily influenced by growing conditions [32]. In addition to weather conditions, soil nutrient status may have contributed to the year-to-year differences in biomass production. In particular, the lower nitrate nitrogen content in 2025 than in 2024 (Table 1) may have further limited vegetative growth and biomass accumulation, since nitrogen plays an important role in sweet sorghum growth and productivity [25, 33]. Phosphorus and potassium contents showed comparatively smaller changes between the two years. However, because all cultivars were evaluated at the same experimental site under identical growing conditions, soil nutrient status cannot explain the observed differences among cultivars.

The retention of soluble sugars after harvesting is of practical importance for bioethanol production. During sweet sorghum storage, soluble sugar content gradually decreases due to tissue respiration and microbial growth, resulting in a reduction in the amount of fermentable carbohydrates [19]. In the present study, total sugar losses ranged from 21.8 to 30.7%, with the most pronounced losses occurring within the first 24 h of storage, which is consistent with previously published data [34]. The preliminary results indicate a general decline in sugar content during juice storage and highlight the importance of minimizing the interval between harvesting and processing. However, because independent biological replicates were not included, the observed differences among cultivars should be considered preliminary and cannot support firm conclusions about genotypic differences in sugar degradation.

In the preliminary trial, ethanol conversion efficiency reached 74.3% of the theoretical yield, which is consistent with previously published data on the fermentation of sweet sorghum juice. Depending on the yeast strain used and the process conditions, the efficiency of sugar conversion to ethanol can range from 61.9 to 98.9% [20]. The value obtained falls within the range typical of sugar-containing feedstocks and is comparable to the values achieved in bioethanol production from sugarcane (*Saccharum officinarum*) juice and sugar beet (*Beta vulgaris*) [35]. The 25.7% difference between theoretical and actual yields may be attributed to incomplete conversion of fermentable sugars by yeast and losses during distillation. Consequently, practical ethanol yield per hectare is expected to be lower than the theoretical value, indicating the need to optimize fermentation and distillation conditions. Because the experiment involved one cultivar and a single batch without independent fermentation replicates, its reproducibility and differences in conversion efficiency among cultivars could not be evaluated.

5. CONCLUSIONS

The preliminary findings indicate the suitability of sweet sorghum as a bioethanol feedstock in Northern Kazakhstan. During the two study years, green biomass yield was influenced more strongly by the Year factor, whereas soluble sugar content was influenced more strongly by genotype. Among the studied cultivars, Sevilya was distinguished, combining a soluble sugar content of 15.2–17.9 °Brix and a sugar yield of 1.04–2.44 t/ha. In the storage experiment, soluble sugar content gradually decreased (r = -0.91), with the most intensive losses occurring within the first 24 hours and total losses ranging from 21.8 to 30.7% over 10 days, supporting timely feedstock processing, although cultivar-specific differences require confirmation using independent biological replicates. The single-batch fermentation trial using Sevilya juice achieved 74.3% of the theoretical ethanol yield; however, conversion efficiency was not compared among cultivars. Future studies should evaluate whether cultivar selection, reduced feedstock storage time, and optimization of fermentation and distillation conditions can improve bioethanol yield.

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