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Review of Breeding Maize Varieties for Biofuel Production

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Abstract Maize is an important crop for both food and energy worldwide. In recent years, research has focused on breeding maize varieties suitable for biofuel production. This study summarizes some of the progress made in breeding "dual-purpose maize." The grain and stalk yield of maize are usually positively related. There is a self-pollinating variety called Quality Protein Maize (QPM) that performs well. It has a good lignocellulose structure, high yield, and an ideal cellulose/lignin ratio, making it very suitable for biofuel production. The research also discusses the application of metabolic engineering and genomics in breeding. Techniques like marker-assisted selection, phenomics, and doubled haploid breeding are mentioned. The study talks about the sustainability of traditional and modern maize varieties in terms of environmental and farming management, even with low input, to meet the demand for both food and energy.
Keywords Maize breeding; Biofuel; Dual-purpose varieties; Stalk yield; Genetic diversity; QPM; Sustainable agriculture

1 Introduction

Traditional fossil fuels are becoming scarcer and causing serious environmental pollution. Because of this, many countries are actively looking for new and cleaner energy sources. Biofuels are a renewable energy source that can help reduce greenhouse gas emissions and ease the problem of energy shortages. This is why biofuels have gained a lot of attention. Especially around the world, the production and use of biofuels has become one of the key methods to promote green development and achieve carbon neutrality (Serrano et al., 2014; Choudhary et al., 2020).

Maize is a high-yield, adaptable, and biomass-rich crop that is widely used as a bioenergy crop worldwide. In addition to its food and feed uses, maize kernels and stover can also provide a large amount of raw materials for biofuel production (Lorenz et al., 2010; Choudhary et al., 2020; Pratikshya et al., 2025). Maize kernels are mainly used to produce bioethanol, while agricultural waste such as stover can be used as a feedstock for second-generation biofuels (Choudhary et al., 2020; Munaiz et al., 2021). Maize has rich genetic diversity and a good breeding base, which provides strong support for the breeding of specialized varieties with high yield, high biomass and high conversion efficiency (Lorenz et al., 2010; Munaiz et al., 2021). With the development of technology, such as molecular breeding, gene editing and metabolic engineering, the breeding goals and methods of maize are becoming more and more diverse (Barrière et al., 2016; Li et al., 2022).

This study mainly sorted out the breeding progress and current challenges of maize in the field of biofuels, reviewed the changes in the global energy landscape and the development background of the biofuel industry, summarized the main goals of maize for biofuel breeding, and introduced the application of different methods such as traditional breeding, molecular markers, gene editing and metabolic engineering, analyzed the main problems currently existing, and looked forward to future research directions.

2 Types of Biofuels and Maize Utilization

2.1 Overview of first-generation (starch-based) and second-generation (lignocellulosic-based) biofuels

Biofuels can be roughly divided into two types based on the raw materials and production methods: first-generation and second-generation. First-generation biofuels are made from food crops, usually by fermenting starch or sugars into ethanol. Maize is commonly used for this because it has a high starch content. In the U.S., maize ethanol is produced in large quantities and makes up most of the biofuel market (Ranum et al., 2014; Skoufogianni et al., 2019).

Second-generation biofuels, on the other hand, are made from non-food materials, like crop residues and forestry waste. In the U.S., maize stover (maize stalks and leaves) is a major source of second-generation biofuels because it is an important agricultural waste product (Dhugga, 2007; Jones et al., 2017; Choudhary et al., 2020). A key advantage of second-generation fuels is that they do not use food, so they make better use of waste materials and reduce dependence on food crops (Dhugga, 2007; van der Weijde et al., 2013; Choudhary et al., 2020).

The technology for first-generation fuels is already well-developed, with the main process being starch hydrolysis followed by fermentation, which is quite efficient. However, because the raw material is food, it competes with food supplies. Second-generation fuels are made from lignocellulose, such as maize stover, switchgrass, and miscanthus. The production process is more complicated and requires pretreatment and enzymatic hydrolysis, which demands more advanced technology (van der Weijde et al., 2013; Zhuang et al., 2013).

2.2 Maize grain and stover in ethanol and other biofuel production

Maize kernels contain a lot of starch, which is a good material for making fuel ethanol. In countries such as the United States where maize is grown a lot, about 40% of maize is used to make ethanol (Ranum et al., 2014; Skoufogianni et al., 2019). Maize ethanol not only provides energy, but also drives many related industries, such as biorefining, feed production and by-product utilization.

Maize stalks contain a lot of cellulose and hemicellulose. After pretreatment and enzymatic decomposition, these substances can be turned into sugars, which can then be fermented to produce ethanol or other biofuels (Dhugga, 2007; Choudhary et al., 2020; Pratikshya et al., 2025). The cellulose, hemicellulose and lignin content in the stalks will affect the fuel conversion efficiency (Dhugga, 2007; Pratikshya et al., 2025).

Different parts of maize, such as leaves, stems and kernels, have different gas and alcohol production capabilities during fermentation. Choosing the right raw materials and fermentation methods can increase yields (Ibrahim et al., 2025). Collecting maize stalks can also reduce farmland waste, but if too much stalks are collected, the soil may deteriorate, such as reducing fertility or destroying the structure, so it must be handled scientifically.

2.3 Advantages and limitations of maize and other bioenergy crops

Compared with bioenergy crops such as sweet sorghum, sugarcane, switchgrass, and Miscanthus, maize has high yield, strong adaptability, and the agricultural planting and processing system is already mature. In addition to being a staple food crop, maize also has considerable biomass yield and good conversion efficiency, especially in temperate regions such as North America and Europe (van der Weijde et al., 2013; Skoufogianni et al., 2019). Maize can also be used as food and energy at the same time, and it is easy to integrate into the existing agricultural system (Chen et al., 2013; Skoufogianni et al., 2019).

Using maize grain to produce ethanol competes directly with food supply (Ranum et al., 2014; Choudhary et al., 2020). If too much maize stover is removed, it can negatively impact the soil, possibly causing degradation and affecting the ecosystem (Jones et al., 2017; Skoufogianni et al., 2019). Maize stover has a lot of lignin, which makes the biomass more difficult to process and convert (Dhugga, 2007; Choudhary et al., 2020). Maize can produce about 3.0 to 5.4 thousand liters of ethanol per hectare, but miscanthus can produce more than double that amount, using less land and water (Zhuang et al., 2013).

3 Genetic Traits Related to Biofuel Production

3.1 High starch content and fermentability

The starch content in maize kernels is key to whether it can be used to produce first-generation bioethanol. This is because starch is easy to convert into sugar, which can then be fermented. Quality Protein Maize (QPM) inbred lines not only have high yields but also high starch and cellulose content, making them ideal for biofuel production (Pratikshya et al., 2025).

However, whether starch ferments well depends not just on how much starch there is but also on its structure. Certain genetic traits can make the starch easier to break down by enzymes, making it easier to convert.

Some high-oil maize varieties accumulate more triglycerides in the kernels, which could be used to produce biodiesel. But these types of maize usually have less starch than regular maize (Li et al., 2022).

Researchers are working on how to increase both oil and starch levels without affecting yield or plant health. They are using molecular markers and genomic prediction methods to find genes that help improve both starch content and fermentability (Gesteiro et al., 2023; Pratikshya et al., 2025).

3.2 Cell wall composition and lignin content for straw ethanol production

Lignocellulose is mainly made of cellulose, hemicellulose, and lignin. Lignin makes plants harder to break down, so it’s harder to convert into sugar. Breeding efforts now focus on reducing lignin content or changing its structure to improve straw digestibility and saccharification efficiency (Barrière et al., 2016; Choudhary et al., 2020; Pratikshya et al., 2025).

The brown midrib (bm) mutation is valuable because it has low lignin content, making it useful for increasing straw utilization (Choudhary et al., 2020).

Different maize varieties have very different cell wall compositions. Some local varieties or inbred lines have more cellulose and less lignin in their straw, which makes them better for biofuel production. For example, QPM straw has these characteristics, which also lead to higher saccharification efficiency and ethanol yield (Pratikshya et al., 2025).

Scientists have discovered some key regions and genes through GWAS and QTL mapping that are related to cell wall formation and digestibility, such as transcription factors such as ZmMYB and ZmNAC, and some enzymes that control lignin synthesis (Barrière et al., 2016).

3.3 Biomass yield and harvest index

Maize biomass includes both kernels and straw. The harvest index (HI) is the proportion of total biomass that becomes kernels. To make ethanol from straw, it’s important to increase overall biomass without reducing kernel yield.

In temperate maize, over time, both kernel yield and straw have increased, but the harvest index hasn’t changed much (Lorenz et al., 2010).

Different types of maize vary greatly in biomass and harvest index. Hybrids usually have higher yields and a higher HI, while local varieties tend to have a lower HI (Munaiz et al., 2021). Some local varieties from Europe actually produce more straw and have better fiber quality.

Kernel and straw yields are usually positively related, and because these traits are highly heritable, it’s possible to improve both traits through phenotypic selection and genomic prediction (Lorenz et al., 2010; Munaiz et al., 2021; Gesteiro et al., 2023).

3.4 Traits related to drought tolerance and nutrient use efficiency

Some traditional maize varieties grow well in low-fertilizer and low-water environments, producing high biomass and energy content (Serrano et al., 2014). These drought-tolerant traits are important for biofuels because they can reduce costs and environmental pressure.

In order to improve drought resistance and nutrient utilization efficiency, breeding mainly focuses on finding gene loci that can enhance water and fertilizer absorption and stress resistance. The development of genomics and phenomics has helped discover these key genes and molecular markers (Choudhary et al., 2020).

4 Breeding Strategies For Maize Varieties For Biofuels

4.1 Conventional breeding methods

Breeders observe the appearance of maize (such as yield, straw and grain quality, etc.) and conduct multi-generation hybridization and screening. Grain yield and straw yield usually increase together, which shows that maize can provide raw materials for both food and biofuels (Lorenz et al., 2010; Gesteiro et al., 2023). The heritability of these traits is generally not low, and traditional breeding methods are still effective. Some local varieties and high-diversity maize resources can also be used in breeding to find materials suitable for different regions or different uses (Munaiz et al., 2021).

Traits such as cellulose and lignin in straw are controlled by many genes and are affected by the environment. It is difficult to achieve good results quickly by selecting based on appearance alone. In addition, traditional breeding cycles are long and slow, and it is difficult to meet the needs of the biofuel industry. Therefore, conventional breeding methods are often used together with molecular techniques to form diversified breeding methods (Munaiz et al., 2021; Gesteiro et al., 2023).

4.2 Marker-assisted selection (MAS)

MAS is a breeding method that can select target traits at the genetic level. Researchers can locate genes related to these traits, such as genes that control biomass, straw digestibility or lignin content. Through GWAS and QTL mapping technology, scientists have found many genetic pathways related to maize straw (Barrière et al., 2016; Gesteiro et al., 2023). Transcription factors such as ZmMYB and ZmNAC are in the same region as the QTL for cell wall digestibility, which provides clear genetic targets for MAS (Barrière et al., 2016).

The advantage of MAS is that it can screen out plants with good genes in the early stage of breeding, thereby shortening the time and improving efficiency. For traits such as cellulose content that are greatly affected by the environment, MAS is more stable than traditional methods (Barrière et al., 2016; Choudhary et al., 2020). MAS can also be combined with conventional breeding, gene editing and other technologies to form a stronger breeding system and accelerate the breeding of biofuel-specific varieties (Choudhary et al., 2020).

4.3 Application of genomic selection (GS) in complex trait improvement

Genomic selection (GS) does not need to find each key gene separately, but predicts the breeding potential of individuals by establishing a relationship model between the entire set of genes and traits. This method performs well in improving straw saccharification efficiency and other aspects (Gesteiro et al., 2023). Compared with traditional QTL or MAS methods, GS can discover the cumulative effects of more small-effect genes, which is very helpful for controlling complex traits.

GS can screen materials in advance and on a large scale, without the need for too many field trials and trait determinations, saving time and cost. With the development of maize genomic data and molecular marker technology, GS is increasingly used in maize biofuel breeding (Barrière et al., 2016; Gesteiro et al., 2023).

4.4 The role of hybrid breeding in improving bioenergy performance

Through hybridization, hybrid vigor can be used to make maize perform better in terms of yield, straw quality and resistance (Lorenz et al., 2010; Munaiz et al., 2021). Hybrids not only have high grain and straw yields, but also high harvest ratios, which can meet the needs of food and fuel at the same time. Breeders can also select materials with more cellulose and less lignin to improve the biofuel utilization value of straw (Pratikshya et al., 2025).

Molecular technology and genomic selection are also used in hybrid breeding, which can help select good inbred lines and hybrid combinations more quickly (Wan et al., 2019; Pratikshya et al., 2025). Some new technologies, such as male sterility and double haploidy, also make breeding easier and improve seed purity and production efficiency (Wan et al., 2019; Mitiku, 2022).

5 Biotechnology and Genetic Engineering in Maize Biofuel Breeding

5.1 Controlling lignin and cellulose content with genetic engineering

Lignin is an important part of the cell wall. It helps plants stay strong and resist stress. But if there is too much lignin, it makes it harder for enzymes to break down the plant material, which also makes it tough for cellulose and hemicellulose to be broken down. To solve this, scientists use genetic engineering to control how lignin is made. They target key enzyme genes in the process, like using antisense RNA or RNA interference to reduce the expression of the Caffeoyl-CoA O-methyltransferase gene (Abdel-Rhman, 2015).

One method involves using a particle bombardment technique to insert an antisense fragment of the 4CL gene into maize. This reduces lignin content and increases the amount of bioethanol produced from the straw (Abdel-Rhman, 2015). Some natural brown midrib (bm) mutants have also been used to breed maize varieties with lower lignin content (Choudhary et al., 2020).

Besides controlling lignin, scientists are also trying to adjust the cellulose content and its crystallization using genetic engineering. They've even introduced the genes for hydrolytic enzymes into maize, so the maize can produce its own cellulase. This reduces the need for added enzymes during the biomass conversion process (Abdel-Rhman, 2015).

5.2 Improving biofuel traits with CRISPR/Cas9 gene editing

Compared to traditional genetic modification methods, CRISPR/Cas9 is faster and more accurate. It can directly modify specific parts of DNA, like knocking out, inserting, or adjusting genes. In controlling lignin and cellulose content in maize straw, this technology can directly target the key genes involved in lignin synthesis (Barriere et al., 2009).

Scientists have also used CRISPR/Cas9 to adjust genes related to starch synthesis, making the starch structure and ratio in maize kernels more suitable. This improves fermentation efficiency and increases ethanol production (Niu et al., 2023) (Figure 1).

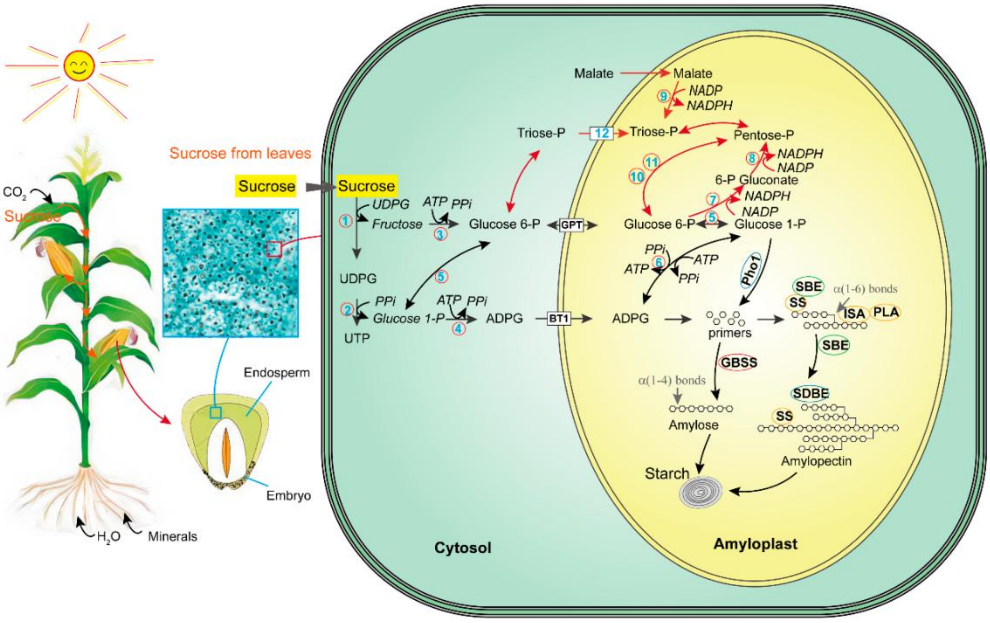

Figure 1 Starch biosynthesis in maize endosperm. Black arrows show the conventional biosynthesis of starch: glucose-1-P is converted to ADPG, which is transported into the amyloplast and polymerized into starch. Red arrows show the oxidative pentose phosphate pathway, which provides NADPH and pentose sugars for starch synthesis. 1, sucrose synthase; 2, UDP-glucose pyrophosphorylase; 3, hexokinase; 4, AGPase; 5, phosphoglucomutase; 6, complexes including AGPase, starch synthase IIa (SSIIa), SSIII, starch branching enzyme IIb (SBEIIb), and SBEIIa; 7, glucose-6-phosphate dehydrogenase; 8, 6-phosphogluconate dehydrogenase; 9, malic enzyme; 10, transaldolase; 11, transketolase; 12, triose-P/P translocator and P/phosphoenolpyruvate translocator. (Adopted from Niu et al., 2023)

CRISPR/Cas9 doesn't just edit one gene. It can adjust several genes at the same time. For example, scientists can simultaneously adjust genes related to biomass, straw digestibility, and stress resistance. This helps them create new maize varieties with a combination of desirable traits (Barriere et al., 2009).

6 Evaluation of the Biofuel Suitability of Maize Lines

6.1 Screening methods for starch yield and fermentability

Screening maize lines suitable for biofuels is usually carried out by combining morphological and biochemical analyses. Morphological screening includes measuring plant height, ear characteristics, grain yield, etc., while biochemical analysis mainly detects the starch content of grains. By measuring the starch content of different maize inbred lines or hybrids, lines with high yield potential can be preliminarily screened (Pratikshya et al., 2025). Further fermentability evaluation is usually carried out in small-scale fermentation trials under laboratory conditions to measure the ethanol yield per unit mass of grain under standard enzymatic hydrolysis and fermentation conditions.

In the breeding process, statistical methods such as principal component analysis (PCA) and correlation matrix are often used to screen and cluster maize lines with excellent biofuel characteristics, which can help identify lines with better performance in terms of starch content and fermentation efficiency.

6.2 Evaluation of lignocellulose degradability

To evaluate the lignocellulose degradability of maize lines, it is first necessary to analyze the cellulose, hemicellulose and lignin content in the straw. The contents of cellulose (28.05%~37.05%), hemicellulose (7.06%~15.81%) and lignin (20.65%~28.35%) in the straw of different maize inbred lines and hybrids are significantly different (Pratikshya et al., 2025). Generally, strains with high cellulose and low lignin have better enzymatic hydrolysis efficiency and higher ethanol yield.

Under laboratory conditions, commonly used evaluation methods include enzymatic saccharification rate determination and simulated fermentation test. Through standardized pretreatment and enzymatic hydrolysis, the releasable reducing sugar content and final ethanol yield in the straw can be determined (Choudhary et al., 2020; Pratikshya et al., 2025).

6.3 Comparison of laboratory scale and field scale evaluation

The evaluation of the suitability of maize biofuels includes not only small-scale biochemical and fermentation tests in the laboratory, but also large-scale yield and quality determination in the field. Laboratory scale evaluation is simple to operate, short cycle and low cost, and is suitable for the initial screening of a large number of materials in the early breeding stage. By measuring starch content, cellulose and lignin content, enzymatic saccharification rate and other indicators in the laboratory, potential lines can be quickly screened out (Choudhary et al., 2020; Pratikshya et al., 2025). Laboratory results are often difficult to fully reflect the performance in the field environment, especially under the influence of climate, soil and management factors.

Field-scale evaluation is closer to actual production conditions and can comprehensively examine the growth performance, yield, harvest index and biochemical characteristics of maize lines in different ecological environments (Serrano et al., 2014; Munaiz et al., 2021). Field trials can test the yield stability, straw yield and quality of different lines in multiple locations for many years, and can evaluate their adaptability to low-input conditions (such as reduced fertilization and irrigation) (Serrano et al., 2014). Field evaluation can also be combined with harvest time to monitor the dynamic changes of biomass, ash content, energy value and other indicators (Serrano et al., 2014; Wojcieszak et al., 2022).

6.4 The role of high-throughput phenotyping

The high-throughput phenotyping platform can automatically measure the morphological, physiological and biochemical characteristics of a large number of maize samples in a short time (Choudhary et al., 2020). Through high-throughput imaging and near-infrared spectroscopy (NIRS) technology, the content of starch, cellulose and lignin in maize kernels and straw can be quickly determined (Choudhary et al., 2020). High-throughput phenotyping can also be combined with molecular markers, genomic selection and other technologies to promote the precise breeding of maize biofuel varieties. High-throughput phenotyping improves screening efficiency and provides rich phenotypic data for genetic analysis and gene localization of complex quantitative traits (Choudhary et al., 2020).

7 Environmental and Agronomic Considerations

7.1 Sustainable development of maize as a biofuel

Nowadays, many people are concerned about whether maize as a biofuel is sustainable. If it is cultivated in an intensive way, such as planting two crops a year, it will not occupy more arable land and can increase the production of maize ethanol. In central and western Brazil, farmers often plant a crop of maize after the soybean harvest. In addition, using renewable energy to process it can reduce greenhouse gas emissions and increase income and job opportunities (Moreira et al., 2020).

In Europe, the large-scale use of maize to generate biogas has brought some problems, such as too concentrated sludge, which affects surface and groundwater, and a lot of greenhouse gas emissions (Herrmann, 2013). Maize cultivation may also lead to a decrease in soil organic matter and a decrease in biological species. To make this system more sustainable, it is necessary to arrange crop rotation properly, handle sludge properly, use a systematic approach to evaluate soil carbon flow and emissions, and scientifically analyze changes in land use.

7.2 Conflict between food and fuel

Maize can be eaten and used as fuel, so some people worry that using too much maize for fuel will affect food supply and even raise food prices. If double-season planting or crop rotation is adopted, fuel raw materials can actually be increased without affecting food production (Moreira et al., 2020; Costantini and Bacenetti, 2021). For example, in South America, the rotation of beans and maize not only has higher energy output per unit area, but also good economic benefits and little impact on food supply (Costantini and Bacenetti, 2021).

The land, climate and economic conditions in different places are different, and one method cannot be applied to all places. The effectiveness of the biofuel system also depends on crop yields, land use and policy support (Nakamya, 2022).

7.3 Issues in soil, water and carbon emissions

Some traditional maize varieties can grow well and produce high biomass regardless of how they are fertilized, sprayed or watered (Serrano et al., 2014). If harvested at the right time, not only will the yield be high, but the content of ash, nitrogen, potassium, chlorine, etc. in the straw can also be reduced. Combined with reasonable fertilization and crop rotation, the organic matter in the soil can be maintained and soil degradation can be prevented (Herrmann, 2013; Hasanain et al., 2025). Although maize has a high demand for water, choosing the right variety and managing irrigation well can improve water use efficiency. In terms of carbon emissions, using maize to make ethanol is also effective in energy use and emission reduction, especially when high-yield, low-input planting methods are used, and it is more obvious when processed with renewable energy (Moreira et al., 2020; Nakamya, 2022). However, ethanol processing and planting itself are still large carbon emitters, and carbon footprints must be reduced through technology and management (Nakamya, 2022).

7.4 Intercropping and straw management potential

Crop rotation or intercropping patterns such as beans and maize can make better use of land resources and increase energy output and benefits (Costantini and Bacenetti, 2021). By planting a diverse crop structure, not only can the soil be improved, but it can also make the soil more nutritious and reduce pests (Hasanain et al., 2025). Intercropping can also help farmers spread risks and enhance their ability to resist climate change.

In terms of straw, if straw is returned to the field and biogas residue is used properly, it can improve soil nutrients, reduce the use of chemical fertilizers, and reduce environmental pollution (Herrmann, 2013; Hasanain et al., 2025). Using no-tillage, conservation tillage or covering with residues can also reduce water evaporation, prevent soil from being washed away, and increase carbon storage in the soil (Hasanain et al., 2025). For compound crops like maize and wheat, if combined with technologies such as straw processing, precision fertilization and water-saving irrigation, there is room for improvement in yield, efficiency and environmental performance.

8 Case Studies

8.1 Breeding methods for energy maize in the USA

In the United States, researchers have conducted many field trials and found a strong connection between maize grain yield and stover dry matter (SDM), with a correlation coefficient of 0.67. They also focused on improving the saccharification efficiency (how well the stover breaks down into sugars). There was a big difference in saccharification rates among over 100 maize varieties. Some had only 20%, while others reached 33%. This trait also had a relatively high genetic heritability (H^2 could be up to 0.71).

Different maize varieties had different ratios of cellulose and lignin in their stover. Materials with high cellulose and low lignin release sugar more easily and have better saccharification. Some parent lines (NAM parental lines) and certain inbred lines performed well in both saccharification efficiency and cellulose content, making them easy to categorize.

After harvesting the grain, the remaining stover is fermented to produce sugar for biofuel production. This approach makes agricultural production more economically valuable and environmentally friendly. Especially in the Midwest of the United States, where maize and soybean crop rotation systems are common, maize stover is particularly well-suited for second-generation biofuels.

8.2 EU bioenergy maize projects

In some European countries, such as Spain, the Mediterranean, and along the Atlantic coast, local maize landraces are gaining attention. These varieties evolved in less-than-ideal environments, producing high stover yields and accumulating more cellulose. These landraces have now become important materials for biofuel breeding.

Researchers evaluated more than 100 old European maize varieties and found that they performed very stably in different regions and years. Some varieties, such as "Amylomaize-Cudillero" and "Lusitano-Asturias", can achieve a dry weight of 10 tons/hectare of straw without nitrogen fertilization.

These old varieties generally have a higher cellulose content and a lower lignin ratio, which makes them easier to enzymatically hydrolyze and is also conducive to the saccharification process. For example, the "Cudillero" variety has a high straw yield, with a cellulose content of 38% and a lignin content of less than 15%, making it very suitable for biorefining (Munaiz et al., 2021).

8.3 Progress in tropical and subtropical regions

In tropical and subtropical regions, maize breeding has also made significant progress in the direction of biofuels. These areas often have dry weather, high temperatures, and poor soil, making it difficult to grow high-yield maize. Some tropical hybrids, especially quality protein maize (QPM), show good stover quality, with high cellulose, low lignin, and high saccharification efficiency (Prasanna et al., 2021) (Figure 2). Under drought or nitrogen-limited conditions, these materials maintain more than 90% of their stover yield, while regular varieties often lose more than 30%.

Now, many tropical maize breeding programs use doubled haploid (DH) technology and marker-assisted selection (MAS). Researchers use MAS to introduce genes that help with cell wall breakdown and stover yield into high-quality tropical maize backgrounds. The selected varieties outperform the control varieties in dry matter accumulation and saccharification efficiency, with an average improvement of 15% to 20%.

9 Challenges and Future Directions

9.1 Genetic bottlenecks and insufficient germplasm resources

Although we have found some maize materials suitable for biofuels through screening and biological analysis in recent years, such as high-cellulose, low-lignin quality protein maize (QPM), the genetic resources of maize are still not rich enough (Pratikshya et al., 2025). This resource limitation not only affects the improvement of complex traits such as biomass, cellulose content, and saccharification efficiency, but also makes it more difficult for maize to cope with environmental changes (such as climate warming and soil deterioration).

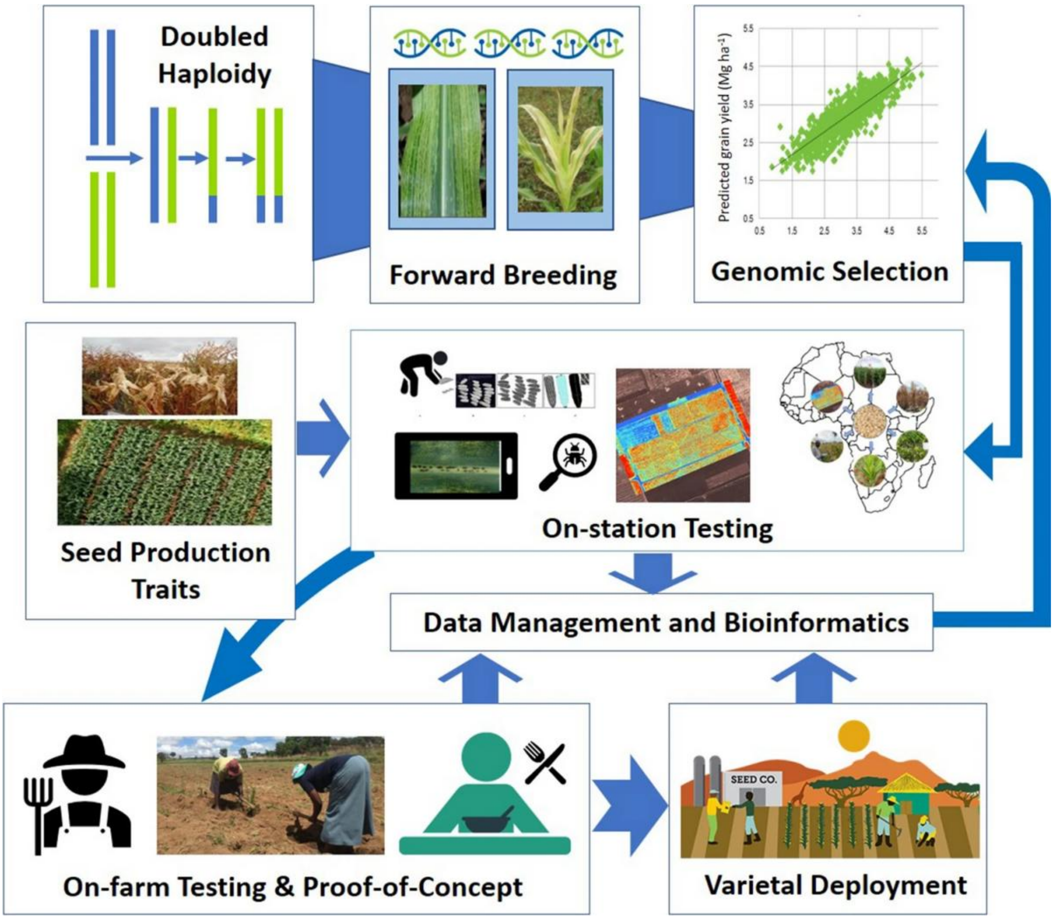

Figure 2 Schematic depiction of the maize breeding pipeline of CIMMYT for developing and deploying elite multiple stress-tolerant tropical maize germplasm for sub-Saharan Africa, Asia, and Latin America (Adopted from Prasanna et al., 2021)

In order to solve this problem, we can make more use of wild species, local varieties and heterologous germplasm in the future, introduce new genes into maize breeding, and expand the gene pool. We can also use some multivariate statistical methods, such as principal component analysis and correlation analysis, to find out which varieties have better biofuel traits.

9.2 The role of multi-omics in trait mining

It is difficult to fully understand the source of these complex traits by relying solely on traditional appearance selection or single gene markers. But now, we can use genomics, transcriptomics, metabolomics and phenomics to study the control mechanisms of maize biomass, cellulose, lignin and other related traits (Pratikshya et al., 2025). Technologies such as genome-wide association analysis (GWAS) and whole genome selection (GS) can help us find gene loci closely related to biofuel traits.

As long as enough planting data in different locations and environments are accumulated, researchers can have a more comprehensive understanding of the performance of maize under various ecological conditions and its genetic mechanisms (Khan et al., 2025; Pratikshya et al., 2025). In the future, as sequencing technology becomes faster and cheaper, automated phenotyping technology becomes more and more advanced, and with the support of big data analysis, the integration of multi-omics will definitely play a greater role in trait discovery, functional verification and molecular design breeding, and will also make maize biofuel breeding more accurate and intelligent.

9.3 Demand for dual-purpose maize for food and fuel

Some places are now vigorously developing the maize biofuel industry, but this has also brought some problems, such as people's concerns about the problem of "grain and fuel competing for land", leading to land and resource shortages (El-Araby, 2024; Khan et al., 2025). Therefore, it is very important to develop dual-purpose maize varieties that can be eaten as food and used as fuel.

There is a certain positive correlation between maize grain and straw yield, which means that if the grain yield is high, the straw may also be high, which can be used as a reference for breeding dual-purpose varieties (Pratikshya et al., 2025). In addition to high yield and good quality, this variety must also be disease-resistant and stress-resistant, and the straw must have more cellulose and less lignin, so that it can be better converted into biofuels (Khan et al., 2025; Pratikshya et al., 2025). In addition, it is also necessary to pay attention to the fact that climate change may affect the nutrition and biomass of maize, and a balance should be struck between stress resistance, nutritional value and fuel efficiency (Khan et al., 2025).

9.4 Breeding and industrial processing should be linked

Some high-yield maize varieties grow well in the field, but when used for biofuels, they do not perform well, and the saccharification rate and ethanol yield do not meet expectations. This may be due to the composition, structure or difficulty in degrading the straw (Sokan-Adeaga et al., 2024; Pratikshya et al., 2025). Moreover, the current industrial processing technology is updated very quickly, and the requirements for raw materials are getting higher and higher.

In order to solve this problem, breeding work should be more closely integrated with industrial experiments. After selecting good varieties from the field, they can be sent to laboratories or pilot plants for saccharification, fermentation and other tests to see which varieties are both high-yielding and suitable for industrial conversion (Ambaye et al., 2021; Sokan-Adeaga et al., 2024). At the same time, it is recommended that breeding experts, enterprises and scientific researchers cooperate more, share information and integrate technology, so as to speed up the process of bringing good varieties to the market.

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Conflict of Interest Disclosure

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