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CRISPR/Cas9 in Poplar Lignin Biosynthesis: Advances and Future Prospects

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Abstract The CRISPR/Cas9 system has emerged as a revolutionary tool for genome editing, offering unprecedented precision and efficiency in modifying genetic material. This systematic review focuses on the application of CRISPR/Cas9 technology in the biosynthesis of lignin in poplar species, highlighting recent advances and future prospects. Lignin, a complex polymer in the cell walls of plants, plays a crucial role in providing structural integrity and resistance to pathogens. However, its recalcitrance poses challenges for industrial processes such as pulping and biofuel production. Recent studies have demonstrated the potential of CRISPR/Cas9 to target and modify genes involved in lignin biosynthesis, thereby reducing lignin content and altering its composition to enhance industrial utility. Several research efforts have successfully employed CRISPR/Cas9 to edit lignin biosynthesis genes in poplar. For instance, the efficient knockout of the phytoene desaturase gene in *Populus alba* × *Populus glandulosa* using a single guide RNA (sgRNA) has shown promising results in generating targeted mutations with high efficiency. Similarly, the application of CRISPR/Cas9 in *Populus tomentosa* Carr. has demonstrated the system's capability to create precise genomic edits, resulting in significant phenotypic changes. Moreover, studies have evaluated the efficiency of various guide RNAs (gRNAs) in poplars, identifying key factors that influence gene editing success, such as GC content and the accessibility of the seed region. The review also discusses the broader implications of CRISPR/Cas9 technology in plant research, including its potential to enhance disease resistance, improve nutritional content, and develop drought-tolerant varieties Despite these advancements, challenges such as off-target effects and the need for efficient delivery methods remain. Future research directions include the development of high-fidelity Cas9 variants and the optimization of delivery systems to minimize off-target modifications and enhance editing efficiency.

Keywords CRISPR/Cas9; Poplar; Lignin biosynthesis; Genome editing; Phytoene desaturase; Guide RNA; Gene knockout; Plant biotechnology

1 Introduction

Lignin is a complex phenolic polymer that is integral to the structural integrity of plant cell walls, particularly in woody species like poplar (*Populus* spp.). The biosynthesis of lignin involves a series of enzymatic reactions that convert phenylalanine into monolignols, which are then polymerized into lignin. Key enzymes in this pathway include caffeoyl shikimate esterase (CSE), cinnamoyl-CoA reductase (CCR), and 4-coumarate:CoA ligase (4CL) (Tsai et al., 2019; Jang et al., 2021; Meester et al., 2021). These enzymes are crucial for the formation of guaiacyl (G) and syringyl (S) lignin units, which contribute to the rigidity and resistance of the plant cell wall.

Lignin's recalcitrance to degradation poses a significant challenge for the efficient conversion of lignocellulosic biomass into biofuels and other bio-based materials. Reducing lignin content or altering its composition can enhance the saccharification efficiency, thereby improving the yield of fermentable sugars (Meester et al., 2020; Jang et al., 2021; Meester et al., 2021). For instance, CRISPR/Cas9-mediated knockout of the CSE gene in hybrid poplar has been shown to reduce lignin content by up to 29.1%, significantly improving saccharification efficiency without affecting plant growth (Jang et al., 2021). Similarly, vessel-specific lignin biosynthesis has been employed to mitigate growth defects while maintaining high saccharification yields (Meester et al., 2021).

The CRISPR/Cas9 system has revolutionized genetic engineering by enabling precise, targeted modifications in the genome. This technology utilizes a guide RNA (gRNA) to direct the Cas9 endonuclease to specific DNA sequences, where it introduces double-stranded breaks that can be repaired to create mutations or insertions (Bortesi and Fischer, 2015; Fan et al., 2015; Arora and Narula, 2017). In poplar, CRISPR/Cas9 has been successfully used to edit genes involved in lignin biosynthesis, such as CSE, CCR, and 4CL, demonstrating its potential for improving lignocellulosic biomass for biofuel production (Tsai et al., 2019; Meester et al., 2020; Jang et al., 2021; Meester et al., 2021).

This systematic review aims to consolidate recent advancements in the application of CRISPR/Cas9 technology for modifying lignin biosynthesis in poplar. By synthesizing current research, this review will offer valuable insights into the potential of CRISPR/Cas9 technology to transform lignin biosynthesis in poplar, paving the way for more efficient and sustainable biofuel production.

2 Review on Research Background

2.1 Lignin biosynthesis pathway in poplar

Lignin is a complex aromatic polymer found in the cell walls of plants, providing structural integrity and resistance to microbial attack. In poplar, lignin biosynthesis involves the phenylpropanoid pathway, where monolignols such as p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol are synthesized and subsequently polymerized into lignin. Key enzymes in this pathway include phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), and caffeoyl shikimate esterase (CSE), among others (Figure 1) (Meester et al., 2021; Jang et al., 2021; Vries et al., 2021).

2.2 Previous methods used for lignin modification

Traditional methods for lignin modification in poplar have included genetic engineering techniques such as overexpression or suppression of lignin biosynthetic genes. For instance, downregulation of 4-coumarate:CoA ligase (4CL) and caffeoyl shikimate esterase (CSE) has been shown to reduce lignin content and improve saccharification efficiency (Tsai et al., 2019; Vries et al., 2021). However, these methods often result in growth defects and other undesirable phenotypic changes, such as collapsed vessels and reduced biomass yield (Meester et al., 2021; Jang et al., 2021).

2.3 Introduction to CRISPR/Cas9 technology

CRISPR/Cas9 is a revolutionary genome-editing tool that allows for precise, targeted modifications of DNA. The system consists of two main components: the Cas9 nuclease, which introduces double-stranded breaks in DNA, and a single guide RNA (sgRNA) that directs Cas9 to the specific genomic location (Figure 2) (Bortesi and Fischer, 2015; Fan et al., 2015; Bruegmann et al., 2019). This technology has been successfully applied in various plant species, including poplar, to achieve targeted gene knockouts and modifications (Fan et al., 2015; Bruegmann et al., 2019).

2.3.1 Mechanism of CRISPR/Cas9

The CRISPR/Cas9 system operates by utilizing the sgRNA to bind to a complementary DNA sequence in the genome. The Cas9 nuclease then introduces a double-stranded break at this location. The cell's natural repair mechanisms, either non-homologous end joining (NHEJ) or homology-directed repair (HDR), subsequently repair the break, often resulting in insertions or deletions (indels) that can disrupt gene function (Figure 3) (Bortesi and Fischer, 2015; Fan et al., 2015; Bruegmann et al., 2019).

2.3.2 Advantages of using CRISPR/Cas9 over traditional methods

CRISPR/Cas9 offers several advantages over traditional genetic engineering methods for lignin modification in poplar:

(1) Precision: CRISPR/Cas9 allows for highly specific targeting of genes, reducing off-target effects and unintended genetic changes (Fan et al., 2015; Bruegmann et al., 2019).

(2) Efficiency: The system can generate homozygous mutants in the first generation, significantly speeding up the breeding process (Fan et al., 2015).

(3) Versatility: Multiple genes can be targeted simultaneously, enabling complex trait modifications (An et al., 2020).

(4) Minimal Growth Penalty: CRISPR/Cas9-mediated knockouts of lignin biosynthetic genes have shown reduced lignin content without significant growth penalties, making it a promising tool for biofuel production (Jang et al., 2021; Vries et al., 2021).

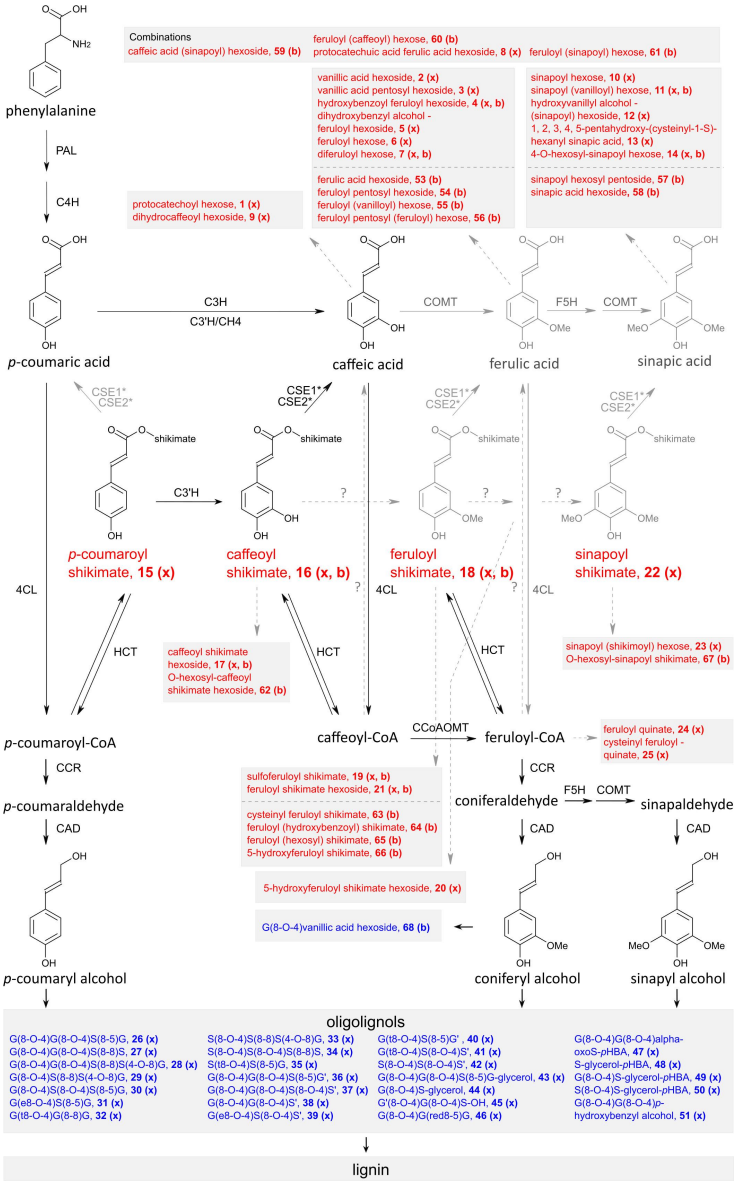

Figure 1 The general phenylpropanoid and monolignol-specific pathways, with the changes in phenolic metabolism indicated for *cse1 cse2* xylem and/or bark extracts compared with WT (Adopted from Vries et al., 2021)

Image caption: Red and blue names indicate metabolites that are up or down, respectively, in *cse1 cse2* xylem and/or bark extracts as compared to WT. Characterized metabolites uniquely detected in bark or xylem profiles are indicated with a (b) or (x), respectively. Co-occurring metabolites are indicated by (x, b). Metabolites that are framed in a box belong to the same class. The flow through the pathway towards the conventional monolignols is in black in WT, whereas (putative) alternative flows are in grey. Successive arrows show two or more metabolic steps. Solid arrows show enzymatic conversions that are validated by experimental evidence, whereas dashed arrows show suggested conversions (Saleme et al., 2017; Van Acker et al., 2017). *The catalytic activity of CSE1 and CSE2 is suggested *in planta* and proven *in vitro* in this work. Each metabolite is indicated with a unique number, corresponding to the metabolites mentioned in the manuscript. CAD, CINNAMYL-ALCOHOL DEHYDROGENASE; CCoAOMT, CAFFEOYL-CoA *O*-METHYLTRANSFERASE; CCR, CINNAMOYL-CoA REDUCTASE; CHS, CHALCONE SYNTHASE; COMT, CAFFEIC ACID *O*-METHYLTRANSFERASE; F5H, FERULATE 5-HYDROXYLASE; PAL, PHENYLALANINE AMMONIA-LYASE (Adopted from Vries et al., 2021)

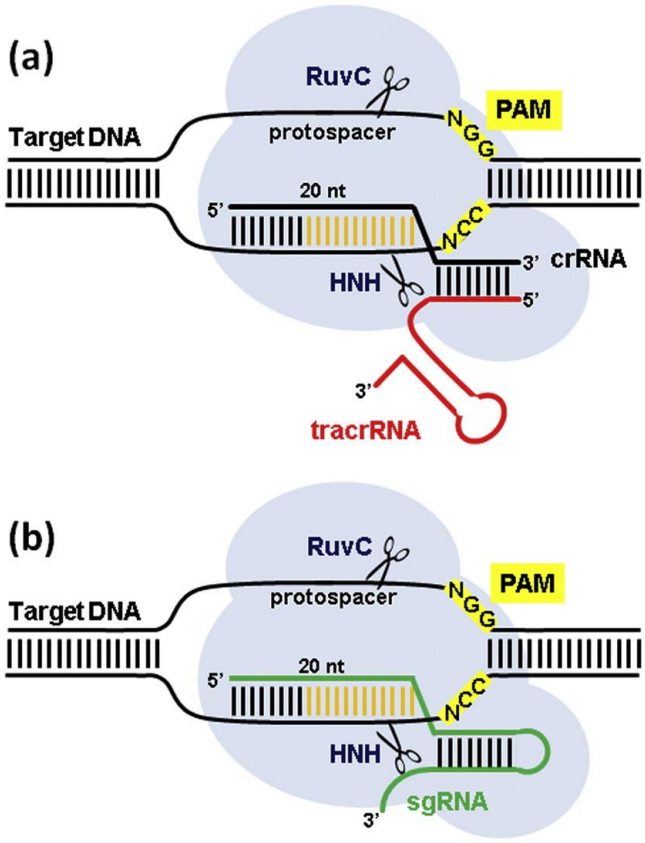

Figure 2 RNA-guided DNA cleavage by Cas9 (Adopted from Bortesi and Fischer, 2015)

Image caption: (a) In the native system, the Cas9 protein (light blue) is guided by a structure formed by a CRISPR RNA (crRNA, in black), which contains a 20-nt segment determining target specificity, and a trans-activating CRISPR RNA (tracrRNA, in red), which stabilizes the structure and activates Cas9 to cleave the target DNA (protospacer). The presence of a protospacer-adjacent motif (PAM, in yellow), i.e., an NGG (or less frequently NAG) sequence directly downstream from the target DNA, is a prerequisite for DNA cleavage by Cas9. Among the 20 RNA nucleotides determining target specificity, the so-called seed sequence of approximately 12 nt (in orange) upstream of the PAM is thought to be particularly important for the pairing between RNA and target DNA. (b) Cas9 can be reprogrammed to cleave DNA by a single guide RNA molecule (gRNA, in green), a chimera generated by fusing the 3' end of the crRNA to the 5' end of the tracrRNA (Adopted from Bortesi and Fischer, 2015)

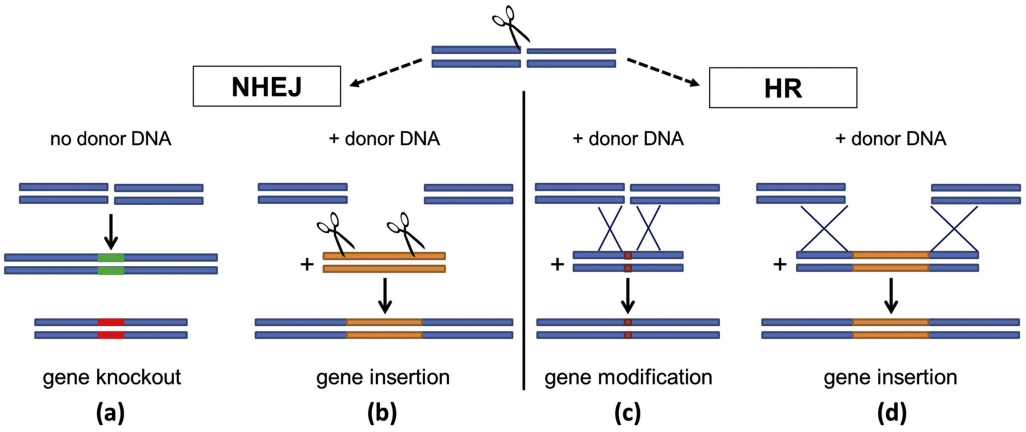

Figure 3 Genome editing with site-specific nucleases (Adopted from Bortesi and Fischer, 2015)

Image caption: Double-strand breaks induced by a nuclease at a specific site can be repaired either by non-homologous end joining (NHEJ) or homologous recombination (HR). (a) Repair by NHEJ usually results in the insertion (green) or deletion (red) of random base pairs, causing gene knockout by disruption. (b) If a donor DNA is available, which is simultaneously cut by the same nuclease leaving compatible overhangs, gene insertion by NHEJ can also be achieved. (c) HR with a donor DNA template can be exploited to modify a gene by introducing precise nucleotide substitutions or (d) to achieve gene insertion (Adopted from Bortesi and Fischer, 2015)

In summary, the CRISPR/Cas9 system represents a powerful and efficient tool for modifying lignin biosynthesis in poplar, offering significant advantages over traditional genetic engineering methods. This technology holds great promise for improving lignocellulosic biomass for biofuel production and other industrial applications.

3 Recent Advancements in CRISPR/Cas9 Applications in Poplar Lignin Biosynthesis

3.1 Gene knockouts

3.1.1 Knockout of *4CL1* gene and its effects on lignin composition

The knockout of the 4-coumarate: CoA ligase 1 (*4CL1*) gene in poplar has revealed significant insights into lignin biosynthesis. In a study by Tsai et al. (2019), CRISPR/Cas9-mediated knockout of *4CL1* in *Populus tremula* × alba resulted in a preferential reduction of syringyl (S) lignin, while guaiacyl (G) lignin levels were maintained. This alteration in lignin composition was accompanied by the upregulation of *4CL5*, a low-affinity paralog of *4CL1*, which helped sustain lignification. Interestingly, the knockout did not significantly affect biomass recalcitrance, suggesting a compensatory mechanism in lignin biosynthesis.

3.1.2 Knockout of *CSE* genes to reduce lignin content and improve saccharification

The caffeoyl shikimate esterase (*CSE*) genes are crucial targets for reducing lignin content in poplar. Research by Jang et al. (2021) demonstrated that CRISPR/Cas9-mediated knockout of *CSE1* and *CSE2* in hybrid poplar led to a significant reduction in lignin deposition, up to 29.1%, without affecting plant morphology or growth. This reduction in lignin content resulted in a 25% increase in saccharification efficiency, highlighting the potential of *CSE* gene knockouts for improving lignocellulosic biomass for biofuel production. Another study by Vries et al. (2021) confirmed the importance of *CSE1* and *CSE2* in lignification, showing that double mutants had a 35% reduction in lignin content and a fourfold increase in cellulose-to-glucose conversion.

3.2 Gene activation and base editing

3.2.1 Use of dCas9 for gene activation in poplar

While the provided data does not include specific examples of dCas9-mediated gene activation in poplar, the potential for such applications is significant. The CRISPR/Cas9 system, particularly the use of deactivated Cas9 (dCas9) fused with transcriptional activators, can be employed to upregulate target genes involved in lignin biosynthesis. This approach could be used to enhance the expression of genes that promote desirable lignin traits or improve overall biomass quality.

3.3 Multigene targeting

3.3.1 Efficient multiplex genome editing approaches

Recent advancements in CRISPR/Cas9 technology have enabled efficient multigene targeting in poplar. A study by Triozzi et al. (2021) described a robust and standardized CRISPR/Cas9 strategy for simultaneous editing of multiple genes in *Populus tremula* × *alba*. This approach utilized the Golden Gate MoClo cloning system to introduce mutations in two genes, *YUC4* and *PLT1*, simultaneously. The high efficiency of this system was demonstrated by the successful generation of double mutants, with biallelic mutations detected in a significant proportion of transgenic roots. This multiplex genome editing capability is crucial for dissecting complex genetic pathways and improving lignin biosynthesis in poplar.

In summary, the application of CRISPR/Cas9 in poplar lignin biosynthesis has made significant strides, particularly in gene knockouts, which have shown promising results in reducing lignin content and improving saccharification efficiency. Future research should explore gene activation techniques and further refine multigene targeting approaches to fully harness the potential of CRISPR/Cas9 in optimizing lignin biosynthesis for bioenergy applications.

4 Case Studies and Experimental Results

4.1 Case study on 4CL1 knockout

The 4-coumarate:coenzyme A ligase (*4CL*) gene plays a crucial role in the lignin biosynthesis pathway. In a study on switchgrass, a CRISPR/Cas9 system was developed to target the *Pv4CL1* gene, which is preferentially expressed in highly lignified stem tissues. Specific guide RNAs were designed to target *Pv4CL1*, and the

construct was introduced into switchgrass calli. Out of 39 transgenic plants regenerated, four were confirmed to have tetra-allelic mutations. These *Pv4CL1* knockout plants exhibited an 8%~30% reduction in total lignin content and a 7%~11% increase in glucose release, along with a 23%~32% increase in xylose release, demonstrating improved saccharification efficiency without a significant growth penalty (Figure 4) (Park et al., 2017).

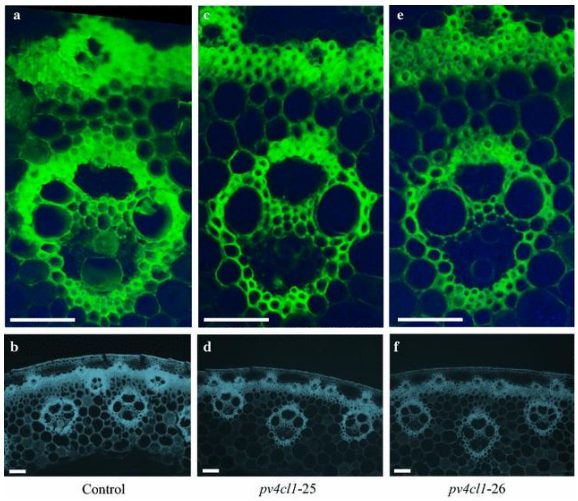

Figure 4 Defined tetra-allelic gene disruption of the 4-coumarate:coenzyme A ligase 1 (*Pv4CL1*) gene by CRISPR/Cas9 in switchgrass results in lignin reduction and improved sugar release (Adopted from Park et al., 2017)

Image caption: Histological staining for lignin deposition in control a, b, *pv4cl1*-25 c, d and *pv4cl1*-26 e, f. Second internode was sampled at R1 stage. Scale bars: 50 μm. Thickness of the cross section is 30 μm (Adopted from Park et al., 2017)

4.2 Case study on CSE gene knockouts

Caffeoyl shikimate esterase (CSE) is another key enzyme in lignin biosynthesis. In hybrid poplar (*Populus alba* × *P. glandulosa*), CRISPR/Cas9 was used to knockout the *CSE1* and *CSE2* genes. Three single guide RNAs were designed, resulting in transgenic poplars with either CSE1 (CSE1-sg2), CSE2 (CSE2-sg3), or both genes (CSE1/2-sg1) mutated. The CSE1-sg2 and CSE2-sg3 poplars showed up to a 29.1% reduction in lignin deposition and up to a 25% higher saccharification efficiency compared to wild-type controls. These knockouts did not exhibit significant morphological differences or growth penalties in long-term field tests, indicating that precise editing of *CSE* genes can enhance lignocellulosic biomass without adverse effects on growth (Figure 5) (Jang et al., 2021).

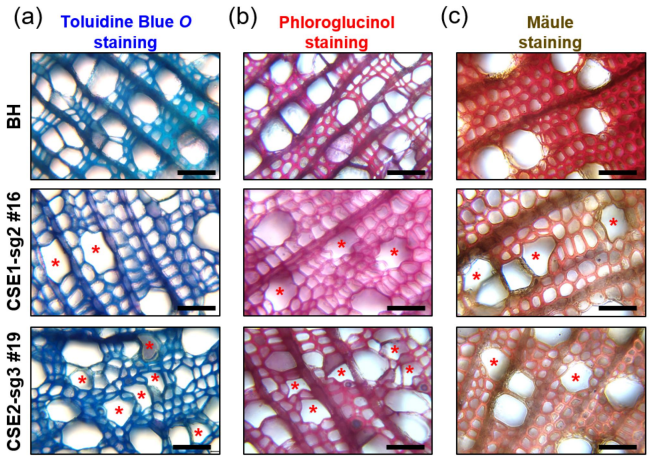

Figure 5 Transgenic CSE-CRISPR hybrid poplars have irregularly shaped xylem vessel cells (Adopted from Jang et al., 2021)

Image caption: Stem anatomy of hybrid poplars (8-month-old LMO field grown) was assessed by (a) toluidine blue, (b) phloroglucinol-HCl, and (c) Mäule staining. Collapsed irregular vessels are marked with asterisks. Scale bars represent 50 μm (Adopted from Jang et al., 2021)

4.3 Case study on gene activation

Gene activation using CRISPR/Cas9 has also been explored in poplar species. In one study, the CRISPR/Cas9 system was employed to target the phytoene desaturase gene (*PDS*) in *Populus tomentosa* Carr. Four guide RNAs were designed to target distinct genomic sites of the *PtoPDS* gene. Following Agrobacterium-mediated transformation, transgenic poplar plants exhibited an albino phenotype, indicating successful gene knockout. Mutation efficiency was estimated at 51.7%, with 30 out of 59 PCR clones being homozygous mutants. This study demonstrated the potential of the CRISPR/Cas9 system for precise genome editing and effective creation of knockout mutations in woody plants (Figure 6) (Fan et al., 2015).

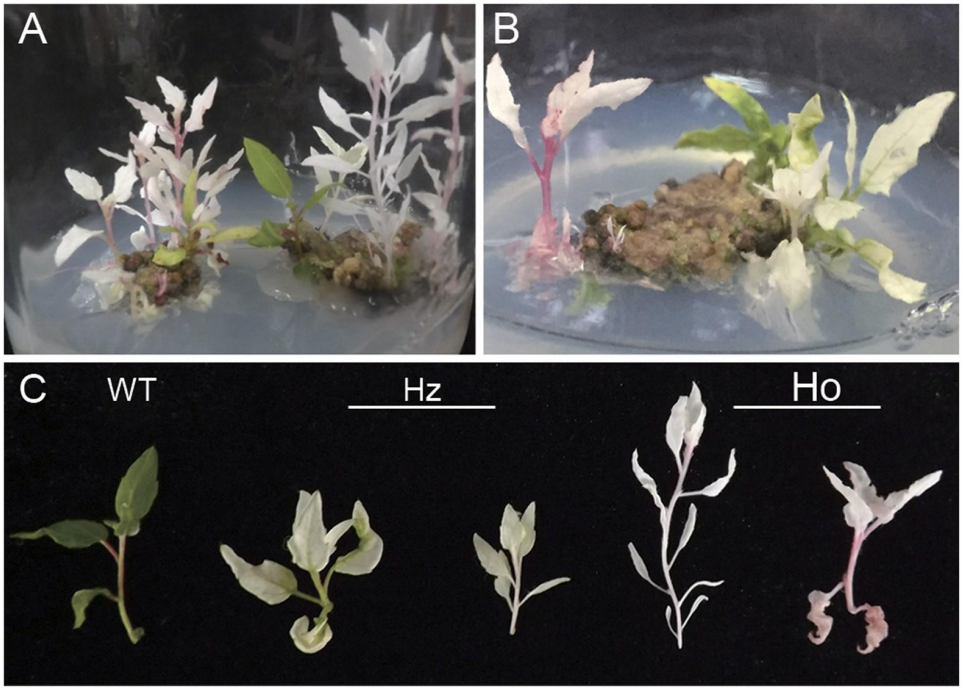

Figure 6 Efficient CRISPR/Cas9-mediated targeted mutagenesis in *Populus* in the first generation (Adopted from Fan et al., 2015)
Image caption: Albinism phenotype of transgenic poplars. (A, B) Albino phenotype of the regenerated poplar shoots generated on the CRISPR/Cas9-*PtoPDS* transformed leaf discs. (C) Representative CRISPR/Cas9 directed *PtoPDS* plants (T0). WT, Nontransgenic wild-type poplar; Ho, biallelic homozygous mutant; Hz, biallelic heterozygous mutant. Both Ho and Hz plants show an albino phenotype (Adopted from Fan et al., 2015)

In another study, the CRISPR/Cas9 system was used to edit the PDS gene in a hybrid poplar (*Populus alba* × *Populus glandulosa*). A single guide RNA was designed, and the construct was delivered via Agrobacterium-mediated transformation. Among 110 transgenic lines, 82 showed either an albino or pale green phenotype, indicating a 74.5% phenotypic mutation efficiency. This confirmed the effectiveness of CRISPR/Cas9-mediated genome editing in hybrid poplar, with no off-target modifications detected (Bae et al., 2021).

These case studies collectively highlight the versatility and efficiency of the CRISPR/Cas9 system in modifying lignin biosynthesis pathways in poplar species, paving the way for improved lignocellulosic biomass for biofuel production.

5 Challenges and Limitations

5.1 Technical challenges

5.1.1 Off-target effects and strategies to minimize them

The CRISPR/Cas9 system, while highly efficient for targeted genome editing, is not without its technical challenges. One of the primary concerns is the occurrence of off-target effects, where the Cas9 nuclease induces double-stranded breaks at unintended genomic locations. This can lead to unintended mutations, which may have deleterious effects on the organism.

Several strategies have been developed to minimize off-target effects. For instance, the design of highly specific guide RNAs (gRNAs) is crucial. Studies have shown that the structure of gRNAs, including their GC content and the accessibility of the seed region, significantly influences their specificity and efficiency (Bruegmann et al., 2019). Additionally, the use of high-fidelity Cas9 variants, such as SpCas9-HF1 and eSpCas9, has been shown to reduce off-target activity while maintaining on-target efficiency (Bortesi and Fischer, 2015).

Another approach involves the use of alternative CRISPR systems, such as CRISPR/Cas12a, which has been demonstrated to have a broader targeting range and higher specificity in poplar (An et al., 2020). Optimizing transformation conditions, such as co-cultivation temperature, can also enhance the efficiency and specificity of genome editing (An et al., 2020).

5.2 Biological and ecological concerns

5.2.1 Potential ecological impacts of genetically modified poplar

The introduction of genetically modified (*GM*) poplars into the environment raises several biological and ecological concerns. One major issue is the potential for gene flow from GM poplars to wild relatives, which could lead to unintended ecological consequences. For example, the knockout of lignin biosynthetic genes, such as *CSE* and *4CL1*, has been shown to alter lignin composition and saccharification efficiency in poplar (Tsai et al., 2019; Jang et al., 2021). These modifications could affect the plant's interactions with its environment, including its susceptibility to pests and diseases, and its role in the ecosystem.

Moreover, the long-term ecological impacts of GM poplars are not fully understood. Field tests have shown that some GM poplars do not exhibit significant differences in growth compared to wild-type controls over multiple seasons (Jang et al., 2021). However, the potential for unforeseen ecological effects necessitates thorough risk assessments and long-term monitoring of GM poplar plantations.

5.3 Regulatory and ethical issues

5.3.1 Current regulatory framework and ethical considerations

The regulatory framework for the use of CRISPR/Cas9 in genetically modifying poplars varies by country and is often complex. In many regions, GM organisms are subject to stringent regulations that require extensive safety assessments before they can be released into the environment. These regulations are designed to ensure that GM plants do not pose risks to human health or the environment.

Ethical considerations also play a significant role in the deployment of GM poplars. Public perception of GM technology can influence regulatory decisions and the acceptance of GM products. Ethical concerns include the potential for unintended consequences, the impact on biodiversity, and the socio-economic implications for communities that rely on traditional forestry practices.

In conclusion, while CRISPR/Cas9 offers powerful tools for improving poplar lignin biosynthesis, addressing the technical, biological, ecological, regulatory, and ethical challenges is crucial for the responsible development and deployment of GM poplars.

6 Future Directions and Prospects

6.1 Improved CRISPR/Cas9 techniques

Advances in CRISPR technology have significantly enhanced its specificity and efficiency, which are crucial for precise genome editing in poplar lignin biosynthesis. Recent studies have demonstrated the potential of CRISPR/Cas12a, a novel CRISPR effector protein, which broadens the targeting range and enables large-fragment deletions, thus offering a more efficient alternative to the traditional CRISPR/Cas9 system (An et al., 2020). Additionally, optimizing the design of guide RNAs (gRNAs) based on factors such as GC content and purine residues has been shown to improve the efficiency of gene editing in poplars (Bruegmann et al., 2019). These advancements are pivotal for achieving higher specificity and minimizing off-target effects, thereby enhancing the overall effectiveness of CRISPR/Cas9-mediated genome editing (Ding et al., 2016; Arora and Narula, 2017).

6.2 Integration with other biotechnologies

Combining CRISPR with other genetic and genomic tools can lead to enhanced outcomes in poplar lignin biosynthesis. For instance, integrating CRISPR/Cas9 with traditional plant breeding techniques and other genome editing platforms like zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) can provide a more comprehensive approach to genetic modification (Wang et al., 2015). Moreover, the use of CRISPR ribonucleoproteins (RNPs) has emerged as a solution to various limitations associated with plasmid-based CRISPR systems, offering a more efficient and precise method for gene editing (Arora and Narula, 2017). Such integrative approaches can facilitate the development of poplar varieties with optimized lignin content and improved traits for biofuel production and other industrial applications (Fan et al., 2015; Wang et al., 2015).

6.3 Field applications and commercialization

The prospects for commercial deployment of CRISPR-edited poplar in biofuel production and other industries are promising. Field tests have shown that CRISPR/Cas9-mediated knockouts of lignin biosynthetic genes in hybrid poplar can lead to significant reductions in lignin content without affecting plant growth, thereby enhancing saccharification efficiency (Jang et al., 2021). This improvement in biomass quality is crucial for sustainable biofuel production. Furthermore, the successful application of CRISPR/Cas9 in other crops, such as potato, underscores its potential for broader agricultural and industrial applications (Wang et al., 2015). As the technology continues to advance, it is expected that CRISPR-edited poplars will play a vital role in the bioenergy sector, contributing to the development of more efficient and sustainable biofuel production systems (Liu et al., 2015; Chung et al., 2017).

By leveraging these advancements and integrating CRISPR with other biotechnologies, the future of poplar lignin biosynthesis research looks promising, with significant potential for commercial applications and contributions to sustainable biofuel production.

7 Concluding Remarks

The application of CRISPR/Cas9 technology in poplar lignin biosynthesis has shown significant promise in enhancing the efficiency of lignocellulosic biomass processing. Studies have demonstrated that targeted gene knockouts, such as those of the CSE genes, can lead to substantial reductions in lignin content without adversely affecting plant growth (Jang et al., 2021). Additionally, the use of CRISPR/Cas12a has expanded the range of possible genetic modifications, enabling more precise and efficient genome editing in poplar species (An et al., 2020). These advancements underscore the potential of CRISPR/Cas systems to revolutionize the field of plant biotechnology, particularly in the context of biofuel production and sustainable agriculture.

Key findings from recent research indicate that CRISPR/Cas9-mediated gene editing can effectively reduce lignin content in poplar, thereby improving saccharification efficiency (Jang et al., 2021). The technology has been successfully applied to create homozygous mutants in a single generation, demonstrating its efficiency and precision (Arora and Narula, 2017; Bruegmann et al., 2019). Moreover, the development of CRISPR/Cas12a systems has further enhanced the capability to induce large-fragment deletions and multigene knockouts, which are crucial for comprehensive genetic studies in woody plants (An et al., 2020). These studies collectively highlight the versatility and effectiveness of CRISPR/Cas systems in modifying lignin biosynthesis pathways in poplar.

Continued research and development in CRISPR/Cas9 technology are essential to fully realize its potential in lignin biosynthesis and beyond. The ability to fine-tune gene editing techniques, such as optimizing guide RNA design and minimizing off-target effects, will be critical for achieving desired phenotypic outcomes without unintended consequences (Liu et al., 2015; Chung et al., 2017). Furthermore, exploring the use of CRISPR/Cas systems in other economically important tree species could lead to broader applications in forestry and agriculture (Wang et al., 2015). Ongoing advancements in this field will not only enhance our understanding of plant biology but also pave the way for innovative solutions to global challenges in energy and sustainability.

The potential of CRISPR/Cas9 to revolutionize lignin biosynthesis in poplar is immense. By enabling precise and targeted modifications of lignin biosynthetic genes, CRISPR/Cas9 can significantly improve the efficiency of biomass conversion processes, making biofuel production more viable and sustainable (Fan et al., 2015; Jang et al., 2021). The technology's ability to create specific gene knockouts and introduce beneficial traits without compromising plant growth or health is particularly advantageous (Ding et al., 2016; Bruegmann et al., 2019). As research progresses, the integration of CRISPR/Cas9 with other biotechnological approaches could lead to the development of poplar varieties with optimized lignin content and enhanced industrial utility, thereby transforming the landscape of renewable energy and sustainable agriculture.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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