# ASSESSMENT OF SUGAR AND LIGNOCELLULOSIC POTENTIAL OF SUGARBEET GENOTYPES FOR IMPROVING BIO-ETHANOL RECOVERY

## \*Sweta Srivastava

Research scholar, Amity Institute of Biotechnology, Amity University <u>sweta.srivastava@gmail.com</u>

## **Rachana Singh**

Assistant Professor, Amity Institute of Biotechnology, Amity University rsingh1@lko.amity.edu

### Seema Paroha

Professor, Department of Biochemistry, National Sugar Institute, Kanpur seemaparoha@rediffmail.com

### **AK Mall**

Principal Scientist, Crop Improvement, Indian Institute of Sugarcane Research, Lucknow <u>ashutoshkumarmall@gmail.com</u>

### A.D. Pathak

Director, Crop Improvement, Indian Institute of Sugarcane Research, Lucknow pathakashwini@rediffmail.com

### Abstract

Beta vulgaris L. converts sun energy to chemical energy. It's a biofuel crop due to its high sugar content. Sugarbeet is a 5-6-month, 20%-sugar tropical crop. Sugarbeet can withstand varied climates and soils. It can cultivate hundreds of hectares of salinity- and frost-resistant land. Firstgeneration bioethanol employs sugar-based raw materials; second-generation uses lignocellulose. Lignocellulosic biomass is the most plentiful renewable resource. lignocellulosic bioethanol might reach 442 billion liters per year. Bioethanol lowers garbage costs. Cheap trash reduces ethanol costs. Sugarbeet root yields 95-100 liters per ton. This project will examine sugar beet genotypes for bioethanol production. Variety affects ethanol production and quality. This experiment at National Sugar Institute employed 7 sugar beet cultivars to generate alcohol and evaluate bioethanol-dependent sugar beet features. Indian Institute of Sugar Cane Research conducted the field trial. Fermentable sugar, dissolved solids, alcohol percentage, and dissolved sugar are biochemical needs. Lab-made ethanol from sugar beets. Total soluble solids were measured using the brix-Sindle method, pol% with a polarimeter, and total reducing sugar with the Lane-Eynon method. Heat-milled Subhra, LKC-2006, LKC-2010, SZ-35, and PAC-60006 Pulp juice produced 1.5 L with distilled water. IISR Comp 1 has 22.12 Brix, SZ-35 15.83, LS-6, Subhra, LKC-2006, and LKC-2010 19.86, 19.92, 17.96, 20.56, and 19.36 Brix. LS-6, IISR Comp I, Subhra, LKC- 2006, LKC-2010, SZ-35, and PAC-60006 contain 15.06, 13.64, 16.34, 12.52, 16.18, 12.37, and 15.21 g/100 ml reducing sugar. LS-6, IISR Comp I, Subhra, LKC-2006, and LKC-2010 have 0, 0.50, 0.23, 0.46, and 0.54 g/100 ml residual sugars. Sugarless wort is in SZ-35 and PAC-60006. LS-6, IISR Comp. L, Subhra, LKC-2006, LKC-2010, SZ-35, and PAC-60006 contain 15.02, 13.14, 16.11, 12.06, 15.64, 12.37, and 15.21 g/100 ml fermentable sugar. Sugar beet bioethanol replaces gasoline. To ferment sugar beet and byproducts into ethanol, comprehend morphological and physiological linkages. This innovative study generates ethanol from fresh sugar beet root. Root quantitative and qualitative features affect ethanol production.

Keywords: Sugar beet; S.cerversiae; Sugar; Lignocellulose and Bio-ethanol

## Introduction

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 (Prasad et al., 2007). 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 (Naik et al., 2010). The political crisis is a major issue with relying on fossil fuels. Therefore, the search for a sustainable and renewable energy source for our industrialized economies and consumer societies is ongoing (Mabee et al., 2005). So, as a sustainable and renewable energy source, bio-ethanol is a desirable choice.

Sugarbeet (Beta vulgaris L.) is a very effective solar energy to chemical energy converter that may be used by people and other animals. Because of its high sugar content, it is one of the most crucial crops used to produce biofuels. In the United States and Europe, sugar beet is currently utilized to produce bioethanol (Anwar et al., 2014). In India, Shree Renuka Sugars in Karnataka processes sugar beet juice specifically for the generation of bioethanol (Pathak et al., 2014). Despite the fact that sugarcane is also used to make bioethanol, sugar beet has a number of advantages over sugarcane, including a shorter lifespan, higher sucrose content, higher temperature tolerance, and resistance to saline and alkaline conditions. The crop also requires a lot less water than sugarcane does. Bio-ethanol is becoming more and more important in our daily lives (Mall et al., 2021).

Because of the ongoing fossil fuel depletion, the economic crisis, and growing environmental concerns, there is an increasing need for bioethanol production from renewable sources for use in transportation. In the transportation industry, it is the most widely used renewable fuel. It is a flexible fuel energy source that can be used either in its pure form or after being blended with gasoline or diesel. It is well knowledge that biomass is used in the manufacturing of ethanol (Formann et al., 2020). A sustainable and "green" bio-source of organic raw materials, India annually produces roughly 625 million tons of biomass from agricultural waste, of which 150 million tones are used for industrial purposes. Costs associated with waste disposal are decreased by producing bioethanol from biomass. Wastes are inexpensive, which lowers the price of producing ethanol as well (Mohanty & Abdullahi, 2016). Since lignocellulosic

#### Ann. For. Res. 65(1): 6936-6946, 2022 ISSN: 18448135, 20652445

biomass is the most plentiful resource that can be reproduced on Earth, it is a desirable material for the production of bioethanol fuel. Bioethanol production from lignocellulosic biomass might reach 442 billion liters annually. Costs associated with waste disposal are decreased by producing bioethanol from biomass. Wastes are inexpensive, which lowers the price of producing ethanol as well. Per ton of root, 95–100 liters of ethanol may be recovered from sugar beet roots (Nair et al., 2022). Hence, the present study focused on the assessment of Sugar and Lignocellulosic Potential of Sugarbeet Genotypes for Improving Bio-Ethanol Recovery.

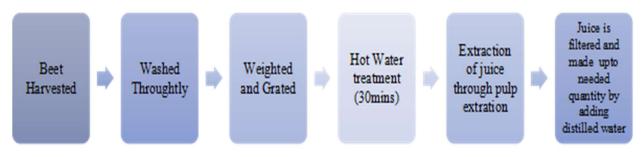
## **Materials and Method**

The type of variety utilized for ethanol production affects both the quality and output of ethanol. This experiment used 7 distinct sugar beet varieties, including LS6, IISR Comp I, SZ-35, PAC-60000, shubra, LKC-2006, and LKC-2010 for the manufacture of alcohol in the current study, to assess some characteristics of varieties of sugar beet that depend on production of bioethanol. At the National Sugar Institute, Kanpur, Uttar Pradesh the study was conducted. At the farm of the Indian Institute of Sugar Cane Research, the field experiment was conducted for a period of one year from November 2017- November 2018. The researched criteria include biochemical characteristics including fermentable sugar, total dissolved solid, alcohol percentage, and total dissolved sugar, among others. In a laboratory, ethanol was made from fresh sugar beet. Beet juice's total soluble solid content was determined using the brix-Sindle method, its pol% content was determined using a polarimeter, and its total reducing sugar content was estimated using the Lane-Eynon method.

## Extraction of juice from beets

To do this, 1 kilogram of grated beet was used, and juice was extracted using boiling water. A muslin cloth filter was used to filter the pulp after it had been rinsed several times in hot water. A total of 5 liters of juice were produced in the end. The extracted juice was used for every analysis.

The juice was expressed from the beets in following manner:



## **Ethanol production**

The pH of the juice was adjusted to 4.5 and sterilized at 15 p.s.i.g for 15 minutes before adding nutrients such ammonium sulphate and potassium hydrogen phosphate. The sugar beet juice was then inoculated with a yeast strain (*Saccharomyces cerevisiae*) that had already been attenuated through a few transfers. Fermentation was therefore conducted for 48 hours, and the cultured wash was evaluated for residual sugars & alcohol concentration in order to quantify fermentation effectiveness and ethanol yield.

## **Results and Discussion**

A 1 kg of each of the seven varieties of sugar beet (LS-6, IISR Comp I, Subhra, LKC-2006, LKC-2010, SZ-35, and PAC-60006) was ground before being treated with hot water. The pulp was then pressed to extract the juice, the volume of the thick juice was increased to 1.5 L by adding distilled water. A hydrometer is used to measure Total Dissolved Solids in Juice (measured as Brix value in degrees), and the results show that IISR Comp l (22.12 degree) and SZ-35 (15.83 degree) have the highest and lowest Brix values, respectively. Other kinds, such as LS-6, Subhra, LKC-2006, LKC-2010, and PAC-60006, have brix values that are different from this one: 19.86, 19.92, 17.96, 20.56, and 19.36, respectively (Table 1).

Juice from the types LS-6, IISR Comp I, Subhra, LKC-2006, LKC-2010, SZ-35, and PAC-60006 has a total reducing sugar concentration of 15.06, 13.64, 16.34, 12.52, 16.18, 12.37, and 15.21 g/100 ml. After filtering, the juice or wort is fermented, and the remaining sugars (in g/100 ml) in the wort of the varieties LS-6, IISR Comp I, Subhra, LKC-2006, and LKC-2010 are 0.04, 0.50, 0.23, 0.46, and 0.54, respectively. The wort of the SZ-35 and PAC-60006 cultivars has no residual sugar. Consequently, the fermentable sugar in various types of wort the respective g/100 ml values for LS-6, IISR Comp. L, Subhra, LKC-2006, LKC-2010, SZ-35, and PAC-60006 are 15.02, 13.14, 16.11, 12.06, 15.64, 12.37, and 15.21.

Table 1: Representing the quantity, final volume of juice and Brix (Total Dissolved Solids) of different varieties (LS-6; IISR Comp l; Subhra; LKC-2006; LKC-2010; SZ-35 and PAC-60006)

S1.	Particulars	LS-6	IISR	Subhra	LKC-	LKC-	SZ-35	PAC-
No			Comp 1	Subira	2006	2010	52 00	60006
1.	Quantity of Sugar beet	1 Kg						
2.	Final Volume of thick Juice	1.5 liters						
3.	Brix (Total Dissolved Solids)	19.86	22.12	19.92	17.96	20.56	15.83	19.36
4.	Total Reducing Sugar Content of thick juice (g/100 ml)	15.06	13.64	16.34	12.52	16.18	12.37	15.21

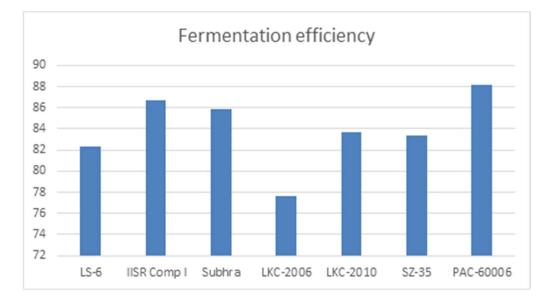
of sugar beet.

5.	Residual Sugars	0.04	0.50	0.23	0.46	0.54	ND	ND
	after							
	fermentation							
	(g/100 ml)							
6.	Fermentable	15.02	13.14	16.11	12.06	15.64	12.37	15.21
	sugars in wort							
	(g/100 ml)							

For various sugar beet cultivars (including LS-6, IISR Comp l, Subhra, LKC-2006, LKC-2010, SZ-35, and PAC-60006), the theoretical and actual ethanol percent (v/v) are, respectively, 9.6, 8.3, 9.9, 7.6, 9.8, 7.56, and 7.9, 7.2, 8.5, 5.9, 8.2, 6.3, and 8.2. (Table 2) (Fig.1)

Table 2: Representing the theoretical ethanol percent (v/v) and actual ethanol percent (v/v) of different varieties (LS-6; IISR Comp 1; Subhra; LKC-2006; LKC-2010; SZ-35 and PAC-60006) of sugar beet.

Sl. No	Particulars	LS-6	IISR Comp l	Subhra	LKC- 2006	LKC- 2010	SZ-35	PAC- 60006
1.	Theoretical Ethanol percent (v/v)	9.6	8.3	9.9	7.6	9.8	7.56	9.30
2.	Actual Ethanol percent (v/v)	7.9	7.2	8.5	5.9	8.2	6.3	8.2



# Fig.1 Representing the fermentation efficiency of different varieties (LS-6; IISR Comp l; Subhra; LKC-2006; LKC-2010; SZ-35 and PAC-60006) of sugar beet.

For various sugar beet types, the ethanol yield is 118.5, 108.0, 127.5, 88.5, 123.0, 94.5, and 123.0 alcoholic liters per ton (fig. 2) While ethanol output in bulk liter age (l/ton) for several sugar beet types is 124.73, 113.68, 134.2, 93.157, 129.4, 99.43, and 129.47 (fig. 3).

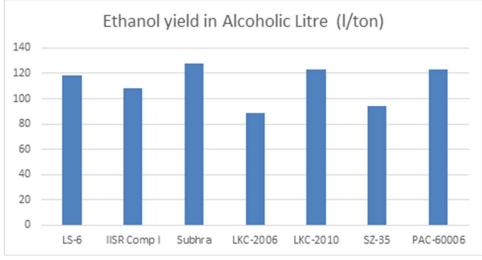


Fig 2 Representing the ethanol yield in alcoholic liter (l/ton) of different varieties (LS-6; IISR Comp l; Subhra; LKC-2006; LKC-2010; SZ-35 and PAC-60006) of sugar beet.

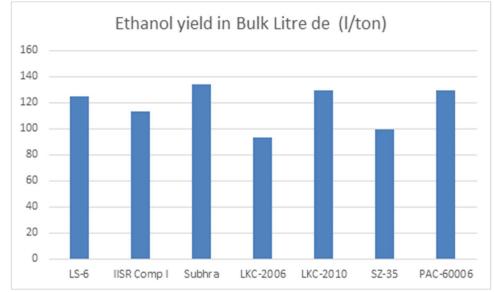


Figure 3: Representing the ethanol yield in bulk liter de (l/ton) of different varieties (LS-6; IISR Comp l; Subhra; LKC-2006; LKC-2010; SZ-35 and PAC-60006) of sugar beet.

The grade of raw sugar produced from the various varieties did not differ, and ethanol production was therefore correlated with the volume of sugar produced for each variety, as per the qualitative examination of the raw sugar acquired from various kinds. The fodder types' impurity levels for potassium and nitrogen were on par with those of other kinds. Regarding the qualities we evaluated, no discernible difference between the two test years was found (Bušić et al., 2018).

S. cerevisiae was used in this work for fermentation, and sucrose, a form of sugar that may be converted to bioethanol, was the sugar used. Therefore, the fresh beet root's cellulose tissue cannot play a significant role in the generation of ethanol (Azhar et al., 2017). This makes sense given the strong link shown in this study between ethanol output and white sugar. Among the 10 beet types, there were variations in ethanol yields ranging from 32 to 43%. Both morphological characteristics and chemical composition of samples of beet root are associated with genotype's influence on ethanol production, with a larger correlation seen for chemical composition factors such impurities and sugar concentration (Duraisam et al., 2017). In contrast to how ethanol production fell as nitrogen and potassium impurities rose, ethanol production rose as sugar content rose. To further assess the influence on ethanol fermentation yields, more study is required, testing a large variety of cultivars under numerous different growth circumstances.

## Conclusion

A viable alternative to gasoline is the fermentation-based manufacture of bioethanol from sugar beet. It is crucial to understand the relationships between a few morphological and physiological features and the generation of ethanol in order to ferment sugar beet and by-products into ethanol. The uniqueness of this study in comparison to prior research is the ability to make ethanol from the freshly available sugar beet root. It was shown that differing ethanol production potentials of various types were highly associated with root qualitative and quantitative features. In fact, the study focused on sugar beet variety development, specifically for the ethanol production. According to a study of the experimental data, fresh root-based ethanol synthesis is more effective than fermentation of raw sugar. The varieties of sugar beet generated more of the ethanol per hectare to that of the kinds of fodder beet among all the variations that were studied. The root yield and sugar content of sugar beet cultivars were higher than those of fodder beet, and these two traits are fundamental to the generation of ethanol. On ethanol yield, which is closely related to chemical makeup of roots, including sugar content, crude syrup purity, potassium impurity and some morphological traits like dry matter and root length, genotype variation impacts have been discovered. Additionally, as sugar content and root output increased, ethanol production did as well.

## References

Prasad, S., Singh, A., Jain, N., & Joshi, H. C. (2007). Ethanol Production from Sweet Sorghum Syrup for Utilization as Automotive Fuel in India. Energy & Fuels, 21(4), 2415–2420. https://doi.org/10.1021/ef060328z

Naik, S. N., Goud, V. V., Rout, P. K., & Dalai, A. K. (2010). Production of first and second generation biofuels: A comprehensive review. Renewable and Sustainable Energy Reviews, 14(2), 578–597. https://doi.org/10.1016/j.rser.2009.10.003

Mabee, W. E., Gregg, D. J., & Saddler, J. N. (2005). Assessing the Emerging Biorefinery Sector in Canada. Applied Biochemistry and Biotechnology, 123(1-3), 0765–0778. https://doi.org/10.1385/abab:123:1-3:0765.

Anwar, Z., Gulfraz, M., & Irshad, M. (2014). Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review. Journal of Radiation Research and Applied Sciences, 7(2), 163–173. <u>https://doi.org/10.1016/j.jrras.2014.02.003</u>.

Pathak, A. D., Kapur, R., Solomon, S., Kumar, R., Srivastava, S., & Singh, P. R. (2014). Sugar Beet: A Historical Perspective in Indian Context. Sugar Tech, 16(2), 125–132. https://doi.org/10.1007/s12355-014-0304-7.

Mall, A. K., Misra, V., Santeshwari, Pathak, A. D., & Srivastava, S. (2021). Sugar Beet Cultivation in India: Prospects for Bio-Ethanol Production and Value-Added Co-Products. Sugar Tech, 23(6), 1218–1234. <u>https://doi.org/10.1007/s12355-021-01007-0</u>.

Formann, S., Hahn, A., Janke, L., Stinner, W., Sträuber, H., Logroño, W., & Nikolausz, M. (2020). Beyond Sugar and Ethanol Production: Value Generation Opportunities Through Sugarcane Residues. Frontiers in Energy Research, 8. <u>https://doi.org/10.3389/fenrg.2020.579577</u>.

Mohanty, B., & Abdullahi, I. I. (2016). Bioethanol Production from Lignocellulosic Waste-A Review. Biosciences, Biotechnology Research Asia, 13(2), 1153–1161. https://doi.org/10.13005/bbra/2146.

Nair, L. G., Agrawal, K., & Verma, P. (2022). An overview of sustainable approaches for bioenergy production from agro-industrial wastes. Energy Nexus, 6, 100086. https://doi.org/10.1016/j.nexus.2022.100086

Bušić, A., Marđetko, N., Kundas, S., Morzak, G., Belskaya, H., Ivančić Šantek, M., Komes, D., Novak, S., & Šantek, B. (2018). Bioethanol Production from Renewable Raw Materials and its Separation and Purification: a Review. Food Technology and Biotechnology, 56(3). https://doi.org/10.17113/ftb.56.03.18.5546.

Mohd Azhar, S. H., Abdulla, R., Jambo, S. A., Marbawi, H., Gansau, J. A., Mohd Faik, A. A., & Rodrigues, K. F. (2017). Yeasts in sustainable bioethanol production: A review. Biochemistry and Biophysics Reports, 10(10), 52–61. <u>https://doi.org/10.1016/j.bbrep.2017.03.003</u>.

Duraisam, R., Salelgn, K., & Berekete, A. K. (2017). Production of beet sugar and bio-ethanol from sugar beet and it bagasse: a review. Int J Eng Trends Technol, 43(4), 222-233.