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RESEARCH PAPER

Maize-based bioethanol: A sustainable pathway for crop diversification in Punjab

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Abstract Punjab has long been at the forefront of India's agricultural self-sufficiency, but the dominance of the paddy-wheat cropping system has led to severe ecological challenges, including groundwater depletion and soil degradation. To ensure sustainable agriculture, crop diversification is imperative, with maize emerging as a viable alternative to paddy. Maize's adaptability and multiple end-uses, particularly in bioethanol production, make it a key crop for India's renewable energy strategy. The Government of India's Ethanol Blended Petrol (EBP) program aims to achieve 20% ethanol blending by 2025-26, necessitating a significant increase in maize-based ethanol production. This study evaluates five maize hybrids for their ethanol production potential to support Punjab's shift towards maize-based bioethanol. Hybrids were assessed for starch content, saccharification efficiency, and ethanol yield. Among them, JH21020 demonstrated the highest ethanol yield (0.45 g/g maize) and fermentation efficiency (98.95%) at 15% substrate loading. The findings highlight the importance of optimizing substrate concentrations for enhanced ethanol recovery. With ethanol production capacity in Punjab projected to rise, promoting high-starch maize hybrids and improving post-harvest technologies will be critical. This study underscores maize's potential to drive both agricultural sustainability and biofuel advancements in Punjab, ensuring economic viability for farmers and reducing environmental stress.

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Introduction

Punjab has historically played a pivotal role in ensuring India's self-reliance in food production. Since the Green Revolution, the state's agricultural landscape has been dominated by the paddy-wheat cropping system, which has continuously expanded over the decades. However, this intensive, input-driven cultivation, particularly of paddy, has raised serious concerns about the sustainability of agriculture in the region. Key challenges include groundwater depletion, soil health degradation, nutrient mining, environmental pollution, and a decline in on-farm biodiversity (Chand and Haque, 1998). In light of these challenges, it has become imperative for Punjab to diversify its cropping system, particularly by shifting from paddy to alternative *Kharif* crops, with maize and cotton being the most viable options (Singh, 2016).

Maize, in particular, stands out as the most promising candidate for diversification. Its versatility in end uses—ranging from human food to animal feed, bioethanol production, and various industrial applications—makes it a valuable crop. In India, the demand for maize is expected to rise sharply, driven primarily by its increasing role in bioethanol production (Gupta *et al.*, 2004). As the country moves towards reducing its dependence on fossil fuels and adopting cleaner, renewable energy sources, bioethanol has emerged as a critical element of India's energy strategy (Mahapatra *et al.,* 2021). Ethanol, the most widely produced biofuel, is primarily derived from crops rich in sugar or starch, such as maize and sugarcane. Its ability to blend with gasoline allows ethanol to significantly reduce greenhouse gas emissions, supporting global efforts for cleaner energy (Dhugga, 2007).

The Government of India's Ethanol Blended with Petrol (EBP) program, aimed at reducing reliance on imported fossil fuels, mitigating air pollution, and saving foreign exchange, has set ambitious ethanol blending targets. The deadline for achieving 20% ethanol blending in petrol (E20 fuel) has been brought forward from 2030 to 2025-26, with a target of 30% blending by 2029-30 (Sanini and Hussain, 2023). This requires a substantial increase in ethanol production, which is expected to be met by both molasses and grain-based sources, with maize playing a key role. Maize, owing to its year-round availability, high productivity, low water requirement, and minimal impact on food security, is well-suited for bioethanol production. Currently, maize occupies 9.7 million hectares in India, with an average yield of 3.12 tons per hectare (Rakshit *et al.*, 2023).

Punjab, as a traditional maize-growing state, is uniquely positioned to capitalize on this opportunity for crop diversification. Historically, maize and cotton were the major Kharif crops, but the Green Revolution shifted the focus towards paddy and wheat, exacerbating sustainability concerns due to groundwater depletion, straw burning, and declining soil health (Kaur and Chauhan, 2024). With the state's ethanol production capacity projected to increase from 2260 KLD to 3860 KLD, there will be a corresponding need for approximately 37 lakh tons of maize (https://www.indiastat.com).

To fully harness this opportunity, a collaborative effort among government bodies, industry, research institutions, and farmers is essential. Strengthening local maize production and supply chains will be key to sustaining the ethanol industry's growth. Punjab Agricultural University (PAU) has already introduced several high-yielding maize hybrids, but to meet the growing demand, approximately six lakh hectares must be dedicated to maize cultivation. This calls for the adoption of modern farming practices, optimal plant density, and post-harvest technologies such as dryers and silos to reduce losses. PAU's research on long-duration maize hybrids specifically developed for ethanol production promises to significantly enhance maize productivity in the state.

The development of maize hybrids with high ethanol production potential is a critical step in ensuring that maize becomes an economically viable and sustainable source of biofuel. The initial screening of maize inbreds with high starch content has led to the creation of hybrids that have demonstrated grain yields of 8-9 tons per hectare. These hybrids, after thorough evaluation, have shown great promise in ethanol production. By advancing maize breeding and cultivation, Punjab can position itself as a key player in the biofuel market, contributing to both agricultural and energy sustainability.

Maize, which once had record acreage of 5.77 lakh hectares in Punjab during 1975-76, now covers only 93.3 thousand hectares, with an average yield of 17.78 quintals per acre (4.39 t/ha). However, the crop offers a viable alternative for Punjab's grain-based distilleries, especially with the Union government's decision to procure maize at the Minimum Support Price (MSP). This policy will not only prevent distress sales by farmers but also encourage the expansion of maize cultivation under improved management practices, allowing high-yielding hybrids to reach their full potential. The development of these hybrids with high ethanol production potential is a timely and necessary step towards capitalizing on the growing demand for maize-based bioethanol.

Materials and methods

Punjab Agricultural University (PAU), Ludhiana is rich reservoir of genetic resources of maize including inbreds of different heterotic pools. The inbreds from MS and tuxpeno pools were evaluated for their seed yield potential and starch content (data not presented) and used to raise F_1s through hand pollination in spring 2022. The hybrids (listed in Table 1) having starch content more than 65% were used in present study, to evaluate their ethanol production potential at flask scale.

Maize flour preparation and analysis

Maize flour was prepared from well dried kernels of maize hybrids. Flour samples were analysed for reducing

Table 1. A set of five maize hybrids were used for study

Hybrid	Pedigree	Starch content (%)
PMH 14	PML 368 x PML 1224	65.2
JH21020	PML 1230 x PML 920	65.0
JH21021	PML 503 xPML 1231	65.4
JH20088	LM 13xPML 1231	66.3
JH20068	LM 21 x PML 22	66.7

sugars using Miller's (1959) method, and starch content was determined using the method of Clegg (1956).

Microorganisms and enzymes

Saccharomyces cerevisiae strain MTCC 11815, an indigenous strain from PAU, was used for ethanolic fermentation. Commercial alpha-amylase and glucoamylase enzymes were procured from Ultreeze Enzymes Pvt. Ltd, Surat, Gujarat, with activities of 23 kU/ml and 37 kU/ml, respectively.

Enzymatic saccharification process

The saccharification process involved two stages: liquefaction and saccharification. For liquefaction, maize flour was prepared at two substrate loadings: 15% and 20% wherein 22.5 g and 30 g of maize flour were mixed with 150 ml of 0.2 M sodium-acetate buffer (pH 5.2), respectively. The mixture was subjected to gelatinized at 100°C and an initial "pre-cooking" dose of 2.55 ml of alpha-amylase was added to the mixture and incubated at the same temperature for 30 minutes to initiate the breakdown of starch. Following this step, a "post-cooking" dose of 5.1 ml of alpha-amylase was introduced, and the mixture was incubated at 60°C for an additional 30 minutes to further hydrolyze starch into dextrins that was tested by adding KI/I_2 reagent for complete decolorization. For saccharification, 1.1 ml of glucoamylase was added to the liquefied starch, and the mixture was incubated at 45°C for 48 hours to convert dextrins into fermentable sugars.

Alcoholic fermentation process

The saccharified samples were subjected to ethanol fermentation in 250 ml capacity Erlenmeyer flasks under already optimized conditions of inoculum concentration of 8% (v/v), an initial sugar concentration of 14°B (Brix), and a fermentation temperature of 28 ± 2°C. Additionally, diammonium hydrogen phosphate (DAHP) was supplemented at a concentration of 0.1% (w/v) to support yeast growth. To start the fermentation, a freshly prepared 24-hour-old inoculum of *S. cerevisiae* MTCC 11815 was added to the saccharified worts @ 8% (v/v) and incubated at 28 ± 2°C until the ©Brix value dropped to a constant level. At the end of the fermentation, yeast cells settled at the bottom of the fermentation flasks. Samples were aseptically collected at 24-hour intervals, stored at 4°C, and analyzed for reducing sugars (Miller, 1959). The final ethanol concentration in the fermented broth was determined using the method of Caputi and Wright (1969).

Results and Discussion

The results from Table 2 provide a comparative evaluation of different maize hybrids for their saccharification and ethanol fermentation performance under varying substrate loadings. The saccharification process, which converts starch into fermentable sugars, showed considerable variation across hybrids. After 48 hours, the Brix values–an indicator of sugar concentration–ranged from 12.5 to 16°B, reflecting the differential efficiency of sugar conversion among the hybrids. Hybrid JH21020 and JH20068 displayed the highest Brix values of 13.5 and 16°B at a 15% and 20% substrate loading, respectively indicative of superior sugar release during saccharification.

The saccharification efficiency, calculated as the percentage of starch converted into sugars, ranged from 70.8% to 83.85%. The highest efficiency was recorded for JH21020 (83.85%), at a 15% substrate loading, underscoring its potential for optimal starch conversion. In contrast, hybrids JH20088 and JH20068 exhibited comparatively lower saccharification efficiencies, likely due to higher substrate loading (20%). This observation suggests that optimizing substrate concentration is critical to maximizing the saccharification process. Earlier, Gawande and Patil (2018) optimized a maize grain concentration of 14% for obtaining a maximum ethanol of 4.2% (w/v). Similarly, Sakib and Haque (2024) reported a corn concentration of 15.8-16.3 as optimum for maxiumum ethanol recovery.

Following saccharification, ethanol fermentation was carried out for 72 hours using *S. cerevisiae* MTCC 11815 Ethanol concentrations ranged from 6.4% to 6.85% (v/v) across hybrids, demonstrating successful fermentation in all cases. JH21020, at 15% substrate loading, again outperformed others with an ethanol concentration of 6. 10.27 ml/ 150 ml of fermentation and a fermentation efficiency of 98.9%. The fermentation efficiency of the other hybrids ranged from 90.1% to 98.9%, indicating high conversion rates of sugars into ethanol.

Ethanol yield, a key indicator of biomass-to-ethanol conversion efficiency, ranged from 0.341 to 0.368 g

Table 2. Comparative evaluation of maize hybrids for their ethanol potential at flask scale.

Parameters	Maize hybrids				
	PMH 14	JH21020	JH21021	JH20088	JH20068
Starch content (%)	65.2	65	65.4	66.3	66.7
% Substrate loading (Total starch, g/150 ml)	15 (22.5)	15 (22.5)	15 (22.5)	20 (30)	20 (30)
Theoretical starch (Th. Sugars)	14.67 (16.14)	14.625 (16.1)	14.715 (16.19)	19.9 (21.9)	20 (22)
*Brix post Saccharification	13	13.5	12.5	15.5	16
Saccharification efficiency, % (Brix basis) [Brix/Th sugars*100)	80.5	83.85	77.6	70.8	72.7
%Ethanol (v/v) using *S. cerevisiae*@ 5% v/v	6.80	6.85	6.72	6.50	6.40
Ethanol produced in ml (for 150 ml), v/v (w/v)	10.20 (8.16)	10.27 (8.22)	10.08 (8.06)	13.0 (10.4)	12.8 (10.24)
Theoretical ethanol possible (ml/150 ml)	10.416	10.384	10.448	14.122	14.207
Fermentation efficiency (%)	97.9	98.9	96.5	92.05	90.1
Ethanol yield (g/g maize)	0.363	0.365	0.358	0.347	0.35

Volume of reaction mixture 150 ml
*[Alpha amylase 1.7 ml/100 ml (precooking dose), 3.4 ml/ 100 ml post cooking); reaction time, 60 min., temperature (100 C precooking, 60 C post cooking) Glucoamylase 1.1 ml/100 ml, temperature 45 C, time 48 hours]
Time taken for completion of fermentation (hrs), 72 h
Yield (% alc. produced *1.50/substrate loading*100)

ethanol per gram of maize. JH21020, with an ethanol yield of 0.368 g/g maize at 15% substrate loading, emerged as the most promising hybrid for bioethanol production. This further reinforces its superior performance in both saccharification and fermentation processes. Hybrids JH20088 and JH20068, which were subjected to 20% substrate loading, showed lower ethanol yields (0.341 and 0.347 g/g maize in respect of JH 20068 and JH20088, respectively), supporting the hypothesis that excessive substrate loading may hinder efficient fermentation. Earlier Walia et al (2014) observed pH 5.8, temperature 31©C and substrate concentration of 16% as optimum for obtaining maximum ethanol of 7.46% (w/v). Sakib and Haque (2024) reported optimum concentrations of pH 5.24-5.52 and temperature 30-31 for obtaining maximum ethanol from corn.

The results also indicate that a 15% substrate loading was generally more effective than 20%, both in terms of saccharification efficiency and ethanol yield. The higher substrate loadings appeared to introduce limitations, such as substrate inhibition, which negatively impacted the overall efficiency of both saccharification and fermentation processes. Earlier, Adewumi et al (2022) observed that high substrate concentration has a more pronounced effect on bioethaniol production than 5-hydroxymethyl furfural in acid catalyzed hydrolysis of starch biomass.

Conclusion

The study highlights that maize hybrid JH21020 followed by PMH 14, particularly at a 15% substrate loading, delivered the most favorable outcomes in terms of saccharification efficiency, ethanol concentration, fermentation efficiency, and overall ethanol yield. These results suggest that optimizing substrate loading is crucial for maximizing bioethanol production from maize. The superior performance of JH21020 makes it a promising candidate for biofuel applications, especially in the context of Punjab's drive towards crop diversification and the growing demand for ethanol in the energy sector.

In the broader context of Punjab's agricultural landscape, maize emerges as an ideal crop to replace paddy, addressing the state's critical water and environmental challenges. Maize's lower water requirements, shorter cropping cycle, and its ability to support ethanol production, coupled with policy support such as procurement at the Minimum Support Price (MSP), make it a key player in the state's crop diversification strategy. As ethanol production capacity in Punjab is expected to rise significantly in the coming years, ensuring a steady supply of high-yielding, ethanol-efficient maize hybrids like JH21020 will be critical to meeting demand.

This study emphasizes the importance of developing specialized maize hybrids with high starch content and ethanol production potential to support Punjab's emerging bioethanol industry. Collaborative efforts between government bodies, research institutions, industry stakeholders, and farmers will be vital to sustaining this growth and ensuring Punjab remains a key player in India's biofuel market.

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