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Comparative Study of Sugar Beet Genotypes and Fermentative Microorganisms for Ethanol Yield Enhancement

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Abstract

Beta vulgaris L. (Sugarbeet) is a biofuel crop due to its high sugar content. Morpho-physiological and biochemical composition of sugarbeet genotypes influences the quality and output of ethanol. This study evaluates the morpho-physiological and biochemical parameters of different sugar beet genotypes (LS-6, IISR Comp-1, Shubra, LKC 2006, LKC 2010, SZ-35, and PAC 60006) for their potential in bioethanol production. Significant variations in root length, leaf number, fresh weight, and biochemical components such as sodium (Na^+), potassium (K^+), and alpha-amino nitrogen were observed. LKC 2006 emerged as the top performer in terms of root development and biomass accumulation, while SZ-35 and PAC 60006 showed efficient fermentation with *Saccharomyces cerevisiae* and *Enterobacter hormaechei*. Ethanol yield analysis revealed that Shubra and LKC 2010 were the highest producers in both alcoholic (AL) and bulk (BL) liters, with *Enterobacter hormaechei* proving the most effective fermentation agent. These findings highlight the crucial role of genotype selection and microbial strains in optimizing bioethanol production, offering insights into the biofuel industry's efficiency improvements.

Keywords: Sugar beet; Ethanol Production; Fermentation Efficiency; Sugar; Bio-ethanol

Introduction

In today's energy landscape, bioethanol plays a crucial role as a renewable fuel alternative, helping to tackle environmental challenges and promote energy sustainability. The production of bioethanol from agricultural waste reduces reliance on fossil fuels and lowers carbon emissions by approximately 80% compared to traditional fossil fuels (Segovia et al., 2022). The addition of bioethanol to gasoline enhances fuel quality and reduces manufacturing costs (Gaith et al., 2024). The global bioethanol market is expected to experience significant growth due to its increasing affordability and widespread use across various industries (Ifeanyi et al., 2023). Bioethanol reduces dependence on volatile fossil fuel markets by providing a stable and renewable energy source (Abdulsalam et al., 2024). The use of bioethanol in energy production is further enhanced by its role as a fundamental molecule for the synthesis of petrochemicals (Anekwe et al., 2023). In India, sugar and ethanol production relies entirely on sugarcane, placing immense pressure on the crop to meet the high demands of the population (Mall et al., 2018a). Introducing sugar beet as a complementary crop can help alleviate this burden and support future requirements (Mall et

al., 2018b).Sugar beet is grown across 41 countries worldwide, bridging a total area of 8.1 million hectares (Mehdikhani et al. 2011; Kumar et al., 2022). The top ten sugar beet-producing countries include the Russian Federation, France, Germany, the United States, Turkey, Poland, China, Egypt, Ukraine, and the United Kingdom (FAO 2019). The annual use of sugar beet has been enhanced by approximately 1.5% predominantly in countries where the population is more such as India and China (Biancardi et al., 2010). According to USDA (2008), sugar beet accounts for 40% of the global sugar trade. However, it has been reported that sugar beet contributes approximately 30% to global sugar production (Pan et al. 2019). The domestication of sugarbeet as a leafy vegetable and a root crop dates back to prehistoric times. However, its cultivation as a commercial crop is relatively recent in India (Panella and Kaffka, 2011). Sugar beet is primarily a temperate crop, but it has recently been successfully adapted for cultivation in tropical countries like India (Ford-Lyod and Williams 1975). This crop shows significant potential in Indian agro-climatic conditions, driven by the growing demand for bioethanol and sugar (Pathak et al., 2017).

Sugar beet not only contributes to sustainable energy initiatives but also provides economic benefits through integrated bioprocess systems that optimize resource utilization (Mario et al., 2024). Due to its high sugar content, it is one of the key crops used in biofuel production. In the United States and Europe, sugar beet is currently being utilized to produce bioethanol (Lakshana et al., 2022). In India, Shree Renuka Sugars in Karnataka processes sugar beet juice specifically for the production of bioethanol (Pathak et al., 2014). Although sugarcane is also used for bioethanol production, sugar beet offers several advantages, including a shorter lifespan, higher sucrose content, greater temperature tolerance, and resistance to saline and alkaline conditions. As bioethanol continues to gain importance in our daily lives, these benefits make sugar beet an attractive alternative for sustainable biofuel production (Mall et al., 2021).

Scientist have reported significant differences in bioethanol production across various sugar beet genotypes, concluding that those with higher root yield and sugar content are more promising for ethanol production (Srivastava et al., 2008). Kosaric et al. (1983) compared two types of yeast, *Saccharomyces cerevisiae* and *Saccharomyces diastaticus*, for bioethanol production from fresh sugar beet roots and reported that *Saccharomyces cerevisiae* have greater efficiency in ethanol production. From our previous study, it has been reported that among 13 sugarbeet root endophytes *Enterobacter hormaechei* and *Enterobacter cloacae* have greater fermentaion potential (Srivastava et al., 2024). Pati et al., 2022 have also reported for the first time that *Enterobacter hormaechei* RF2 is highly ethanol-tolerant and genetically stable, making it a promising bioethanol producer. Indeed, ethanol production from renewable materials and biomass largely depends on the physico-chemical properties of the sugarbeet genotypes, the efficiency of microorganisms, and fermentation conditions such as initial sugar concentration, pH, temperature, microbial density, and fermentation time (Zhan et al., 2003).

Therefore, the study aimed to compare and assess the ethanol production potential of endophytes isolated in a previous study with the standard *Saccharomyces cerevisiae* strain. Additionally, the study sought to evaluate the effect of various sugar beet genotypes and their related characteristics on ethanol production, with the goal of identifying high-yielding sugar beet genotypes and their corresponding microbial strains.

Materials and Method

Plant Materials and experimental design:

The experimental material comprised of 7 sugarbeet genotypes/varieties (LS-6, IISR comp-1, LKC-2006, SZ-35, PAC 6006, Shubhra and LKC-2010). The field experiment was conducted at the Indian Institute of Sugarcane Research farm over two consecutive years, from November 2017 to November 2018, with the crop being harvested between April and May. Distilled water was used to incubate the seeds in a shaker for 24 hours at 130 rpm in order to promote germination and eliminate germination inhibitors from the seed coats. All the seven varieties of sugar beet seeds were selected after checking its viability. After selection of suitable seeds each variety was sown in rows with basal dose of fertilizers 40:30:20 (NPK). The seeds were sown with inter row spacing of 50 cm and the spacing between the plants were 15 cm, the design of the field experiment was RBD (random block design). The varieties were grown in experimental field (10 rows of 6m length) under favourable conditions. The crop is grown under assured irrigation conditions with at least 8 irrigations upto maturity and harvest of crop. All the cultural operations were followed to reach the ideal crop stand.

Morpho-physiological characteristics of Sugarbeet genotypes:

Biomass production with respect to morphological components viz. shoot and root were studied at maturation in all varieties. The samples were dried in hot air oven at 70°C and weights of shoot and root were recorded. The data were collected on five randomly selected competitive plants and averaged for root length (cm), shoot length/crown size (cm), shoot fresh weight (Kg), shoot dry weight (gm), root fresh weight (kg), root dry weight (gm).

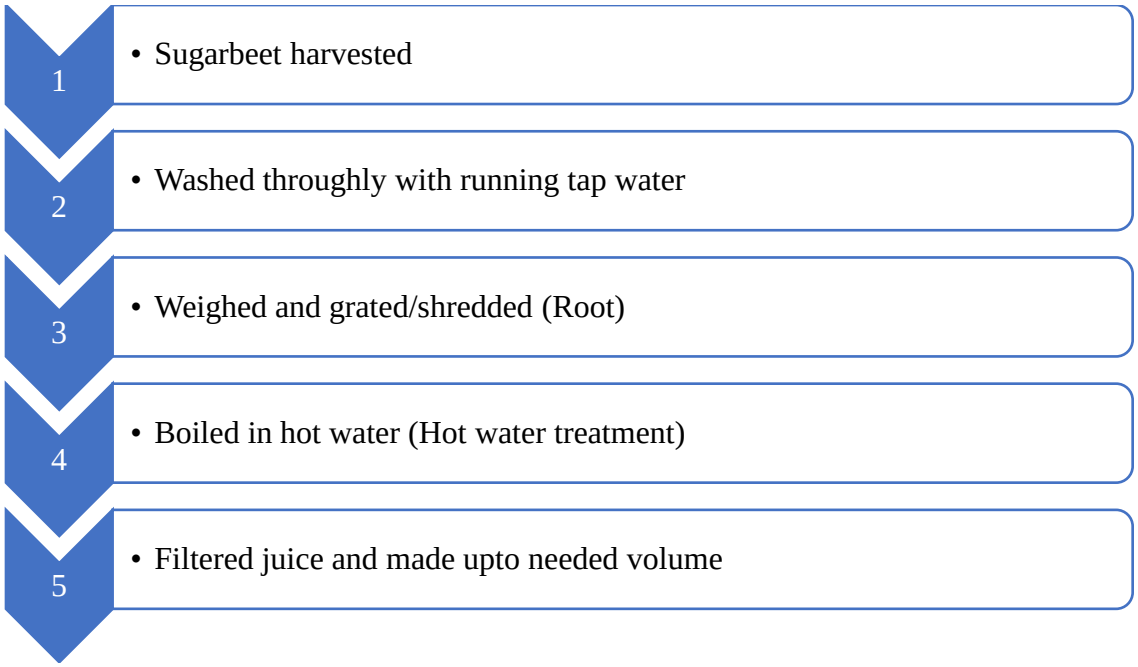
Microorganism's and their fermentation ability:

The sugarbeet roots were harvested during the maturity season to determine the species of microorganism present in sugarbeet root tissue. Fermentation ability of microbes were also analysed and the samples with the highest performance were chosen for further sequencing based on the results and the partial sequence of cloned 16S rRNA genes was sequenced by amplifying the 16S rRNA genes with forward and reverse primers using the BLAST (Basic Local Alignment Search Tool) research tool and published (Srivastava et al., 2024).

Extraction of juice from sugarbeet:

The juice samples were extracted from washed and peeled sugar beet root of all the varieties at maturation stages of growth and development. For preparing sample from all the varieties of sugar beet the juice is extracted from 1 kg of each of the seven shredded sugar beet genotypes, these sugar beet stripes were dipped in water and thermal extraction of juice was done via boiling in the water till they become soft and pulpy. The juice volume was then adjusted to 1.5 liters for subsequent analysis (Mesbahi GR, 2003).

The process for extracting juice from the beets was as follows



Quality indexes/parameters of sugarbeet juices:

Brix (°), sucrose (%), Pol (%), purity (%) in juice and sodium, potassium & alpha amino were recorded. Brix (°) was measured from each plot from fresh roots using hand refractometer. The sucrose percentage in sugar beet root is estimated by polarimetric method (Babaee et al., 2020) process. For Sucrose (%) estimation26 g of fresh shreded sugar beet in sugar flask was taken, then added 177 ml of basic lead acetate solution. Heated on water bath at 70° to 80°C for half hour, cooled and filter with whatman filter paper no.1 to get sugar beet juice for estimation of sucrose. Took the filterate in 200mm polarimetric tube and took the reading for sucrose percentage against reagent blank and purity (%) in juice was computed by using the equation:

Sucrose (%)/Brix (%) × 100

Purity = Pol %/Brix % × 100

Alpha-amino-N is determined by colorimetric method with ninhydrin (Fisher et al., 1963). Took 5 gm of shreded sugar beet and added 100ml of distilled water and homogenised in blender for 3-5 minutes. Then filtered it with whatman filter paper No.1.Took 0.1 ml of clear filterate in dry test tube, and added 1ml of ninhydrin stannous chloride solution (c). Cover with aluminium foil, shake for a while and kept it in boiling water bath for 20 minutes. After the blue colour developed took out the tubes out of boiling water bath and bringed them to room temperature. Then add 5ml of dilute solution (50%) of isopropyl alcohol. Then took the filterate and observed the blue colour using 570mmicron wave length or green filter in a colorimeter against reagent blank.

Potassium is determined by flame photometer (Jankowski et al., 1961). Took 5ml of beet juice extract and diluted it to 50 ml in a volumetric flask. Caliberated the standard potassium solution (50 ppm) and adjusted the galvanometric reading to 100 division using potassium filter. Aspirate the unknown dilutent beet extract and took the galvanometric reading.

Sodium is also determined by flame photometer (Jankowski et al., 1961). Took 5ml of beet juice extract and diluted to 50ml in volumetric flask. Caliberated the standard sodium solution (10ppm) and adjusted the galvanometric reading to 100 divisions using sodium filter. Then aspirated the unknown diluents beet extract and took galvanometric reading.

The reducing sugars were estimated by the method of somogyi (Fales et al., 1961). Suitable aliquots of samples (dilution, if required) were taken in separate test tubes. Distilled water was added to aliquot making final volume of 2.0ml in each test tube. To each tube 2.0ml alkaline copper reagent was mixed and kept in boiling water bath for 10 minutes. After cooling, 2.0ml of arsenomolebedate reagent was added and the volume was made up to 25.0 ml with distilled water in each tube. Absorbance was measured at 540nm against reagent blank.

Total carbohydrate was determined by anthrone method. Took 100mg of sample in a boiling tube and kept it in boiling water bath for 3 hours with 5 ml of 2.5 N-HCL and cooled it to room temperature. Then neutralised it with solid sodium carbonate until effervescence ceases then made the volume to 100ml and centrifuged. After centrifugation collected the supernatant and took 0.5 and 1ml of aliquots for analysis. Prepared standard by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of working standard then made these volumes to 1ml by adding distilled water. Then added 4ml of anthrone reagent in all 8 tubes and kept it on boiling water bath. After cooling read the green to dark green colour at 630nm (Fales et al., 1961). For all of the test, the results were expressed as mg/g for root samples, and mg/ml for juice.

Fermentationof sugar beet juices

Fermentation is a process through which simple sugars can be directly converted into alcohol with the help of fermentable enzymes produced by fermentable microorganisms *Saccharomyces cervisiae*, yeast and two other microorganisms *Enterobacter hormaechie* and *Enterobacter cloacae* isolated from sugarbeet root (Srivastava et al., 2024).

Yeast culture was routinely sub cultured at an interval of 15 days and maintained an YPG (w/v) (yeast extract 1.0%, peptone 2.0%, glucose 2.0% and agar 2.0%) medium at 30°C. The number of viable yeast expressed as colony forming unit per millilitre (108 c.f.u./ml) was estimated.

Fermentation with *S. cervisiae*

The extracted sugarbeet juices so obtained was fortified with ammonium sulphate (2g/litre) and 4 gram/l of di-potassium hydrogen phosphate. To this beet juice we added a strain of *S. cervisiae* (2g/litre) for fermentation maintaining pH 4.8 with hydrochloric acid and kept the medium for fermentation for 72 hours.

Fermentation with *E. hormaecheie*

The cultures of *E. hormaecheiwas* isolated from sugar beet root. The medium (for each 250ml conical flask) contained 100.0 ml of sugarbeet juice of all the varieties were taken. A 5% inoculum (containing 108 cells/ml) was used to inoculate the juice samples. Fermentation process was carried out at 30°C for 72 hours.

Fermentation with *E. cloacae*

Standard culture of *E. cloacea* was obtained from sugarbeet root. The medium (for each 250.0 ml conical flask) contained 100.0 ml sugar beet juice of all the varieties separately. A 5% inoculums (containing 108 cells/ml) was used to inoculate the juice samples. Fermentation process was carried out at 30°C for 72 hours.

Distillation

After fermentation was completed, all ethanol was distilled from the worts using a laboratory distillation setup comprising a distillation flask, a Liebig condenser, a collection flask for ethanol, and a thermometer. The raw spirits, containing 20–23% (v/v) ethanol, were further refined to approximately 43% (v/v) using a distillation apparatus equipped with a bi-rectifier unit and then subjected to chemical analysis.

Statistical analysis:

All the morpho-physiological and quality indexes/parameters measured in the obtained filtrates were analyzed in triplicates. The means, standard deviations, standard error, ANOVA and DMRT for parameter values were calculated. The statistical analysis was conducted using Microsoft Excel 2019 for Windows software and OPSTAT online software.

Results and Discussion

Morpho-Physiological parameters of different genotypes of sugar beet:

Statistical analyses of data in Table (1) indicated significant differences in length, weight and number of leaves per plant. The morpho-physiological parameters of different sugar beet genotypes provide critical insights into their growth potential and adaptability (Figure 1). The data indicates that LKC 2006 outperforms other genotypes across various metrics, including root length, fresh weight, and number of leaves per plant. This genotype's superior root development (26.33 cm) likely contributes to its enhanced nutrient and water uptake, which is crucial for optimal growth (Fageria, 2012).The number of leaves per plant is another vital indicator of photosynthetic capacity, with LS-6 showing the highest count (39 leaves). A greater leaf area generally correlates with increased photosynthesis and biomass accumulation, leading to higher yields (Weraduwage et al., 2015). The relatively lower leaf counts in genotypes like SZ-35 and PAC 600006 may limit their overall productivity, particularly in competitive agricultural environments.Root fresh weight, as observed in LKC 2006 (2.34 kg), signifies the plant's ability to store energy, which is essential for growth and stress response (Wang et al., 2017). The fresh weights of Shubra and LKC 2010 further highlight the importance of selecting genotypes with robust storage capabilities, which can enhance yield potential.Dry weight measurements also play a critical role in evaluating nutrient accumulation and overall plant health. The statistically similar dry weights observed in several genotypes (Shubra, LKC 2006, PAC 600006, and LKC 2010) suggest comparable nutrient storage efficiencies among them, although lower values in SZ-35 and LS-6 indicate potential challenges in nutrient utilization or environmental adaptability (Tayyab et al., 2023).

Moreover, the findings concerning shoot length and fresh weight underscore the significance of biomass in determining overall plant vigor. LKC 2006's remarkable shoot length (10.67 cm) and fresh weight (23.30 g) reinforce its potential as a high-yielding variety (Hussein et al., 2020).

Table 1: Morpho-Physiological parameters of different genotypes of sugar beet:

Sugarbeet genotypes	Root Length (cm)	No. of leaves/ plant	Root fresh weight (Kg)	Root dry weight (kg)	Shoot length (cm)	Shoot fresh weight (gm)	Shoot dry weight (gm)
LS6	25.33 ± 0.26^{ab}	39.00± 0.71^{a}	1.68 ± 0.02^{e}	0.66 ± 0.006^{d}	8.66 ± 0.17^{c}	15.17 ± 0.12^{f}	0.64 ± 0.01^{ab}
IISR Comp-I	23.67 ± 0.12^{c}	27.00± 0.02^{c}	1.77 ± 0.01^{d}	0.77 ± 0.015^{c}	8.33 ± 0.12^{c}	20.10 ± 0.36^{c}	0.62 ± 0.01^{b}
Shubra	25.00 ± 0.27^{b}	23.00± 0.033^{d}	2.12 ± 0.02^{b}	0.98 ± 0.006^{a}	8.67 ± 0.14^{c}	16.07 ± 0.12^{f}	0.63 ± 0.01^{ab}
LKC 2006	26.33 ± 0.26^{a}	26.00± 0.55^{c}	2.34 ± 0.01^{a}	0.97 ± 0.021^{a}	10.67 ± 0.03^{a}	23.30 ± 0.15^{a}	0.69 ± 0.012^{a}
LKC 2010	24.66 ± 0.51b^{c}	33.00± 0.41^{b}	2.05 ± 0.01^{c}	0.93 ± 0.003^{a}	9.33 ± 012^{b}	21.50 ± 0.41^{b}	0.63 ± 0.001^{ab}
SZ -35	22.33 ±	18.00±	1.34 ±	0.87 ±	7.33 ±	17.27 ±	0.58 ±

	0.47^{d}	0.17^{e}	0.01^{g}	0.009^{b}	0.08^{d}	0.29^{e}	0.01^{b}
PAC600006	21.67 ± 0.32^{d}	17.00 ± 0.27^{e}	1.56 ± 0.02^{f}	0.94 ± 0.015^{a}	9.33 ± 0.17^{b}	19.20 ± 0.41^{d}	0.61 ± 0.01^{b}

*Values are mean of values of experiment done in triplicates and ± indicates standard error. Different letters within column indicate significant higher differences as compare to control at ($p \leq 0.05$).

Figure 1: General View of sugarbeet in field

Biochemical content of different types of sugar beet genotypes

Figure 2 illustrates the biochemical composition of various sugar beet genotypes (LS-6, IISR Comp-1, Shubhra, LKC-2006, LKC-2010, SZ-35, and PAC 6006) in terms of sodium (Na^+), potassium (K^+), and alpha-amino nitrogen concentrations expressed in mmol per 100 g of root. Notably, Na^+ concentrations were relatively high across most genotypes, with LS-6, IISR Comp-1, and PAC 6006 exhibiting slightly lower levels compared to others. In contrast, LKC-2010 and SZ-35 displayed the highest Na^+ content, suggesting these genotypes may have superior osmotic adjustment capabilities, which can be crucial for stress tolerance (Lv et al., 2019).

Potassium levels were generally higher than sodium levels across all genotypes, with LKC-2010 and SZ-35 again showing the highest K^+ concentrations. This aligns with findings that potassium plays a vital role in plant physiology, influencing enzyme activation and overall metabolic functions (Pandey et al., 2021; Coelho et al., 2024). LS-6 and IISR Comp-1, having comparatively lower potassium content, may be less efficient in nutrient uptake or retention.

Alpha-amino nitrogen concentrations were consistently lower than both Na^+ and K^+ for all genotypes, with SZ-35 and LKC-2010 exhibiting the highest levels. This observation indicates that these genotypes may have enhanced nitrogen assimilation capabilities, which are essential for protein synthesis and overall plant growth (Ebmeyer et al., 2021). Conversely, LS-6 and PAC 6006 showed the lowest alpha-amino nitrogen levels, potentially impacting their growth and yield negatively.

In summary, LKC-2010 and SZ-35 demonstrate higher nutrient accumulation across all measured biochemical components, indicating their potential for improved growth and resilience. LS-6 and PAC 6006, with consistently lower concentrations, may face challenges in nutrient availability, which could affect their overall performance in agricultural settings (El-Mageed et al., 2022).

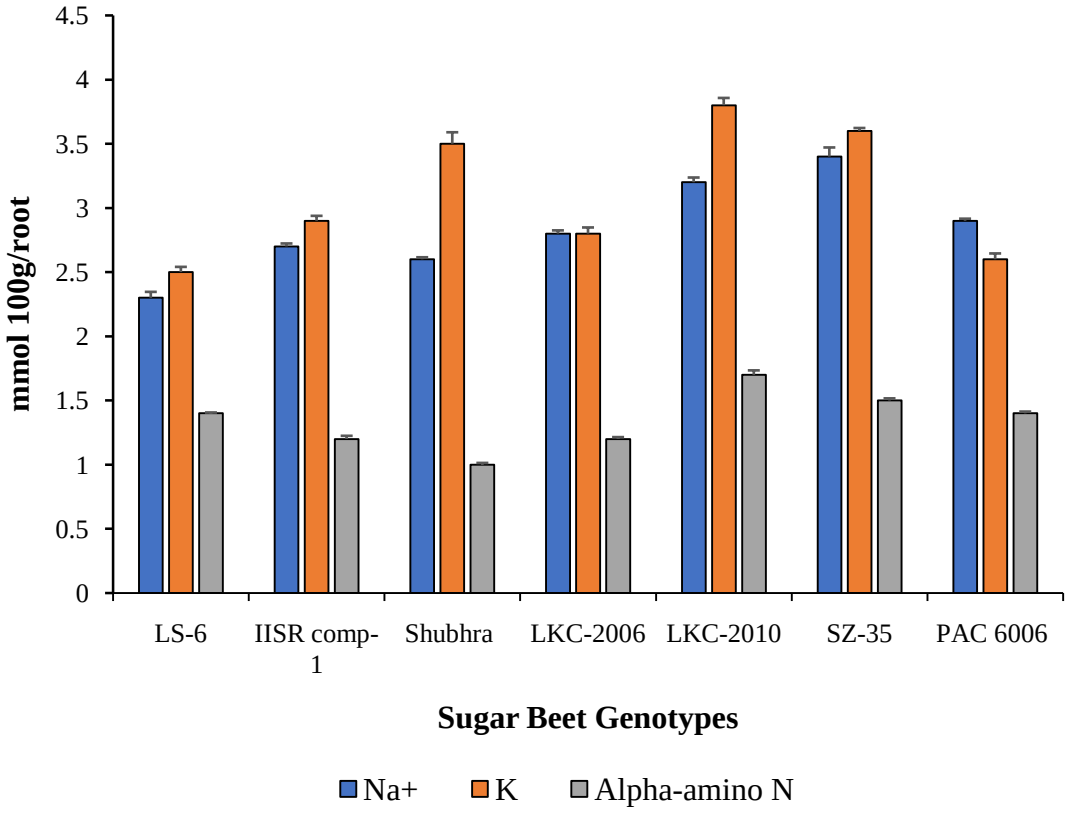


Figure 2: Biochemical content of different types of sugar beet genotypes

Extraction of Juice from sugar beet and evaluation of Brix value, sugar concentrations and ethanol production:

Juice extraction was performed for each of the seven sugar beet genotypes by grating 1 kg of sugar beet. The grated pulp was subjected to boiling water extraction, followed by multiple rinses with hot water to maximize juice recovery. The pulp was then filtered through muslin cloth, and the final juice volume was adjusted to 1.5 liters for consistent analysis across all samples (Table 2).

Brix value (Total Dissolved Solids):

The Brix value, which indicates the concentration of total dissolved solids, showed significant variability among the sugar beet varieties. IISR Comp l had the highest Brix value (22.11), followed by LKC 2010 (20.55), Shubra (19.91), and LS 6 (19.85). SZ 35 had the lowest Brix (15.83). Higher Brix values suggest a higher concentration of sugars, which is advantageous for ethanol production (Islam et al., 2020). IISR Comp l's high Brix (22.11) suggests it has the highest sugar concentration, which would theoretically lead to more efficient ethanol production. The findings highlight the substantial variation in Brix values, sugar concentrations, and ethanol yields among the sugar beet cultivars studied. SZ-35, with the lowest Brix value, and IISR Comp 1, with the highest, demonstrate the range of sugar content that can be expected in different cultivars. This variation significantly impacts the total reducing sugar concentration and, consequently, the fermentable sugar available for ethanol production (Kasegn et al., 2024).

However, its lower actual ethanol yield (7.2% v/v) in table 3 indicates inefficiencies during fermentation, likely due to higher residual sugars (0.50 g/100 ml). SZ 35's lower Brix (15.83) correlates with lower sugar content, which limits its ethanol production potential.

This variability in Brix values aligns with findings by Zabed et al., 2014, who reported that sugar concentration is one of the most important factors affecting ethanol yields from sugar beet varieties. A high Brix value generally translates to more fermentable sugars, but fermentation efficiency and yeast strain performance also play key roles in the final ethanol yield (Gumienna et al., 2014).

Total Reducing Sugar Content

Shubra exhibited the highest total reducing sugar content (16.33 g/100 ml), followed closely by LKC 2010 (16.18 g/100 ml) and PAC 60006 (15.21 g/100 ml). These genotypes were more efficient at converting sugars into fermentable forms, which led to better ethanol yields (Benjamin et al., 2014). LKC 2006, while having lower reducing sugar content (12.52 g/100 ml), had a moderate Brix value (17.95), suggesting a lower overall sugar availability for fermentation (Resende et al., 2018). According to Myat et al. 2016, high reducing sugar content is an important factor for improving ethanol production, as these sugars are more readily fermented by yeast.

Fermentation with *Saccharomyces cerevisiae*:

Residual sugars post-fermentation were minimal across varieties, with LS 6 (0.04 g/100 ml) and SZ 35 (0.03 g/100 ml) being the lowest. Fermentable sugars in the wort ranged from 12.06 g/100 ml (LKC 2006) to 16.10 g/100 ml (Shubra).

Fermentation with *Enterobacter hormaechei*:

Residual sugars were not detected (ND) in LS 6, SZ 35, and PAC 60006. Other varieties showed residual sugars, with LKC 2010 having the highest at 0.44 g/100 ml. Fermentable sugars in the wort ranged from 12.17 g/100 ml (LKC 2006) to 16.14 g/100 ml (Shubra). The fermentation process, using *Enterobacter hormaechei*, revealed differences in residual sugar levels post-fermentation, with LS 6, SZ 35, and PAC 60006 showing no residual sugar, indicating efficient sugar conversion (Zhu et. al., 2023).

Fermentation with *Enterobacter cloacae*:

Residual sugars post-fermentation were lowest in SZ 35 (0.02 g/100 ml) and highest in LKC 2010 (0.50 g/100 ml). Fermentable sugars in the wort varied slightly, ranging from 12.06 g/100 ml (LKC 2006) to 16.12 g/100 ml (Shubra).

Genotype SZ 35 and PAC 60006 demonstrated efficient sugar utilization with minimal residual sugar, particularly with *Saccharomyces cerevisiae* and *Enterobacter hormaechei*. It has been observed that incomplete fermentation is often associated with genotypes that have higher sugar content but lower fermentation efficiency (Stevanato et al., 2019).

Table 2: Brix value (Total Dissolved Solids) and total sugar concentration before fermentation and after fermentation of different varieties of sugar beet.

S. No	Particulars	LS 6	IISR Compl	Shubra	LKC 2006	LKC 2010	SZ 35	PAC 60006
1.	Quantity of Sugar beet	1 Kg	1 Kg	1 Kg	1 Kg	1 Kg	1 Kg	1 Kg
2.	Final Volume of thick Juice	1.5 L	1.5 L	1.5 L	1.5 L	1.5 L	1.5 L	1.5 L
3.	Brix (Total Dissolved Solids)	19.85	22.11	19.91	17.95	20.55	15.83	19.35
4.	Total Reducing Sugar Content of thick juice (g/100 ml)	15.05	13.63	16.33	12.52	16.18	12.36	15.21
Fermentation with Saccharomyces cervisiae								
5.	Residual Sugars after fermentation (g/100 ml)	0.04	0.50	0.23	0.46	0.54	0.03	0.15

6.	Fermentable sugars in wort (g/100 ml)	15.01	13.13	16.10	12.06	15.64	12.33	15.06
Fermentation with *Enterobacter hormaechie*								
7	Residual Sugars after fermentation (g/100 ml)	ND	0.25	0.19	0.35	0.44	ND	ND
8	Fermentable sugars in wort (g/100 ml)	15.05	13.38	16.14	12.17	15.74	12.36	15.21
Fermentation with *Enterobacter cloacae*								
9	Residual Sugars after fermentation (g/100 ml)	0.04	0.49	0.21	0.46	0.50	0.02	0.15
10	Fermentable sugars in wort (g/100 ml)	15.01	13.13	16.12	12.06	15.60	12.32	15.06

*ND-Not detectable

Table 3 Theoretical ethanol percent (v/v) and actual ethanol percent (v/v) of different varieties of sugar beet.

Sl. No	Particulars	**LS 6**	**IISR Comp l**	**Shubra**	**LKC 2006**	**LKC 2010**	**SZ 35**	**PAC6 0006**
1.	Theoretical Ethanol % (v/v)	9.6	8.3	9.9	7.6	9.8	7.56	9.30
2.	Actual Ethanol % (v/v) with *Saccharomyces cervisiae*	7.8	7.2	8.4	6.9	8.2	6.3	8.2
3	Actual Ethanol % (v/v) with *Enterobacter hormaechie*	8.2	7.3	8.6	7.2	8.7	6.5	8.5
4	Actual Ethanol % (v/v) with *Enterobacter cloacae*	7.8	7.2	8.3	6.5	8.1	6.3	7.9

Fermentation efficiency, ethanol (AL) and ethanol (BL) of different varieties of sugar beet

The performance of seven sugar beet genotypes—LS 6, IISR Comp 1, Shubra, LKC 2006, LKC 2010, SZ 35, and PAC 60006—was evaluated across three key parameters: fermentation efficiency, ethanol yield in alcoholic litres (AL) conditions, and ethanol yield in bulk litres (BL) conditions.

Fermentation Efficiency

The efficiency of ethanol production varied across different sugar beet varieties and microbial strains. With *Saccharomyces cerevisiae*, the highest efficiency was observed in LKC-2006 (90.79%), followed by PAC-60006 (88.17%) and IISR Com 1 (86.75%), while the lowest was in LS-6 (81.25%). Similarly, fermentation with *Enterobacter hormaechei* yielded the highest efficiency for LKC-2006 (94.74%), with PAC-60006 (91.40%) and IISR Com 1 (87.95%) also performing well. The lowest efficiency for this strain was recorded for LS-6 (85.42%). In the case of *Enterobacter cloacae*, IISR Com 1 exhibited the highest efficiency (86.75%), followed by PAC-60006 (84.95%) and LKC-2006 (85.53%), while LS-6 again showed the lowest efficiency (81.25%).

Overall, LKC-2006 emerged as the best-performing variety for ethanol production, particularly with *E. hormaechei*, achieving the highest efficiency (94.74%). Conversely, LS-6 consistently exhibited the lowest efficiency across all strains. Notably, PAC-60006 demonstrated strong and consistent performance across all microbial fermentations, with efficiencies exceeding 84%. These results highlight the significant influence of both sugar beet variety and microbial strain on

ethanol production efficiency, with *E. hormaechei* generally providing the best results across most varieties (Figure 3).

Ethanol Yield in AL (Alcoholic litres)

Among the varieties, Shubra showed the highest ethanol yield with all strains, achieving 126 l/ton with *S. cerevisiae*, 129 l/ton with *E. hormaechei*, and 124.5 l/ton with *E. cloacae*. Similarly, LKC-2010 also demonstrated high yields, reaching 123 l/ton with *S. cerevisiae*, 130.5 l/ton with *E. hormaechei* (the highest among all varieties), and 121.5 l/ton with *E. cloacae*.

In contrast, SZ-35 consistently exhibited the lowest ethanol yield, producing only 94.5 l/ton with *S. cerevisiae*, 97.5 l/ton with *E. hormaechei*, and 94.5 l/ton with *E. cloacae*. Similarly, LKC-2006 also showed lower ethanol yields compared to other varieties, especially with E. cloacae (97.5 l/ton).

Overall, the performance of Shubra and LKC-2010 highlights their potential as high-yielding varieties for ethanol production, particularly with *E. hormaechei*, which consistently provided the highest yields across most varieties. The strain *S. cerevisiae* showed slightly lower yields compared to the bacterial strains, while *E. cloacae* offered intermediate performance. This analysis underscores the influence of both microbial strains and sugar beet variety on ethanol yield efficiency (Figure 4).

Ethanol Yield in BL (Bulk litres)

The ethanol yield in BL (l/ton) varied across different sugar beet varieties and microbial strains. Among the varieties, Shubra showed the highest ethanol yield overall, achieving 134.4 l/ton with *S. cerevisiae*, 131.6 l/ton with *E. hormaechei*, and 132.8 l/ton with *E. cloacae*. LKC-2010 also demonstrated consistently high yields, with 131.2 l/ton for *S. cerevisiae*, 131.7 l/ton for *E. hormaechei*, and 129.6 l/ton for *E. cloacae*. Similarly, PAC-60006 performed well, yielding over 126 l/ton across all microbial strains. On the other hand, SZ-35 exhibited the lowest ethanol yields, with 100.8 l/ton for both *S. cerevisiae* and *E. cloacae*, and a slightly higher 129.5 l/ton for *E. hormaechei*. LKC-2006 also produced lower yields compared to other varieties, particularly with *E. cloacae*, where it yielded only 104 l/ton. Overall, *E. hormaechei* showed the highest ethanol yield across most varieties, demonstrating its effectiveness as a fermentation agent. Varieties like Shubra, LKC-2010, and PAC-60006 emerged as top-performing candidates for ethanol production, while SZ-35 and LKC-2006 lagged behind (Figure 5).

Figure 3 Fermentation efficiency of different varieties of sugar beet.

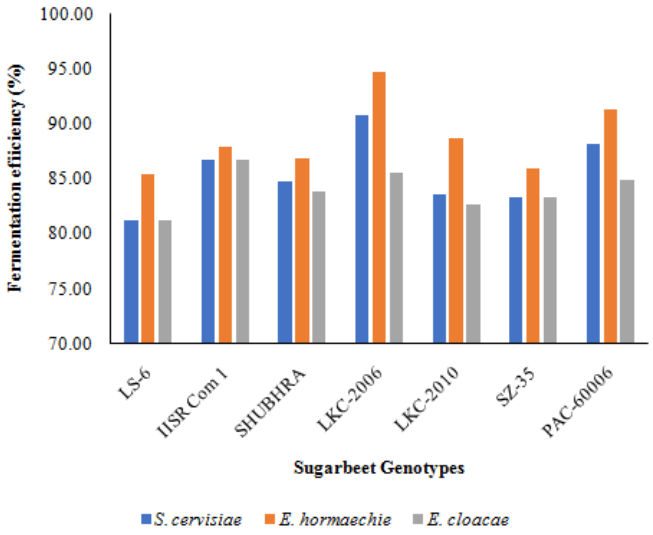


Figure 4 Ethanol yield in alcoholic liter (l/ton) of different varieties of sugar beet.

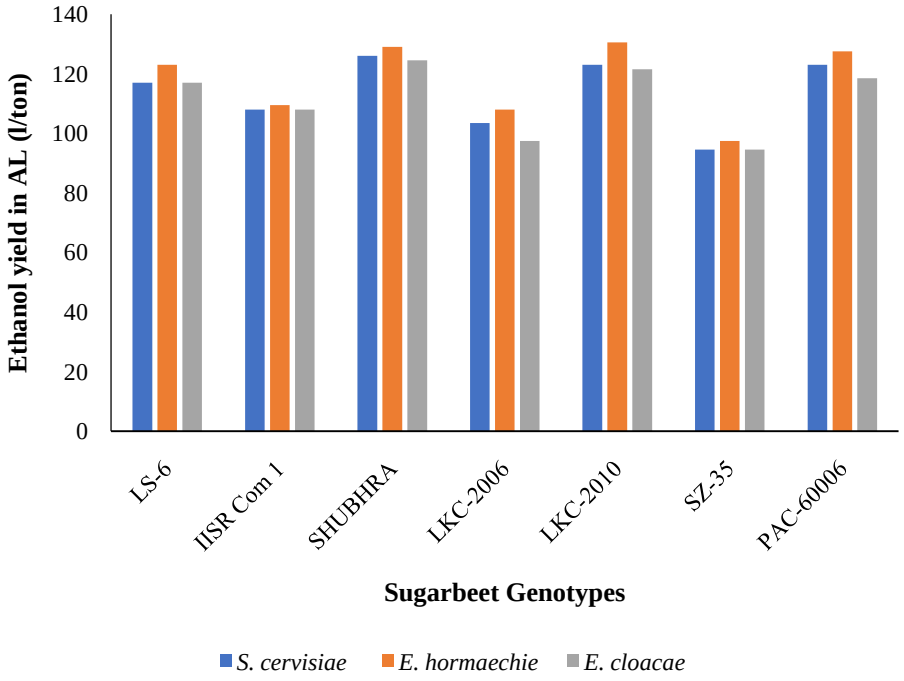
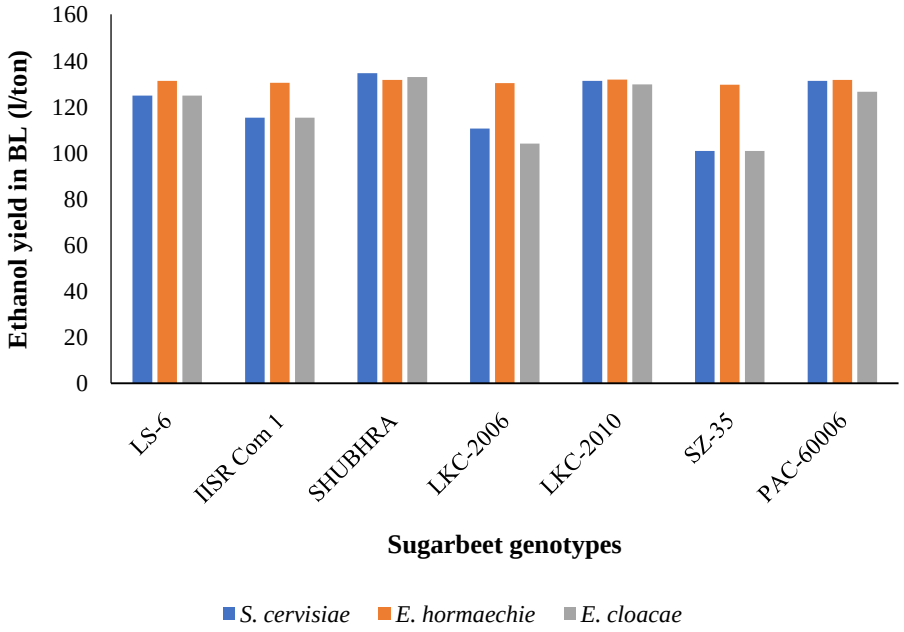


Figure 5: Ethanol yield in bulk liter de (l/ton) of different varieties of sugar beet.



The results highlight significant variations in ethanol production efficiency, ethanol yield in AL (l/ton), and ethanol yield in BL (l/ton) across different sugar beet varieties and microbial strains. Among the tested varieties, Shubra, LKC-2010, and PAC-60006 consistently demonstrated superior performance in terms of ethanol production, with Shubra achieving the highest theoretical ethanol yield and actual ethanol yields across most microbial strains. *Enterobacter hormaechei* emerged as the most effective fermentation agent, delivering the highest ethanol yields in both AL and BL across nearly all varieties, outperforming *Saccharomyces cerevisiae*

and *Enterobacter cloacae*. Conversely, varieties like SZ-35 and LKC-2006 exhibited relatively lower ethanol production and yield, underscoring the importance of variety selection in optimizing fermentation efficiency (Sun et al., 2024).

The findings underscore the combined influence of sugar beet variety and microbial strain on ethanol production. The superior performance of Shubra, LKC-2010, and PAC-60006 with *E. hormaechei* indicates their potential as optimal choices for large-scale bioethanol production. These results provide valuable insights into improving bioethanol yields through strategic selection of sugar beet varieties and fermentation agents, paving the way for enhanced efficiency in the biofuel industry.

The correlation between the biochemical composition, morpho-physiological characteristics, and ethanol production efficiency of sugar beet genotypes reveals distinct relationships that influence their performance as bioethanol feedstocks.

The graph depicting Na^+, K^+, and alpha-amino nitrogen levels shows that genotypes with balanced mineral content and lower alpha-amino nitrogen levels, such as Shubra, LKC-2010, and PAC-60006, generally exhibit higher ethanol yields. Elevated levels of K^+ in genotypes like Shubra and LKC-2010 contribute to better osmotic regulation and fermentation efficiency (Yang et al., 2014). Conversely, SZ-35 displayed lower ethanol yields, correlating with its reduced K^+ and alpha-amino nitrogen levels. Among the genotypes, Shubra, LKC-2006, and LKC-2010 demonstrated superior morpho-physiological traits such as root fresh weight, root dry weight, and shoot fresh weight, all of which support higher sugar content and, consequently, better fermentation potential. For instance:

- LKC-2006 had the highest root fresh weight (2.34 kg) and shoot fresh weight (23.30 g), correlating with its strong ethanol production efficiency and yield.
- Shubra exhibited high root dry weight (0.98 kg) and a balanced Na^+/K+ ratio, enabling better fermentation, as reflected in its high theoretical and actual ethanol yields (Neto et. al., 2017)

In contrast, SZ-35, with its smallest root length (22.33 cm), root weight (1.34 kg fresh, 0.87 kg dry), and shoot parameters, had the lowest ethanol yield across all microbial strains. This poor performance aligns with its unfavorable biochemical profile and limited sugar availability for fermentation (Woo-Yong Song et.al., 2015). The genotypes with higher K^+ levels and superior morpho-physiological attributes, such as Shubra, LKC-2010, and PAC-60006, consistently outperformed others in ethanol yield in both AL and BL. *Enterobacter hormaechei* facilitated the highest ethanol yields across all genotypes, likely due to its better utilization of fermentable sugars. The theoretical and actual ethanol yields further underscore the importance of sugar content in determining ethanol production efficiency (Baral et.al., 2019).

Conversely, genotypes with suboptimal root and shoot biomass, like SZ-35, exhibited reduced ethanol yields, irrespective of the microbial strain. The morphological traits and chemical composition of the beet root samples, influenced by genotype, play a crucial role in ethanol yield, with a stronger correlation observed for chemical composition elements (Orlov et al., 2023).

Conclusion:

The dependence on petroleum-based fossil fuels, which run out quickly trying to keep up with the world's ever rising demands, is a growing worldwide problem today. Additionally, fossil fuels have a direct effect on the atmosphere (Hossain et al., 2023). Fossil fuels have been known to produce greenhouse gas emissions that are bad for the environment. Burning petroleum-based fuels elevates atmospheric CO_2 levels, which directly contributes to global warming (Mofolasayo, 2023). The production of bioethanol from sugar beet by fermentation offers a

practical alternative. To effectively convert sugar beet and its by-products into ethanol, it is crucial to understand the relationships between specific morphological and physiological characteristics and ethanol production. Due to its short development period and high potential for ethanol production, sugar beet is a highly profitable crop for farmers, particularly in areas with saline soils. It also helps with soil reclamation and income diversification (Mall et al., 2022). By decreasing sugar surpluses, raising ethanol yields, and supplying a reliable source of income, using sugar beet for ethanol production can help stabilize the Indian sugar business. The integration of biochemical and morpho-physiological data confirms that sugar beet genotypes like Shubra, LKC-2010, and PAC-60006 are ideal for bioethanol production due to their superior root characteristics, balanced mineral composition, high sugar availability and efficient fermentation performance with *E. hormaechei*. Conversely, genotypes such as SZ-35 and LS-6, with lower biomass and less favorable biochemical profiles, are less suited for ethanol production. The findings of this study underline the importance of selecting sugar beet genotypes with favorable biochemical and morpho-physiological traits for maximizing ethanol production. Their consistent performance across various ethanol production parameters makes them highly desirable for biofuel applications.

These results emphasize the critical role of genotype selection, combined with optimal microbial strains, to enhance fermentation efficiency and achieve sustainable bioethanol production. This study provides valuable insights into the integrated approach needed to optimize sugarbeet characteristics and fermentation processes, paving the way for more efficient biofuel production in the future.

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