# Bioengineering energy crops: Multi-omics and Genome editing strategies to enhance polysaccharides composition in biomass

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**ABSTRACT:**

The utilization of cellulosic biomass as a raw material for the production in biorefineries is widespread due to its cost-effectiveness, recyclability, and availability. However, existing biorefineries are still expensive and inefficient. This is largely due to the recalcitrant nature of lignocellulosic biomass, which necessitates expensive pretreatment processes to degrade the plant cell wall and lignocellulolytic enzymes to convert cellulose to glucose. Multi-omics and genome editing are possible approaches to lower the cost of operating biorefineries while also increasing the amount of energy extracted from biomass. It has been reported that genetic engineering is a successful method for enhancing agricultural plants' productivity, biomass yields, and specific traits. This chapter examines the development of various genetic modification approaches to modify plant cell walls. It proposes the application of genetic modification of various plants, as a potential solution to the high costs and limited yields associated with biorefineries also utilizing agricultural waste as a feedstock for generation energy. Overall, the development of reliable and effective lignocellulosic biomass conversion procedures into bioproducts should be facilitated by the combination of various molecular biology and multi-omics approach that concurrently gather structural and chemical information regarding the biomass.

**Keywords: Lignocellulose, biorefinery, omics, Crispr/cas9, engineering crops**

## I. INTRODUCTION

The population has increased dramatically during the past century. The estimated worldwide population will rise above 9 billion by the year 2050 and reach a peak of 9.73 billion in 2064 [1], [2]. In tandem with the increasing growth of the global population, resource availability and quality of the environment are constantly declining. Global warming is a result of environmentally hazardous and current energy consumption practices. One of the biggest hurdles to progress over the next few years will be the ability of the world to satisfy the needs of a rapidly expanding population. As the global population expands, there is going to be a larger demand for materials involving energy, food, and water [3]. This challenge might call for innovative ideas and long-term strategies to guarantee that the requirements of everyone are addressed without compromising future generations' well-being. As the world's energy needs continue to rise, our growing concern about environmental contamination (global warming and agricultural waste generation) and the decreasing availability of fossil fuels has driven us to switch towards alternate renewable energy sources. Future energy demands must be met with eco-friendly and environmentally sustainable energy which is generated from renewable resources. As a result, efforts are being made to find bio-based solutions that will increase energy security, reduce our dependence on fossil fuels, eliminate greenhouse gas emissions, and minimize waste generation.

### A. Lignocellulose biomass

As of now, corn grain, sugar cane, etc. generally known as "first-generation feedstocks," have been used to produce the majority of biofuel and biorefined products. Such crops compete with food production for land, biological conversion of these crops to biofuel is expensive, and it only replaces a small percentage of the production of fossil fuels. This has pushed researchers to focus on developing second-generation feedstocks (2Gen biofuel), which include organic wastes and cellulosic biomass [4]. Cellulosic biomass, such as agricultural waste and industrial waste, is typically an abundant and affordable supply that is accessible in nearly every country. Lignocellulosic agricultural waste is produced worldwide on an annual basis at a rate of 140 billion metric tonnes [5]–[7]. The development of "second generation" biomass-derived biofuels also faces numerous significant difficulties, including increasing biomass yield per hectare per year, maintaining sustainability while reducing agricultural inputs, and avoiding conflict with food production. Given these factors, there has been a lot of

attention paid to turning lignocellulosic biomass into fermentable sugars. In the end, ethanol made from lignocellulosic materials has the potential to provide the majority of the world's transportation fuel demands while having a significantly less impact on the food supply, requiring fewer agricultural inputs, and emitting less net carbon dioxide than fossil fuels [8], [9]. Biomass made from lignocellulosic materials continues to draw interest on a global scale, providing an environmentally friendly alternative to fossil fuels for the production of biofuels of the 2nd generation as well as other biobased materials without affecting global food security [10].

Lignocellulose, often known as lignocellulosic biomass (LB), implies plant-derived dry matter (biomass) [11]. LB is one of the most abundant and rich sources of renewable biomass found on the earth [12]. It primarily consists of cellulose, hemicellulose, and lignin, as well as, to a lesser extent, pectin, proteins, and minerals. Cellulose and hemicellulose, two carbohydrate polymers, and lignin, a non-carbohydrate phenolic polymer, contribute to the majority of lignocellulosic biomass [13]. Lignin links with cellulose fibres to reinforce and harden the cell walls of plants, thus making LB a complex structure.

Cellulose is the major structural polysaccharide of the cell wall. It makes up about 30–50% of the dry weight of lignocellulose and is made up of d-glucose units linked via β (1→4) linkage that is connected in linear chains [14], [15]. Cellulose derived from not food-related energy crops can be broken down into glucose monomers due to the activities of microbial cellulases and then converted to biofuels or other compounds with added value [13].

Hemicellulose, which makes up between 15 and 30 per cent of plant cell walls, is the second major polysaccharide component of lignocellulose. The binding of cellulose microfibrils to reinforce the cell wall is one of the primary functions of hemicelluloses, which occur embedded in the cell walls of plants. Hemicellulose, particularly differs from cellulose in the sense that it has a random and amorphous structure, and is composed of several heteropolymers, such as xylan, xyloglucan, arabinoxylan, glucuronoxylan, and glucomannan [16]. A diluted acid or base treatment, and several microbial hemicellulases, may hydrolyse hemicelluloses to produce oligosaccharides, which are then broken down into simple sugars. A complex group of enzymes known as hemicellulases aid in removing side chains while also randomly attacking the hemi cellulosic backbone to liberate oligosaccharides.

Lignocellulose's third major constituent is lignin, it makes up about 15–30% of its dry mass [13]. All vascular plants contain lignin, which is the second-most abundant source of carbon after cellulose [17]. Vascular plants' cell walls and tissues are made stronger and more rigid because of lignin, which also guards the cell wall from microorganisms that would otherwise break down the structural polysaccharides. The most promising possibilities for lignocellulose biomass feedstock at present include rice straw, corn stover, switchgrass, miscanthus, and woody lignocelluloses which include poplar and eucalyptus.

**B. Conversion of lignocellulosic biomass to value-added products**

The bioconversion of lignocellulosic biomass into bio-hydrogen, bioethanol, biopolymer and other biobased products holds an economically viable alternative to fossil fuels [18]. Biomass pretreatment, biomass hydrolysis, fermentation, and recovery (or distillation) are the four sequential procedures used to transform lignocellulosic biomass into bioethanol and other biobased products [19].

The pretreatment technique is used to break down the structure of plant cell walls using hydrolytic enzymes and make it easier for enzymes to reach the complex network of polysaccharides like cellulose, hemicellulose and lignin. There are four categories of pretreatment techniques widely used nowadays: biological pretreatment, physical pretreatment, chemical pretreatment, and physio-chemical pretreatment [20]. The pretreatment is essential for giving hydrolytic enzymes complete access to cellulose and hemicellulose. Some problems with pretreatment are observed in the pretreatment first is the high expense of acid and alkali used during the process, secondly, the pretreatment stage also results in harmful byproducts such as acetic acid and furfurals, which subsequently prevent hydrolytic enzymes and fermentation [11].

The second step in the conversion of lignocellulosic biomass to value-added products is the enzymatic hydrolysis of biomass. In this acids or enzymes are utilized, and cellulose fibres and hemicellulose are hydrolysed and transformed into glucose and fructose monomers. In other words, the enzymatic process which transforms cell wall polysaccharides into fermentable sugars is known as enzymatic hydrolysis. The accessibility of the feedstock and the pretreatment need to be further improved to make the enzymatic hydrolysis process cost-effective. The lignocellulosic biomass's intricate structure, however, also has an impact on the rate of enzymatic hydrolysis. This means that a successful pretreatment reduces the crystallinity of the cellulose, and eliminates hemicellulose, and lignin as per the process requirements while increasing the yields of the hydrolysis process [21]. The lignocellulolytic enzyme aids in breaking down lignocelluloses into simpler sugars. Cellulases and hemicellulases are the two primary lignocellulolytic enzymes employed in bioconversion, which transform lignocellulosic biomass into fermentable sugars. The enzymes involved have been employed in the industrial sector for a long time and are thought to be essential for the saccharification process of feedstocks [22].

The third step in the process of conversion of lignocellulosic biomass to value-added products is the fermentation process, this involves employing yeast or desired microorganism (that may be bacteria or fungi) to transform the glucose monomers into ethanol or other value-added byproducts.

In the final step of bioconversion of LB, the product of the fermentation process is further refined and distilled to separate the byproduct of fermentation which can be intracellular or extracellular depending upon the process. The fermentation products after the distillation can be bioethanol, biopolymer or other value-added products. Figure 1: shows an overview of lignocellulosic biomass its components and biorefinery operations with steps in the conversion of lignocellulosic biomass to value-added product

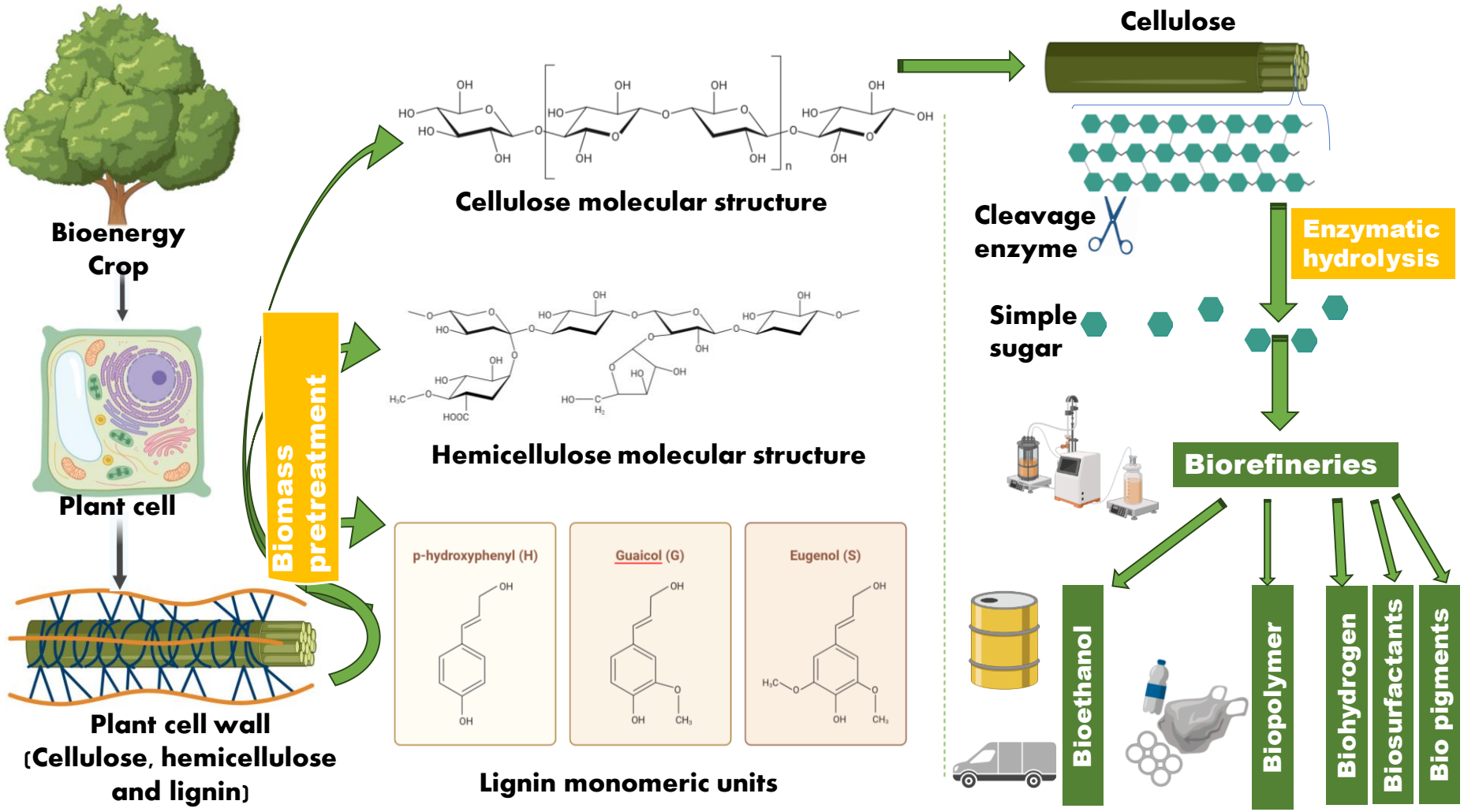


**Figure 1: An overview of lignocellulosic biomass and biorefinery operations.**

### C. Major obstacles in transforming biomass into value-added products

Production of bioethanol and other biorefined products from biomass still faces difficulty in terms of economic, environmental, and energy issues. It continues to be expensive (because energy inputs are needed to break down the links between lignin, hemicellulose, and cellulose), environmentally hazardous (because production waste is generated and left behind after the distillation process), and ineffective (because lignocellulosic biomass has a recalcitrant structure). The structure and composition of lignocellulosic biomass have been found to greatly affect the rate and yield after biomass's hydrolysis [19]. As per the research, Low sugar yield and thereafter lower biofuel yields are caused by high levels of lignin in plant cell walls.[23], [24].

### D. Bioengineering plant cell wall

Reengineering plant cell walls by taking advantage of developments in the field of plant genetic engineering, carbohydrate chemistry, and omics science can give a clear understanding of plant cell wall ultrastructure. Better hold over this can help in lowering the cost of biomass saccharification as well as conversion, while significantly raising biofuel production yield [25]. It has been reported that genetic engineering is an effective method for giving plants particular traits that generate desired expressions of genes that can be further put into use, to improve resistance to biotic and abiotic factors, and improve parameters like grain quality, plant growth, the composition of biomass, and enzymatic digestibility [26]. To maximize value through breeding and genetic engineering, it is important to determine each crop's cell wall composition before adopting it as a feedstock for industrial processing. Plant cell walls can be genetically modified in such a way that it reduces biomass resistance(recalcitrance) during pretreatment and enzymatic hydrolysis and facilitates the easy synthesis of bioethanol and other biobased products. Various genes and protein alterations are carried out at the structural level (plant cell walls) that enhances cell wall polymer synthesis, its breakdown, and regulation which plays a crucial role in biorefineries [27]. Genetic engineering can be used to boost the concentration of cellulose, thereby decreasing the expenses of cellulase enzymes, and eliminating the requirement of various pretreatment processes [28]. The surface area of cellulose can be increased, its microfibril structure and polymerization may be reduced, its efficiency to which enzymes cleave the cellulose polysaccharide into simple glucose can be improved, and as a result, sugar production yields can be increased [25].

This chapter intends to examine current advances in plant cell wall genetic modification with an emphasis on utilizing it as an approach to enhance the characteristics of energy crops and agricultural waste derived from different plants for the generation of biorefined products.

## II. THE SIGNIFICANCE OF TRANSFORMING THE COMPOSITION OF POLYSACCHARIDES DURING BIOREFINERY

The primary components of lignocellulosic or plant biomass are cellulose, hemicellulose, lignin, and pectin, which are found in larger quantities in dicot plants [29]. In biorefinery, various types of biomasses are selected based on their content and structure. Cell walls of woody and grassy biomass are made up of three types of polymers: cellulose (35-40%), hemicellulose as well as other structural polysaccharides together constitutes (20-35%), and lignin (15-20%)[30], [31]. Cellulose microfibrils are anchored within the hemicellulose matrix and form chemical connections with it. Hemicellulose forms a chemical bonding with lignin. There are no linkages between cellulose and lignin [32], [33]. To successfully manage the polymers and produce a more suitable crop for biorefinery, it is important to understand how the cell wall composite frame is synthesized and hydrolysed.

### A. Modifying Cellulose

Cellulose $(C_6H_{10}O_5)_n$ is the most prevalent abundant polysaccharide on Earth and the primary structural component of plant cell walls [19]. Cellulose, which makes up about 40–60% of the weight of LB polymers, is composed of cellobiose as the primary repeating unit and ß-D-glucopyranose units that are connected by ß-(1,4) glycosidic linkages. The cellulose chains, which contain 500–1400 D–glucose units, these cellulose chain structure themselves so they form microfibrils, which are then coiled to form cellulose fibrils and held together by both intramolecular hydrogen bonds and intermolecular van der Waals forces. [34]. It is responsible for giving plants their tough outer layer. It is a significant coating that shields the internal tissues and cells of plants, ensuring that they remain robust both throughout growth and for the duration of their lives. Cellulose is also known as the backbone unit of plants [35].

Cellulose fibrils are incorporated in a lignocellulosic matrix, making them very resistant to enzymatic degradation. Cellulose and hemicellulose bind to produce a cellulose-hemicellulose complex, which also inhibits hydrolysis rates. As a result, alterations to both the composition and the structure of plant cell walls can increase cellulose surface area, it will decrease polymerization and microfibril crystallinity, also it will improve degrading enzyme efficiency. So, ultimately modifying cellulose will increase saccharification yields [36].

### B. Modifying Hemicellulose

Hemicelluloses $(C_5H_8O_4)_n$ are another form of polysaccharide found in plant cell walls. They are responsible for both cell wall structure and cell growth regulation. Unlike cellulose, these types of polymers possess an amorphous structure. The most common hemicelluloses include xylans, xyloglucans, glucomannans, mannans, and galactomannans [37]. These polysaccharides are composed of simple sugars including D-glucose,

D-xylose, D-galactose, D-glucuronic acid, L-arabinose, and D-mannose [38]. Hemicelluloses form bonds with lignin after wrapping around cellulose fibrils. Their structures affect the yield of sugar during enzymatic hydrolysis because they reduce cellulose accessibility to hydrolysis [39], [40]. Because of its complex structure, hemicellulose, in addition to lignin, contributes to biomass recalcitrance. It forms a cellulose-hemicellulose complex structure that must be hydrolyzed by a pretreatment procedure to make cellulose easily available to enzymes, boosts hydrolysis yields, and thereby ethanol yields[25], [41].

In grasses and dicotyledons, the major hemicellulose is xylan, which hydrolyzes to xylose (pentose). However, pentose conversion to ethanol is less effective than hexose conversion, making genetic modification an important technique for reducing xylan and increasing hexose/pentose composition in plant cell walls [42]. Hemicelluloses serve as an internal barrier that restricts enzyme accessibility. It has been suggested that removing hemicelluloses via steam explosion or diluted acid pre-treatment could boost cellulose conversion by increasing the enzymes' accessibility to cellulose [43]–[45].

### C. Modifying Lignin

Hydrophobicity and structural stiffness are properties of lignin. Syringyl (S), p-hydroxyphenyl (H) and guaiacol (G) monomers make up the lignin polymer. In the cell wall, lignin holds hemicelluloses to cellulose. It is generally recognized that lignin impacts the conversion of cellulose to simple sugars negatively and is affected by a number of variables, including total lignin concentration, lignin composition/structure (especially the presence of hydroxyl groups and S and G monomeric units), and more [46]. Primarily, lignin acts as a physical barrier that prevents enzymes from accessing cellulose, hence physically limiting the accessibility of polysaccharides. Secondly, its hydrophobic structural features, which include hydrogen bonds, methoxy groups, as well as polyaromatic structures, make it irreversibly adsorb cellulases along with other enzymes during enzymatic hydrolysis [47], [48].

According to studies, cellulases are reversibly inhibited by phenolic hydroxyl groups, which are lignin-derived substances [49]. By using a chemical treatment with chemicals like hydroxypropylation, free phenolic hydroxyl groups are made unavailable, and it was observed that the inhibitory action of lignin decreased significantly (by 65–91%) [50]. The S/G ratio in lignin plays a crucial role in imparting recalcitrance to the cell wall but modifying the S/G ratio eases the accessibility of cellulolytic enzymes to reach the cellulose [51]. As a result, various alternative methods, to develop transgenic crops having genetic modification, have been studied over time for lowering the degree of lignification of lignocellulosic biomass and to enhance the conversion process for the generation of bioethanol. Successful lignin genetic engineering enhanced the physiological characteristics of biofuels and the accessibility of residual cellulose to the hydrolytic enzymes [52]. Furthermore, it is found that compared to control biomass, transgenic biomass has a high cellulose dosage and doesn't need a thorough pretreatment. Transgenic lines which are not given any pretreatment procedure produce more fermentable sugar as a result of higher saccharification yields than biomass controls with pretreatment [53], [54]. Figure 2 depicts the factors affecting conventional biorefineries and biorefineries with bioengineered crops with controlled factors.

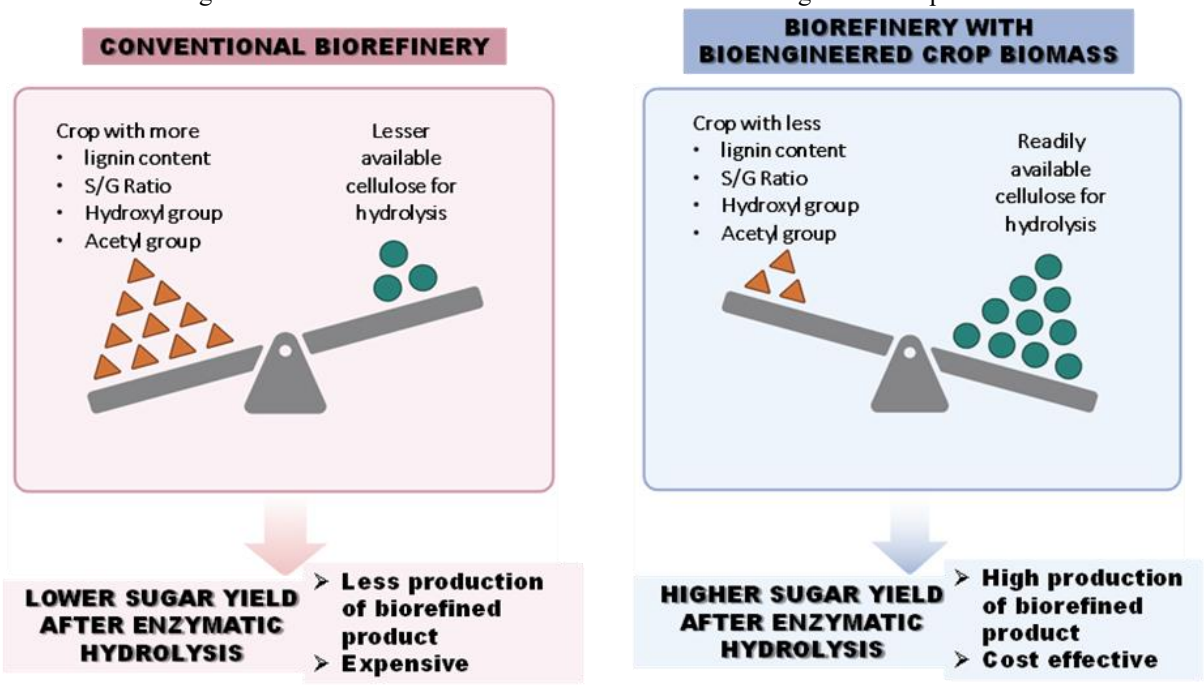


**Figure 2: Factors affecting conventional biorefineries and biorefineries with bioengineered crops**

## III. EXISTING APPROACHES TO ENHANCE POLYSACCHARIDES COMPOSITION

By eliminating the need for pretreatment, increasing the amount of cellulose, lowering the quantity and cost of cellulase enzymes, and engineering fermentative microorganisms to use pentose sugars for improved bioethanol yield, genetic modification is a desirable tool that can play a significant role in overcoming the limitations in biorefineries.

Plants that have been genetically modified to produce more biomass also produce more polysaccharides, which further enhances the productivity of biorefinery. Improved levels of cellulose in cell walls can be achieved by redirecting crop carbon resources from the formation of lignin to cellulose or hemicellulose. For instance, downregulating 4-coumarate CoA ligase increased cellulose content by 15% and decreased lignin content by 45% in aspen (*Populus tremuloides*). Coniferaldehyde 5-hydroxylase was suppressed in aspen, which further decreased the lignin concentration to 52% and increased the cellulose level to 30%. When cinnamoyl CoA reductase was downregulated in tobacco, the amount of glucose and xylose in the cell wall increased significantly, but the amount of lignin decreased [55].

A different strategy to increase plant biomass (and subsequently, cellulose) is by genetic alteration of plant growth regulators.

Delaying flowering is another method for increasing biomass. It was expected that suppressing flowering genes would increase biomass production since delayed flowering leads to improved vegetative development. 'Flowering locus C' (FLC), a floral repressor gene, was discovered in Arabidopsis. FLC is regulated by a number of genes, which in turn leads to an assembly of flower promotion genes known as "floral pathway integrators". FLC suppresses these floral pathway integrators, thus delaying flowering. Plants that overexpress the FLC gene have increased biomass yield and a longer period of vegetative growth [56]–[58].

Some studies attempted to enhance biomass production by increasing the number of essential nutrients deficient in the soil, such as phosphorous. When the only supply of phosphorus in the soil was 2mM (millimolar) phytate, then transgenic Arabidopsis with the purple acid phosphatase gene from *Medicago truncatula* generated two times as much biomass [59]. Some of the existing techniques used for crop improvement programmes are discussed below:

**A. RNAi**

RNA interference, also known as RNAi, is a powerful gene-silencing technique used in various biological systems, including plants. RNAi is being used to understand how genes work. Efforts are made using RNAi to decrease both the overall lignin content and the S/G as a result of RNAi suppression or a knock-out mutation of COMT (Caffeic acid O -methyl transferase (COMT) which converts 5-hydroxy-coniferyl alcohol into synapyl alcohol, which forms a part of the S subunit). Sugarcane, switchgrass, corn, sorghum, and alfalfa are examples of crops with altered lignin levels and composition as a result of COMT suppression/mutagenesis [60]–[63]. As a method of increasing plant biomass miR156, which is conserved in all angiosperms, is a promising option for modification of miRNA levels of expression [64]. Poplar plants with transgenic lines overexpressing miR156 showed shorter internode lengths and a 30% reduction in stem lignin [65]. Plants overexpressing miR156 such as Alfalfa produced more biomass and had shorter internodes and thinner stems [66].

**B. Marker-assisted selection (MAS)**

Marker-assisted selection (MAS) is the process of choosing cultivars based on molecular markers for particular genes of interest. MAS could speed up and simplify the process of choosing target traits for breeders when used as a necessary breeding tool. Because it quickly and effectively identifies desirable features, MAS is an especially attractive alternative. Markers are specific DNA sequences that are positioned close to the target gene. The markers are believed to remain associated with the targeted gene in subsequent generations since they are highly preserved during reproduction processes. Single nucleotide polymorphisms (SNPs), amplified fragment length polymorphisms (AFLP), random amplified polymorphic DNA (RAPD), and restricted fragment length polymorphisms (RFLP) are all types of DNA markers. Over the past few decades, crop biomass yield has improved significantly as a result of marker-assisted breeding [67].

**C. Somaclonal variation**

Molecular changes to DNA that take place during in vitro development ultimately give rise to somaclonal variation, which is equivalent to spontaneous mutations. The regeneration systems, explant tissue type (differentiated or meristematic tissues), medium components (type and degree of concentration of growth regulators), and in vitro culture conditions can all contribute to this phenotypic diversity. Genetic variation can be enhanced by somaclonal variation [68].

**D. Mutation breeding**

Inducing mutations using radiations like X-rays and gamma rays along with a variety of chemicals is another method for creating an entirely novel plant with desired features. Due to the mutation's inherent inability to determine whether a trait is favourable or negative so in order to determine which seeds carry the required features afterwards, a large number of seeds must be subjected to mutation conditions [69].

**E. Somatic hybridization**

A novel hybrid plant can be developed by fusing the somatic protoplasm collected from different plants. After the cell wall has been removed (which is often accomplished by using several enzymes), protoplasm is

accessed. By using normal reproduction, the hybrid plants developed via this technique can produce new generations.

**F. Protoplast Fusion**

A cutting-edge method for transferring genes to produce a desired level of quality and quantity is protoplast fusion. In this method, parasexual hybrid protoplasts are generated by fusing two separate genetically derived protoplasts derived from various somatic cells. It is possible to transfer genes from one species to a different species that reflect beneficial features including increased biomass productivity, enhanced protein quality, and better tolerance to heat and cold. When sexual crossings are not possible, the process of protoplast fusion which is followed by SE (somatic embryogenesis) regeneration can be used [70].

**G. Polyploidy**

When a plant contains more than two sets of chromosomes, it is said to be polyploid. Two fundamental processes give rise to polyploidy firstly meiotic division abnormalities that occur naturally and secondly the mitosis disruption brought on by antimitotic agent cause polyploidy. The most frequent antimitotic for causing polyploidy in plants are colchicine, oryzalin, and trifluralin.

A method of plant breeding is called polyploidy breeding which makes use of polyploid plants in order to produce novel hybrid plants. It can be used to create new plant hybrids with enhanced traits, such as increased disease or pest resistance or higher yields. Additionally, it can be used to create novel crop varieties that are better suited to particular soil types or climates. Polyploidy in plants affects cell size, quantity, and growth vigour; as a result, plants may perform better at accumulating biomass. Two fundamental processes firstly meiotic division abnormalities that occur naturally and secondly the mitosis disruption brought on by antimitotic agents cause polyploidy. Over the years, the biomass and sugar content of sugarcane has significantly increased as a result of polyploidisation [71].

**H. Transgenesis**

Transgenesis is the technique of introducing one or more genes from one plant into a different plant. This often involves isolation of the DNA from one species, altering it, and then its insertion into the new species. There are numerous techniques to 'transform' or introduce a new gene into a plant. Direct gene transfer techniques are based on physical (particle gun bombardment, microinjection, electroporation methods), chemical (lipofection, polyethylene glycol (PEG)-mediated, etc.), and vector-mediated gene transfer techniques are based on Agrobacterium plasmid and plant viruses. There are a number of steps during the transgenesis process, including the isolation of the desired gene, vector construction, transgenesis techniques, transgene integration, and transgene inheritance [72].

**i. Electroporation**

This method of protoplast transformation involves converting plant cells into protoplasts (cells that are without a cell wall), which makes it easier for DNA to enter the cells. This is done by temporarily destabilizing the cell membrane with an electrical impulse [68].

**ii. Microinjection**

A glass pipette used for microinjection is used to inject DNA into the cells. Although this method is extremely beneficial for introducing complete genetic information into plant cells, it isn't as frequently employed in plants as it is in animals, mostly because it is not economical. Costly equipment is needed for the tedious process of microinjection [73].

**iii. Micro-projectile bombardment**

In microprojectile bombardment, also known as biolistics, targeted plant cells or tissues are injected with naked DNA using high-velocity microprojectiles (such as gold micro-particles). This approach has been widely employed, particularly with species like corn and rice in which Agrobacterium cannot be used [74].

**iv. Agrobacterium Mediated transformation**

Agrobacterium Mediated is the preferred approach for transformation it is mediated by the bacterium Agrobacterium tumefaciens, which is due to its extraordinary ability to introduce a DNA fragment via a unique plasmid into the host cell. Because of its ease of use, capacity to transfer a single copy of the desired gene, and effectiveness in transferring a large fragment of DNA within the host cell—all at a very low cost—this technique is widely accepted among researchers. With the aid of plant tissue culture, which allows the rapid regeneration of multiple transgenic plants at one time, the agrobacterium-mediated transformation of genes has been used to modify numerous economically significant crops to enhance yield, disease resistance, and improved nutritional quantity, and biomass composition [75].

Researchers have been able to enhance the multiple traits in plants in the past few decades by utilizing a range of techniques as mentioned above. These approaches for crop improvements have become extremely helpful methods for increasing plant yields, and they are currently being used to boost plant biomass and change the characteristics of plant cell walls to improve the effectiveness of lignocellulosic biomass in biorefineries. But these existing techniques have some limitations such as they are time-consuming, do not allow for accurate genome modification, also it does not allow for manipulation of how genes are regulated in a specific genomic region. Understanding of the functional genomics in an organism is limited by these approaches. But on the other

hand, next-generation techniques such as genome editing and omics approaches have generated considerable interest among agricultural researchers due to their simplicity, accuracy and power, as they provide new possibilities to create improved crop varieties by directly adding beneficial traits or eliminating undesirable traits.

## IV. NEXT-GENERATION STRATEGIES TO BOOST THE COMPOSITION AND CHARACTERISTICS OF POLYSACCHARIDES IN BIOMASS

Most existing breeding approaches are time-consuming, labour-intensive and challenging. Next-generation strategies have made it much easier to create new and improved varieties with improved agronomic characteristics such as resistance to disease, abiotic stress, shelf life and improved crop productivity [76]. With the advent of new plant breeding technologies, it has become possible to precisely understand and alter the plant genome without the use of foreign DNA [77]. So, it is really important to use these strategies to create new and better crop varieties to get around the issues that come with biorefineries while handling the crops produced by traditional breeding methods [78]. Omics technologies can also facilitate the development of agricultural research in the areas of food, health and energy. It also helps in contributing to the preservation, improvement and remediation of the environment in an innovative and time-consuming method. Omics technologies concentrate on the traits of interest with accuracy. They have the potential to improve the production of cellulose in energy crops.

### A. Genome editing strategies for modifying the composition of polysaccharide

#### i. Genome editing techniques

A set of techniques for introducing desired alterations such as insertion, deletion or replacement of DNA at certain genomic loci is referred to as "gene editing". Gene editing uses sequence-specific nucleases (SSNs) to create double-strand breaks (DSBs) at specific genomic sites. The desired changes are being made by subsequent non-homologous end joining (NHEJ), microhomology-mediated end joining (MMEJ), or homology-directed repair (HDR) [79]. The engineering of unique zinc-finger nucleases (ZFNs) [80] or meganucleases [81] has been the research emphasis during the early stages of genome editing in order to generate the necessary DSBs at each specific DNA target site. These nuclease systems required specialised expertise to produce synthetic proteins with DNA-binding domains that could be tailored to specific sequences and coupled to non-specific nucleases for target cleavage, giving researchers unheard-of tools for genetic manipulation. Transcription activator-like effectors (TALEs), a new class of catalytic domains from the *Flavobacterium okeanokoites* (FokI) family of bacterial proteins, have opened up new avenues for precise genome editing. Any DNA sequence of interest can be cleaved with a fair amount of frequency by TALE-based programmable nucleases. The creation of a sophisticated molecular clone for each novel DNA target and its poor efficacy of genome screening in successfully targeted cells, however, pose the biggest obstacles for transcription activator-like effector nucleases (TALEN) techniques [82]. CRISPR/Cas9 (clustered regularly interspaced short palindromic repeat (CRISPR)-associated 9) is a recently identified, powerful gene editing tool having its origin as a bacterial adaptive immune system [83]. This method has emerged as a viable substitute for ZFNs and TALENs to induce targeted genetic alterations since it can be successfully programmed to edit the genome of eukaryotic cells via an RNA-guided DNA cleavage module [83]. Cas9 nucleases are RNA-guided DNA endonucleases that cause DSBs at target locations and are part of type II CRISPR-Cas systems. To cleave the target and non-target strands, respectively, Cas9 possesses two different nuclease domains, HNH and RuvC [84]. A Cas9 nickase (nCas9) that cleaves only one DNA strand is produced when either nuclease domain is inactivated. Dead Cas9 (dCas9) is produced when both nuclease domains are inactivated but still attach to the target DNA [85]. Base editors and prime editors can execute precision genome editing without the need for DSBs using nCas9 [86]. dCas9 functions as a scaffold for enlisting effectors close to certain targets. Type II CRISPR-Cas systems contain Cas9 nucleases, which are RNA-guided DNA endonucleases that cause DSBs at target locations [87], [88]. To cleave the target and non-target strands, respectively, Cas9 possesses two different nuclease domains, HNH and RuvC[84], [88]. A Cas9 nickase (nCas9) that can only cleave one DNA strand is produced by the inactivation of either nuclease domain. Dead Cas9 (dCas9) is produced by inactivating both nuclease domains and continues to attach to the target DNA [85]. Base editors and prime editors can execute precision genome editing without the need for DSBs using nCas9 [86], and dCas9 acts as a scaffold for enlisting effectors close to particular DNA sequences. dCas9 acts as a scaffold to draw effectors close to particular genetic locations. Widespread applications of dCas9 include controlling transcription, modifying epigenetic regulations, imaging living cells, and other activities [89]. In addition to revolutionising plant biology, genome editing (GE) offers a way to address issues with plant architecture, food security, nutrient content, environmental adaptation, disease resistance, and the manufacture of plant-based products. Here we will discuss regarding use of genome editing strategies for the modification of the composition of polysaccharides.

The primary sources of sugar in the cell wall are cellulose and hemicellulose, which are also the most useful components of lignocellulosic biomass for the manufacture of fuels, industrial chemicals, and materials [90]. Due to lignin's protective coating, which provides a constrained surface area for enzymatic and chemical hydrolysis, the use of this sugar source in lignocellulosic materials is subject to a number of limitations [91]. The production of lignocellulosic biomass that is suitable for the pulp, paper, and textile industries as well as biofuel and easily digestible forage was enhanced by manipulating the composition of lignin and lowering its content in plant cell walls [92]. By downregulating/knocking-out the lignin biosynthesis genes and regulatory transcription

factors, various genetic and molecular approaches have been used on lignocellulosic biomass to decrease lignin content and alter its composition.

**ii. Gene editing in plant cell walls**

Forage plants (maize, sorghum, rice, and lucerne) have been the subject of various bioengineering and GE research to reduce lignin while simultaneously increasing cellulose content [93]. Several forage crops have been successfully modified using CRISPR/Cas9-based mutagenesis to create stable mutations in genes involved in lignin production. In a study of rice mutants with frameshift mutations in the p-coumaroyl ester 3′-hydroxylase (C3′H) gene, which is involved in the manufacture of both lignin and chlorogenic acid, Takeda et al. (2018) [94] compared the effects of CRISPR and RNAi. The CRISPR-derived C3′H-knockout mutants were severely undersized and sterile in comparison to the mutants resulting from RNAi-mediated C3′H-knockdown. The study's findings made it abundantly evident how the rice plant's lignin composition and other cell wall components are assembled in response to C3′H suppression. According to reports, these structural changes in rice are very helpful for improving biomass digestibility and saccharification. The same study team examined the effect of conifer aldehyde 5 hydroxylase (OsCAld5H1) gene suppression on rice lignin structure, which modifies the ratio of syringyl (S) to guaiacyl (G) lignin composition [95]. Figure no 3 shows the opportunities and challenges in polysaccharide modification with genome editing techniques.

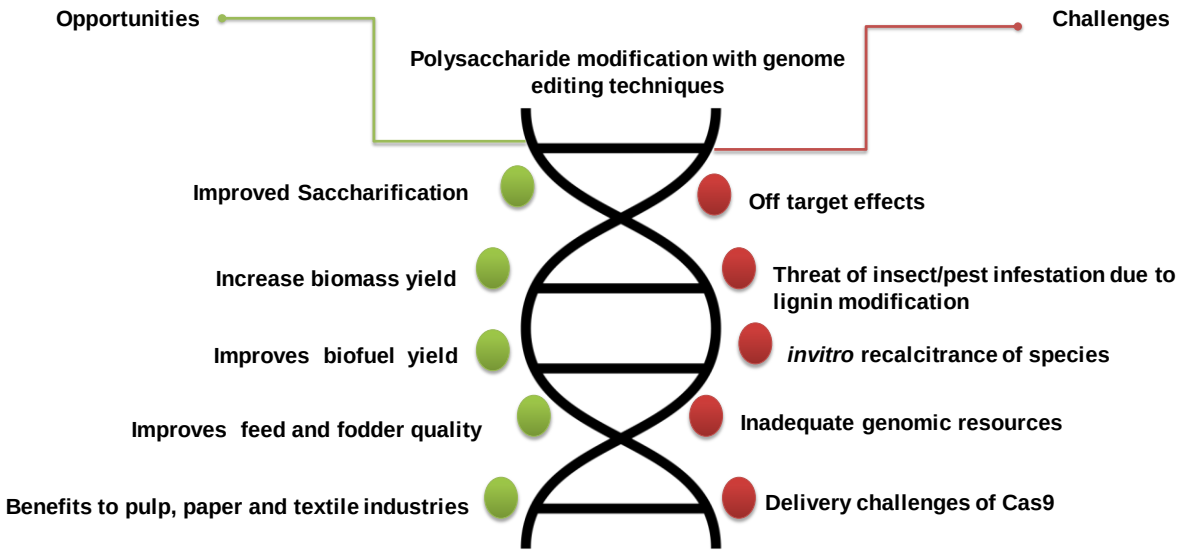


**Fig 3.** Opportunities and Challenges in polysaccharide modification with genome editing techniques

To effectively down-regulate the genes involved in the lignin biosynthesis pathway in poplar species, a CRISPR-based gene knockout and silencing strategy was used. Fan et al. (2015) [96] reported creating the first very effective, stable CRISPR-based genome-edited poplar. The main focus of the GE experiments in poplar is lignin production via phenylpropanoid metabolism and cell wall characteristics. By targeting three 4-coumarate: CoA ligase genes (4CL1, 4CL2, and 4CL5), the mutational effectiveness of CRISPR-Cas9 was examined on the biosynthesis of lignin and flavonoids in the woody perennial *P. tremula* x alba [97]. According to the findings, 4CL1 and 4CL2 are crucial for the production of lignin and flavonoids. Mutations in the 4CL1 gene showed a decrease in lignification, but the 4CL2 gene was found to be important in the formation of chlorogenic acid in leaves. The 4CL gene's CRISPR/Cas9-mediated mutation in the same poplar study resulted in a 20% reduction in lignin content and a 30% fall in the S/G ratio. Importantly, homogeneous reddish-brown wood, a trait linked to lignin deficit, appeared in every independent 4CL1 line.

To lower lignin content, CRISPR-based knockout experiments targeting the MYB transcription factors in poplar trees were carried out. These studies showed that some MYBs (PtoMYB156, PtoMYB115, and PtoMYB170) negatively regulated phenylpropanoid metabolism and secondary cell wall biosynthesis, whereas other MYBs (PtoMYB156, PtoMYB57, and PtoMYB170) increased proanthocyanidin biosynthesis, lignification, and flavonoid accumulation [98]–[101]. PtoDWF4 gene knockout was created in poplar trees using CRISPR/Cas9 approach, demonstrating the gene's critical function in secondary cell wall synthesis and wood development [102].

In order to effectively develop lignin double mutants in biomass crops, genome editing techniques were used. For instance, TALEN was effectively used in sugarcane, a crop that produces the majority of the world's ethanol and accounts for roughly 80% of all sugar produced. P-hydroxyphenyl (H), guaiacyl (G), and syringyl (S)

monomers make up the lignin polymer. Caffeic acid O-methyl transferase (COMT), converts 5 hydroxy coniferyl alcohol to sinapyl alcohol which is part of the S subunit and is mediated via the phenylpropanoid route [103]. The S/G ratio and total lignin concentration are decreased as a result of COMT gene mutations that knockout the gene. The sugarcane COMT gene is targeted with TALEN to enhance cell wall composition and bioethanol synthesis [104]. In order to alter the lignin production in sugarcane, a conserved area of COMT was targeted with a single transcription activator-like effector nuclease (TALEN) pair for multiallelic mutagenesis. Field-grown TALEN-mediated COMT mutants demonstrated up to 19.7% lignin reduction and a much lower syringyl to guaiacyl (S/G) ratio, which led to an improvement in saccharification efficiency of up to 43.8%. Under field conditions, the ability of COMT mutants to produce biomass was not significantly different from that of the original cultivar [105].

The CRISPR/Cas9 method was used to create low-lignin switchgrass. With a 10% mutation efficiency, the CRISPR/Cas9 system was successfully established in switchgrass and the technique enables switchgrass knock-out mutant plants with lower lignin concentration and reduced recalcitrance by precisely targeting the chosen Pv4CL1 gene [106]. Plants having decreased activity of phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate: CoA ligase (4CL), p-coumaroylshikimate 30-hydroxylase (C30 H), p-hydroxycinnamoyl-CoA: shikimate-hydroxycinnamoyl transferase (HCT), caffeoyl CoA O-methyltransferase (CCoAOMT), and cinnamoyl CoA reductase (CCR) often shown a drop in the quantity of lignin [27]. Such kind of genes can be targeted in bioenergy crops with the use of facile genome editing tools.

The lignin biosynthesis pathway contains the enzyme CSE, which was only recently found [107]. The CSE1 and CSE2 are targeted in poplar with the use of CRISPR-Cas9 in two distinct investigations. In *Populus tremula 3* x *Populus alba* cv *717-1B4* double mutants, lignin and biomass yield were reduced, according to one study, although the corresponding single mutants had no influence on lignin or growth [108]. In contrast, the second investigation discovered that *P. alba 3* x *P. tremula var. glandulosa cv 84K* showed up to 16% lower lignin content without biomass reduction in both cse1 and cse2 single mutants [109]. Poplar lines with the LAC14 mutation caused by CRISPR-Cas9 had a greater biomass yield, a higher S/G ratio, and around 7% less lignin [110].

In order to reduce the amount of lignin in barley, Caffeic Acid O-methyltransferase 1 (HvCOMT 1), a gene involved in lignin biosynthesis, is targeted using CRISPR/Cas9 technology. Comparing the mutant to the wild-type (WT), the mutant had a 34% greater fermentable glucose recovery rate and a total lignin content that was 14% lower. The mutant biomass's hydrolysates produced bioethanol with a concentration and yield of 14.3 g/L and 0.46 g/g total sugar, respectively. In comparison to the results from WT (10.7 g/L and 0.41 g/g total sugar), this result was 34% and 12% higher [111].

In various genome modification experiments, the unfavourable traits of lignocellulosic biomass were the focus in order to produce plants that were more suited to bioprocessing. Therefore, in order to reduce the need for pre-treatment techniques and develop plants with comparatively lower lignin concentration, it is required to develop such lignocellulose-rich plants using genome editing techniques like CRISPR. Table1: Gives brief about the polysaccharide modification with the use of genome editing techniques.

**Table No. 1 Polysaccharide modification with genome editing techniques**

| **Crop** | **Gene targeted** | **Effect on polysaccharide composition** | **Editing method** | **Repair mechanism** | **Transformation method** | **Reference** |
|---|---|---|---|---|---|---|
| *P. alba 3 P. x glandulosa* | CSE1, CSE2 | Reduction in lignin content by 29.1%, improved Saccharification Efficiency by 25% than wild type | CRISPR/ Cas9 | NHEJ | *Agrobacterium* mediated transformation | [109] |
| *Populus tremula* × *P. alba* | CSE1, CSE2 | Double mutant of CSE1 and CSE2 shows reduction in lignin content by 35% | CRISPR/ Cas9 | NHEJ | Electroporation | [108] |
| *Saccharum spp.* | COMT | 29-32% decrease in lignin amount, decrease S subunit content with increase hemicelluloses content | TALEN | NHEJ | *Agrobacterium* mediated transformation | [63] |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Oryza sativa* | CAld5H1 | Lignin G unit enriched,considerable S unit production | CRISPR/Cas9 | NHEJ | *Agrobacterium* mediated transformation | [95] |
| *Panicum virgatum* | *4CL-1* | Decrease in cell wall thickness, reduction in lignin content by 8-30%, enhance sugar release | CRISPR/ Cas9 | NHEJ | *Agrobacterium* mediated transformation | [106] |
| *Hordeum vulgare* | COMT1 | 14% decrease in lignin composition and 34% higher fermentable sugar recovery | CRISPR/ Cas9 | NHEJ | *Agrobacterium* mediated transformation | [111] |
| *Oryza sativa* | XYN1 | Decrease lignin content and down regulation of genes responsible for xylan and lignin biosynthesis | CRISPR/ Cas9 | NHEJ | *Agrobacterium* mediated transformation | [112] |
| *Populus tomentosa* | *PtoMYB156* | Elevation in expression of gene responsible for secondary wall biosynthesis (*PAL1, 4CL5, C4H2, COMT2, CCR2, CAD1*), xylan (*GT43B*) and cellulose (*CESA2B*) | CRISPR/ Cas9 | NHEJ | *Agrobacterium* mediated transformation | [98] |
| *Arabidopsis thaliana* | CCR1 | Decrease in lignin content and enhanced saccharification efficiency | CRISPR/ Cas9 | NHEJ | Floral dip method | [113] |
| Sudan grass | COMT | Decrease lignin content and increase in biomass | CRISPR/ Cas9 | NHEJ | Particle bombardment | [114] |
| *Poplus alba* | CESA | Thinner cell wall, increase in hemicelluloses content, reduction in cellulose content, improved **saccharification efficiency** | CRISPR/ Cas9 | NHEJ | *Agrobacterium* mediated transformation | [115] |

**B. Omics studies for modifying the composition of polysaccharide**

**i. Omics science**

Omics refers to the exploration and analysis of large volumes of data that represent the biological system's structure and function at a specific level. It has significantly advanced the methodologies for investigating biological systems. This is largely due to the development of "omics", which combines "top-down" approaches with "bottom-up" strategies to provide a comprehensive tool for effective biological system investigation [116]. The term "omics" has become associated with a lot of different areas of study, and it's become a catchword that gets a lot of attention nowadays. Omics technologies enable the visualization or observation of all alterations that occur when the genetic, nutritional, or environmental conditions of an organism are altered, thus providing insight into changes in plant metabolism due to environmental interactions. Using omics, it is possible to identify the genes that are responsible for the proteins that produce or inhibit the desired characteristics. Once these genes are identified, they can then be modified in the plant or transferred from one species to another to create a transgenic plant with increased polysaccharide composition within the plant cell wall. Omics technology has increased the accessibility of high-throughput screening methods, accelerating the development of crop breeding in the direction

of a precise understanding of the relationships between genotypes and phenotypes and accelerating the rate at which agricultural traits can be improved. Genomics, proteomics metaproteomics, metagenomics, and metatranscriptomics studies in computational biology are primarily concentrating on the molecular basis behind the complex traits and expanding our understanding regarding the primary mechanisms governing crop physiology [117].

**ii. Omics science in Bioengineering energy crops**

The application of multi-omics methods in boosting biomass composition provides a comprehensive understanding of engineering energy crops. It enables a better understanding of the biological pathways behind the traits defining biomass. It also provides crucial information for the selection of varieties of energy crops suitable for biofuel production. The multi-omics studies consist of genomics, transcriptomics, proteomics, metabolomics, and phenomics to study improvement in biomass quality. Many of the multi-omics approaches are becoming a major tool in exploring the biological pathways associated with complex genotypic traits related to biomass. Moreover, it provides comprehensive knowledge about the role of the genes involved in biomass synthesis in energy crops. It helps in bridging genotypes with the phenotypes, and to identify the candidate genes involved in biomass component biosynthesis. These candidate genes can be utilized in several ways, such as reduction in recalcitrance of biomass and extracting biomass with high cellulose or less lignin. Thus, the ultimate goal of the multi-omics approaches is to improve biomass quality as well yield to bioengineer energy crops. Table 2 summarizes multi-omics approaches used for improving biomass quality in energy crops. Figure 3 explains the applications of the multi-omics approaches for bioengineering energy crops.

**Table 2. Multi-omics approaches to study improvement in energy crops**

| Omics Technological Approaches | Subject | References |
|---|---|---|
| Genomics, Phenomics | Cross-talk between genotyping and high throughput phenotyping to analyse crop yield | [118] |
| Metabolomics, Transcriptomics | Study of the genetic architecture of lignin biosynthesis pathway in *Populus*. | [119] |
| Transcriptomics, Proteomics | Lignocellulose degradation by various microbial communities | [120] |
| Metagenomics | Operational taxonomic unit (OTU) analysis determined specific microbial strains well adapted for degrading a particular lignocellulosic substrate. | [121] |
| Genomics, Transcriptomics | Genomic approaches to enhance biomass degradation by the industrial fungus ***Trichoderma reesei*** | [122] |
| Genomics | Genome-Wide Association Study (GWAS) for major biofuel traits in Sorghum using Minicore collection | [123] |
| Transcriptomics, Proteomics and Metabolomics | Metabolic network reconstruction and analysis of lignocellulosic carbon utilization in *Rhodosproidium toruloides* | [124] |
| Omics Technological Approaches: Different multi-omics technologies;<br>Subject: Description of the omics technology;<br>References: Related literature on the omics technology | | |

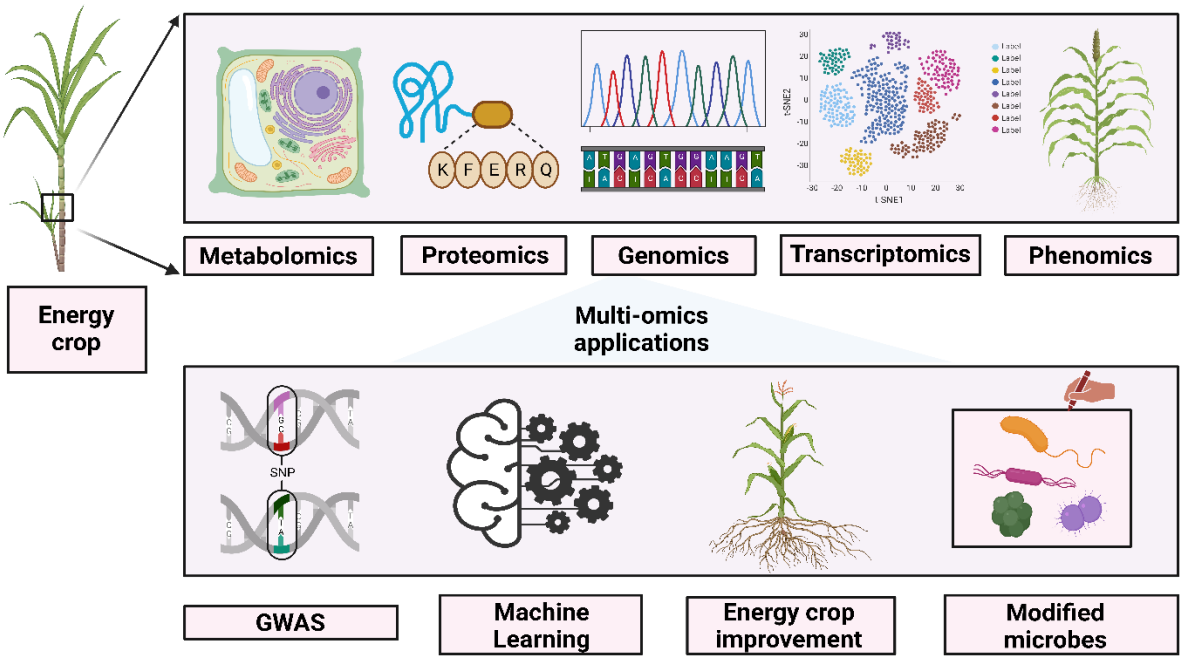


**Fig 3.** Applications of the multi-omics (Metabolomics, Proteomics, Genomics, Transcriptomics and Phenomics) approaches to bioengineering energy crops.

Multi-omics approaches are becoming a standard method for investigating the biological pathways underlying complex genotype traits in energy crops. It is enhancing our understanding of the functions of genes involved in biosynthesis biomass components. It includes searching the candidate gene responsible for desired lignocellulosic biomass that is richer in cellulose, less rich in lignin and weaker recalcitrance. The candidate genes can be used in genome engineering approaches for multiple purposes. Thus, the ultimate goal is to improve biomass quality and yield and optimize the conversion process.

Improvements in plant genetics and genomic technologies are accelerating gene discovery for product development. In this regard, several next-generation sequencing and high-throughput marker genotyping are currently used. In addition, these approaches, together with omics technologies (transcriptomics, genomics, metabolomics, and proteomics), have emerged as potential tools. It helps to understand genomic variation in energy crops at DNA, RNA, and protein level. It also includes the identification of genes affecting the expression of traits associated with biorefining applications. Thus, omics technologies also assist in developing more varieties with traits involved in generating higher value-added products.

The genes involved in the lignin pathway in *Populus deltoides* were identified and characterized by involving genomics, transcriptomics, and phenomics data [125]. They revealed that R2R3-MYB transcription factor MYB125 (Potri.003G114100) is a potential trans-regulator of a wide range of lignin biosynthesis pathway genes. Furthermore, exploring genomic regions associated with complex phenotypic traits is also made possible by GWAS (Genome-wide association studies). The presence of several novel genes including transcription factors that are linked with biomass yield and other bioenergy phenotypes has been reported [126]. They performed GWAS through a 12 K Illumina genotyping array obtained from 714 individuals of a European black poplar (Populus nigra L.). Moreover, significant marker-trait associations were uncovered across eight of the ten sorghum chromosomes, with two main hotspots near the end of chromosomes 7 and 9 [127]. Such studies aimed to identify genetic loci responsible for biomass yield and its related traits for breeding purposes through GWAS. Figure 4 shows ongoing research in multi-omics to improve the biomass of energy crops.

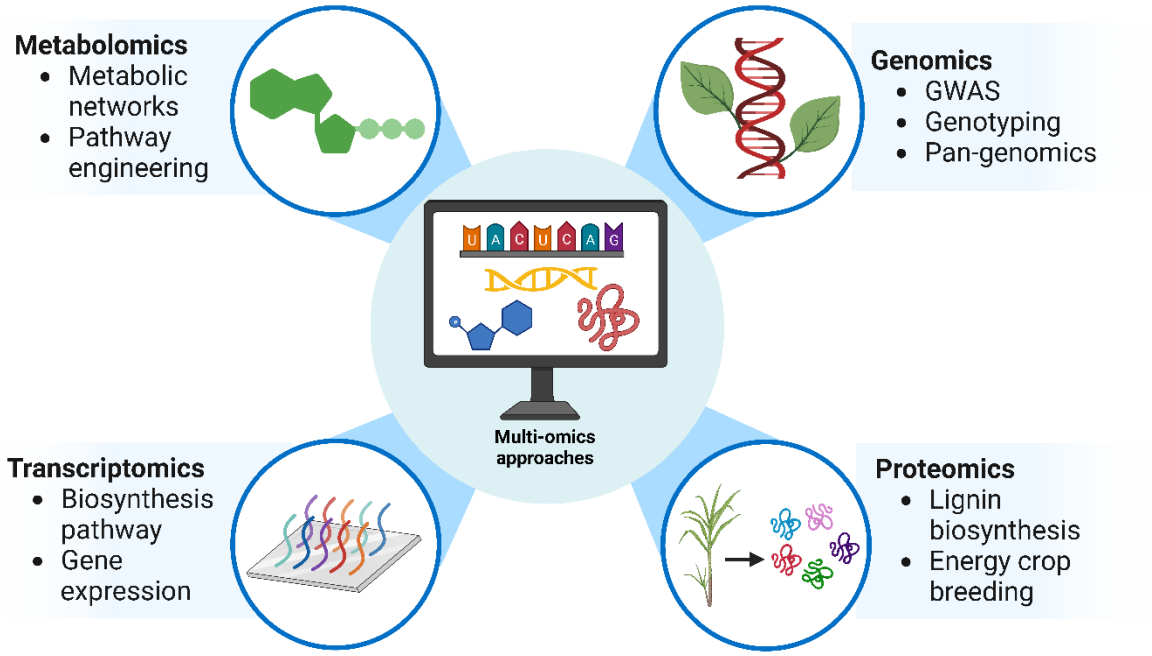


**Fig 4. Ongoing research in the multi-omics domain to improve the biomass of energy crops.**

Advancements in Next-Generation Sequencing (NGS) have led to increased research in multi-omic studies, including big data from various disciplines. The omics platforms are metabolomics, genomics, transcriptomics, proteomics, metagenomics, and phenomics. Researchers have reported evidence on identifying candidate genes that can be used for biomass genome engineering. Moreover, multi-omics approaches are used to enhance bioenergy crop biomass traits and improve microbial strains to degrade cell wall contents. In addition, the progress of research in the omics field provides insight into recognizing multidimensional analysis of how microbes react to a changing environment.

**iii. Machine Learning in plant omics**

The term "big data", "machine learning," and "AI" are just a few of the terms that describe modern computer processes. Big data refers to the utilization of large amounts of data of various types and intricate patterns that cannot be adequately analysed through traditional methods. In the context of big data, artificial intelligence (AI) is used to train a computer to carry out tasks that are beyond the capabilities of humans, particularly when taking into account the time and effort required, which are often involved in making decisions in a wide range of contexts. Machine learning, on the other hand, is the branch of artificial intelligence in which computers are trained to identify relationships from large training datasets. Machine Learning (ML) has been employed in the fields of genomic screening, genomic prediction, and marker-assisted selection.

Recently, machine learning (ML) approaches have been identified to be effective for the future of plant omics integration data research. Recently, computer-aided tools and mathematical models are reported that can be used in systems biology [127]. A comprehensive understanding of cell biology from subcellular levels to the entire organism can be performed by systems biology be a holistic method. It includes genomics, transcriptomics, proteomics, and metabolomics. These omics studies need data handling, annotation of biomolecules, statistical power analysis, data archiving, and sharing. However, a single multi-omics approach cannot handle the full complexity of the living system. Hence, ML methods and multi-omics approaches together can be a potential way to perform precision breeding for energy traits of interest for enhanced biofuel production.

**iv. Artificial Intelligence (AI) in biorefinery**

AI can improve the likelihood of finding true optimal genotypes by concentrating on existing breeding material that has the potential to produce high-quality traits. The integration of AI into agriculture has been slow to take place, but plant breeders and geneticists predicting an agricultural revolution through the application of computer science techniques in agriculture. High-throughput genomics and phenomics, as well as improved breeding, have all been accelerated by the application of AI technologies in agriculture. Furthermore, Artificial Intelligence (AI)-based ethanol fermentation forecasting has been done using yeast phenomics data [128]. Using image processing software, yeast morphological images were acquired using a nonstaining protocol and extracted high-dimensional morphological data. They concluded that the neural network algorithm produced the best performance for predicting ethanol fermentation in biorefinery. However, significant progress has been observed in the multi-omics domain, but more complex mechanisms behind the improvement of biomass composition are

needed to be explored. Therefore, innovative technologies based on multi-omics studies are a future direction to produce bioproducts through energy crop improvement in breeding programs.

## V. CONCLUSION

Increasing the cellulose content of biomass through the transformation of lignocellulose biomass using next-generation technologies will lead to an increase in the available biomass composition. This will thereby enhance the cellulose content and thus improve the production in biorefinery for the production of bioethanol, biopolymers, biohydrogen, etc. Also, such advanced modification can be used to alter the structure and quantity of lignin in the biomass, which may reduce or eliminate the requirement for pretreatment. This will make the cell walls of plants less resistant and it will be simple to hydrolyse the biomass, which will minimize or eliminate the requirement for strong pretreatment techniques. Ultimately this will increase the available space and volume for the enzyme to act thus enhancing lignocellulolytic enzyme efficiency. Also, it will make biorefineries cost-effective by reducing the need for enzymes.

## VI. FUTURE PERSPECTIVES

Conventional technologies along with existing molecular biology techniques have advanced the understanding of the molecular basis of crop physiology. However, further research is required to fully understand the molecular, cellular and metabolic substrates of plant species especially governing the polysaccharide traits. Several attempts have been made to determine the role of a particular molecular process in cell wall expression within the plant breeding and genetics field, but none of these methods has been able to predict metabolic pathways responsible for efficient polysaccharide content across a wide range of species. However, recent studies on plants have demonstrated that genome editing and omics technology that modifies genetic, epizootic, regulatory and metabolic processes which is responsible for a large amount of genetic variation. The omics technology will be easily presented if researchers continue to work on the development of this field in conjunction with machine learning and computational biology and mathematical/statistical models, as well as the use of cutting-edge genome editing techniques such as TALENs and CRISPRs to create hybrids plants with richness in polysaccharide composition. Future research should be focussed on reducing the need for expensive lignocellulolytic enzymes by attempting to genetically modify plants to produce enzymes that break down cell walls. this will further make the biorefinery economically viable by using in-plant enzymes instead of microbial synthesis.

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