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Accelerating wood domestication in forest trees through genome editing: advances and prospects

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Abstract

The high economic value of wood requires intensive breeding towards multipurpose biomass. However, long breeding cycles hamper the fast development of novel tree varieties that have improved biomass properties, are tolerant to biotic and abiotic stresses, and resilient to climate change. To speed up domestication, the integration of conventional breeding and new breeding techniques is needed. In this review, we discuss recent advances in genome editing and Cas-DNA-free genome engineering of forest trees, and briefly discuss how multiplex editing combined with multi-omics approaches can accelerate the genetic improvement of forest trees, with a focus on wood.

Keywords

Forest Tree Breeding, Gene editing, Cas-DNA-free plant transformation

Introduction

Wood has been used for centuries as construction material, a source of energy, and for the production of pulp and paper. More recently, woody biomass is also considered as a promising resource for biorefineries that convert the polysaccharide fraction into fermentable sugars and the lignin fraction into aromatic building blocks for the chemical industries [1, 2]. The continuous demand for wood from plantation forestry requires the development of highly productive tree varieties at industrial scale. Ideally, such trees have optimized wood quality, are resilient to climate change, have an excellent nitrogen and water use efficiency (allowing them to grow on marginal soils), and are armed to resist abiotic and biotic stresses. Because tree breeding cycles are slow, due to the long generation times typical for trees, and because wood quality traits can often be tested only after a decade of growth in the field - the genetic improvement of trees needs to be accelerated by combining conventional breeding with new breeding techniques (NBTs).

The large genetic and phenotypic diversity that is naturally present within populations forms the basis for targeted breeding strategies. For example, a study of 1,100 undomesticated *Populus trichocarpa* trees revealed a wide variability in lignin content and composition, resulting in large differences in the total yield of fermentable sugars upon saccharification [3], opening up possibilities for breeding tree varieties tailored for the biorefinery. When the genes causal to a trait are known, specific alleles can be sought in natural populations and integrated into the breeding program [4, 5]. In the past decades, biomass traits have also been improved using genetic engineering strategies, which typically rely on the overexpression or silencing of one or a few genes. Although transgenic lines that stably express a transgene can be identified [6], it is more difficult to identify stable lines with intermediate levels of RNA interference

(RNAi)-mediated gene downregulation, as RNAi depends on the expression levels of both the endogenous gene and the transgene. For example, intermediate levels of RNAi-mediated downregulation of *CINNAMYL ALCOHOL DEHYDROGENASE* (*CAD*), *CAFFEIC ACID-O-METHYLTRANSFRASE* (*COMT*) and *CINNAMOYL-CoA-REDUCTASE* (*CCR*), all genes involved in lignin biosynthesis, resulted in unequal reduction in the expression of these genes throughout the xylem in poplar, as witnessed by the patchy red xylem coloration that is caused by the downregulation of these genes [7-10]. Recently, the use of the clustered regularly interspaced palindromic repeats (CRISPR)/Cas systems has overcome this limitation and has become the method of choice for precise, stable and heritable genome engineering in plants. In addition, multiplex CRISPR strategies allow to precisely engineer individual or multiple gene family members in a single effort, a goal which was previously cumbersome to achieve via classical technologies [11]. In this review, we focus on recent advances in gene editing and Cas-DNA-free transformation approaches, and briefly discuss the potential of integrating CRISPR systems and multi-omics approaches to accelerate research and breeding in the forestry landscape, as visually represented in Figure 1.

Focus point 1: Adapted and advanced gene editing tools

Use of Cas9 for allele-specific editing

To date, the predominantly used CRISPR/Cas system in plants is the CRISPR/Cas9 system. By employing one single effector endonuclease (e.g., Cas9), this CRISPR system introduces blunt double-strand breaks (DSBs) in the DNA [12], often resulting in either identical (homozygous) or distinct (heterozygous) mutations in both targeted alleles. Biallelic knockout mutations are often desired in reverse genetic studies to discover the function of genes, but frequently provoke dramatic phenotypes that interfere with downstream applications [13, 14]. In this context, the creation of mono-allelic variants or leaky alleles can be advantageous. Forest trees, having highly heterozygous genomes, make good targets for such editing approaches, by offering plenty of options for allele-specific guide RNA (gRNA) design. De Meester et al. illustrated the potential of differential editing of the two alleles by targeting the lignin-biosynthesis gene *CCR2* in *P. tremula x P. alba* [14]. In their study, a strong yield penalty was observed in all biallelic knockout lines. However, one transgenic line harbored one null allele and one weak (or leaky) allele containing a 3-base pair deletion that led to the expression of a CCR2 protein with reduced activity, resulting in normal tree growth, along with a reduced lignin content and an associated increase in saccharification efficiency. Lines that were heterozygous knockout for either of the two *CCR2* alleles had wild-type lignin levels, indicating that the wild-type *CCR2* alleles were haplosufficient and that it was the specific combination of

one knockout allele with the particular weak allele that resulted in the beneficial phenotype [14].

Working with highly heterozygous species also holds the possibility of either finding or even aiming for monoallelic knockout mutants in haploinsufficient genes. In comparison to dosage effects achieved by RNA interference (RNAi), those achieved by CRISPR are stable. Monoallelic gene knockouts in a haploinsufficient gene were already demonstrated in one of the first CRISPR studies in poplar, in which the *PHYTOENE DESATURASE 8* (*PDS8*) gene of *P. tomentosa* was targeted [15]. The authors identified a monoallelic mutated line, which, unlike the biallelic knockout mutants that were white, exhibited a pale green phenotype, implying a gene dosage effect.

Taken together, allele-specific editing can easily be achieved in heterozygous species, including forest trees, and can be exploited to achieve favorable phenotypes.

Use of different Cas systems in trees

Since the development of CRISPR/Cas9 as a genome engineering tool, the original *Streptococcus pyogenes*-derived SpCas9, which utilizes a simple 5'-NGG-3' protospacer adjacent motif (PAM), is widely used in plant genome engineering. Whilst SpCas9 is certainly useful for standard applications, other Cas systems have been investigated in plants as well, to overcome vector size limitations or PAM restraints. One example is Cas12a (formerly known as Cpf1), which creates staggered cuts and utilizes T-rich PAM sequences, such as 5'-TTTV-3' [16, 17]. Recently, three variants of Cas12a [AsCas12a (*Acidaminococcus* sp. BV3L6), LbCas12a (*Lachnospiraceae* bacterium ND2006), and FnCas12a (*Francisella tularensis* subsp. *novicidain* U112)] were employed to edit the *PDS8* gene of *P. alba* × *P. glandulosa* [18]. Using a multi-gRNA approach, editing efficiencies of up to 70% were achieved by employing the AsCas12a editor, which is comparable to the editing efficiencies observed for SpCas9 [19]. The use of these different Cas12a variants broadens the possibilities for gene editing in trees by offering a wider target search window due to the distinct PAM requirements as compared to SpCas9. By further relaxing or even eliminating PAM requirements of the genome editor, as achieved by the SpG and SpRY Cas systems [20, 21], even more gene editing opportunities can be created. These advantages come notably into play when precise gene editing systems such as base editing are applied, which require exact positioning of the gRNA on the target DNA.

Base editing in trees

Introduced in 2016, base editing allows the targeted mutagenesis of specific base pairs at often higher efficiencies than achieved by homology-directed repair [22]. Whilst the first base editors

allowed the conversion of cytosine to thymine (C–T), nowadays, A–G base transitions [23], C–A transversions [24], and C–G transversions [25] can be achieved, with various target windows. Furthermore, subvariants of base editors, such as dual-base editors – which are capable of simultaneously generating C–T and A–G conversions [26] –, RNA base editors [27], or organelle DNA editors such as mtDdCBE which is based on zinc finger technology [28], were added to the base editing toolbox. From this wide collection, only C–T and A–G conversions, achieved by cytosine base editors (CBE) and adenine base editors (ABE), respectively, as well as C–G transversions, achieved by C–G base editors (CGBEs) have been tested in woody plants. Li et al. demonstrated the applicability of ABE and CBE in *P. tremula x P. alba*, by targeting *4-COUMARATE: CoA LIGASE1* (*4CL1*), a gene involved in lignin biosynthesis, and *PII*, involved in coordinating nitrogen and carbon assimilation [29]. In agreement with expectations, the monoallelic *4cl1* mutant lines did not display any altered phenotype, whereas the biallelic mutants showed the red-xylem phenotype previously reported in *4cl1* mutants [30], reflecting the altered lignin composition. In the same year, C–G base editors (CGBEs) were successfully used to edit *PDS1* and *PDS2* in the same poplar hybrid [31].

Prime editing

Besides base editing, another precise CRISPR-based editing method is prime editing (PE). A prime editor is a fusion between a Cas9 nickase and a reverse transcriptase [32]. Different to base editors, PE enables generating all 12 possible base-to-base conversions, as well as (small) specific insertions and deletions. This allows not only to generate knockout alleles, but also to intentionally engineer weak alleles, which can prove to be advantageous for certain traits as described above for *CCR2*. Furthermore, PE enables the intentional introduction of *cis*-regulatory elements in the promoters of genes of interest. The intrinsic heterozygosity, naturally encompassed within trees, allows the design of prime editing gRNAs (pegRNAs) that would specifically redirect the expression of a single allele, yielding ectopic gene expression, while maintaining the endogenous gene functionality via the other, non-edited allele.

In plants, PE was first demonstrated in rice and wheat protoplasts, but with low efficiency [33]. By using two pegRNAs that encode the same edit *in trans* (paired pegRNA approach), the editing efficiency could be increased up to 17-fold in rice protoplasts as compared to classical PE [34]. Furthermore, the low editing efficiency was also addressed by Anzalone et al. in their recent advancement of the PE system, the so-called twin-PE, which they developed in mammalian cells [35]. By utilizing the prime editor protein, together with two distinct pegRNAs, the twin-PE system can replace an endogenous DNA sequence, situated between two PAM recognition sites at the opposing strands of genomic DNA, by a DNA sequence of choice.

Furthermore, the twin-PE system, used together with the site-specific recombinase Bxb1, allows integrating large DNA cargos of up to 5.6 kb into the genome, and targeting chromosomal rearrangements, as illustrated by a 40-kb inversion in human cells [35]. In trees, the twin-PE system could be used to reallocate the promoter of a gene to the coding sequence of another gene by a targeted heterozygous reciprocal translocation, yielding potentially favorable ectopic gene expression of a single allele of the targeted gene. Once established in trees the (twin-)PE system will enable the tuning of enzyme activities, the incorporation of novel genes and the introduction of targeted chromosomal rearrangements to enhance the genetic improvement of forest trees.

The combo system

Next to genome editing applications, CRISPR systems have been repurposed for regulating gene expression in plants [36, 37]. Recently, the CRISPR-Combo system, a tool that allows the simultaneous editing and activation of genes, was developed [38]. This system relies on a single Cas9 protein and an engineered gRNA that recruits transcriptional regulators. Interestingly, the authors correlated gRNA length with the ability for DNA cleavage (i.e. mutagenesis), and demonstrated that 17- to 20-nucleotide (nt) gRNAs enable DSB formation, while 14- to 16-nt gRNAs bind the target DNA but prohibit cleavage of the target strand [39]. Using the CRISPR-Combo system, Pan and co-authors successfully edited the *P. trichocarpa 4CL1* gene and simultaneously activated the morphogenic regulator-encoding gene *WUSCHEL* (*WUS*), to accelerate root and shoot initiation [38]. Given that transformation and regeneration of many tree species or elite clones have not yet been optimized, the application of such a Combo system could be particularly important to improve specific traits, while simultaneously enhancing the regeneration capacity of recalcitrant tree species or elite varieties.

Focus point 2: Cas-DNA-free genome engineering

Classical gene editing strategies often rely on the stable integration of Cas-encoding DNA together with a selectable marker gene into the plant genome. Although these methods allow the swift selection of transformants on selective medium, together with highly efficient gene editing, the continuous presence of the gene editing toolbox (Cas nuclease and gRNA) in the genome of the plant is not desired, as it might generate off-target mutations leading to mosaicism when sequences are present in the plant that are highly similar to the target sequence [40, 41]. In addition, the field-testing and commercialization of gene-edited trees containing Cas-encoding DNA may not be allowed because the presence of the Cas gene combined with the ability of the gene to spread to wild relatives may be seen as a risk factor

for potential environmental harm [42-44]. After all, the potential introduction of DNA, encoding the Cas nuclease and gRNA, into natural populations could alter the frequency of the edited allele in these populations, as the Cas nuclease may dominantly edit both alleles in each successive generation. Although engineering sterility is a promising way to avoid the spread of Cas-encoding DNA [45, 46] the removal of these sequences from the genome of the plant will facilitate gaining regulatory approval for the unconfined field release and commercialization of gene-edited trees [44, 47].

Transient delivery of CRISPR DNA

The elimination of transgenes from the plant genome is readily achieved via Mendelian segregation in sexually propagated crops with short generation times [48, 49]. Unless strategies to promote early flowering are utilized, this method is hardly applicable for clonally propagated plants and/or perennials that require years to reach sexual maturity. Moreover, many commercially relevant tree species are highly heterozygous, and the sexual reproduction of these plants would break up their genetic constitution, leading to the loss of the desired characteristics in the progenies [50].

To overcome these issues, transient delivery methods gained interest [51]. These methods include *Agrobacterium*-mediated transformation followed by regeneration on non-selective medium, polyethylene glycol (PEG)-mediated transfection of protoplasts, and the delivery of transgenes via particle bombardment [52-55]. These methods have been successfully used in trees but often suffer from low editing efficiencies. Moreover, the regeneration of plants from protoplasts can induce genome instability resulting in aneuploidy, while the delivery via particle bombardment is known to cause genomic rearrangements [56, 57]. Recently, DNA has also been delivered into plant cells via a variety of nanostructures that passively traverse through the plant's membrane [58-61]. Even though this technique is still in its infancy and the delivery is currently limited to ~12-kb plasmids, efficient delivery and expression of fluorescent markers was already achieved in tobacco, arugula, wheat and cotton cells, without transgene integration [61, 62].

Although the transient expression of Cas-encoding DNA is a promising strategy to develop Cas-free gene-edited plants, an important drawback inherent to all DNA delivery methods comes from the potential incorporation of DNA fragments into the genomic DNA. After all, endogenous plant nucleases degrade the delivered DNA, resulting in fragments that might integrate into on- or off-target DSBs [63]. Vector DNA integration has been reported for both PEG-mediated transfection of protoplasts and the biolistic delivery of transgenes [53, 56, 64, 65].

DNA-independent genome editing

Several strategies have been developed to overcome the integration of vector DNA by switching to delivery methods that are completely free of foreign DNA [51, 66, 67]. One of the most popular methods is the delivery of Cas proteins together with their gRNA as *in vitro* assembled ribonucleoprotein (RNP) complexes. Typically, RNPs can be transfected into plant protoplasts via PEG-mediated transfection or electroporation, or into calli via biolistic delivery. The use of RNPs to achieve DNA-free genome editing has been demonstrated in a variety of plant species [51, 66, 67], including a limited number of woody species, such as grapevine, rubber tree, apple, chestnut and pine [68-72]. However, no regeneration from gene-edited cells has been reported yet. The regeneration of protoplasts is technically challenging and underdeveloped for most plant species, although regeneration from protoplasts of the model tree *P. tremula* x *P. alba* has been described [73].

One alternative to achieve Cas DNA-free genome editing in woody species exploits the delivery of RNA templates by plant viruses. Typically, the use of virus-mediated gene editing in plants is limited due to restrictions in cargo size, as large foreign sequences make the viral genome unstable, resulting in deletions during replication [74, 75]. Recently, this technical barrier has been overcome by taking advantage of the flexible filamentous structure of potato virus X (PVX) to simultaneously deliver Cas nucleases and gRNAs [76]. In addition, efforts are made to completely alleviate cargo size restrictions by exploiting negative strand RNA viruses, which generally have large cargo capacities and high genomic stability. Two studies report the successful delivery of Cas nucleases and gRNAs, along with highly efficient gene editing, via the sonchus yellow net rhabdovirus (SYNV) and the barley yellow striate mosaic virus (BYSMV) [77, 78]. Interestingly, RNA viruses, like PVX, SYNV and BYSMV, lack a DNA-replication phase, and therefore do not integrate into the host genome. Regenerated plants from infected tissues can thus be considered free of foreign DNA. Although this method promises to be extremely powerful, it is currently limited by the restricted host range of these engineered viruses. Future efforts should therefore be focused on the engineering of viruses with a naturally wide host range, together with the development of hypercompact nucleases, such as the recently discovered CasΦ [79], which could overcome the cargo size limitations.

Focus point 3: Integration of CRISPR systems and multi-omics approaches

Today, multiplex editing approaches allow the simultaneous and stable engineering of multiple genes [18, 80-83]. To accelerate wood domestication in forest trees, efforts must be centered around elucidating the most promising combination of targets. One way to achieve this builds on the wealth of reverse genetic studies that have investigated the function of individual genes [84-86]. Alternatively, promising genes can be derived from multi-omics approaches. For

example, Wang and co-authors combined transcriptomic, metabolic, proteomic, and phenomic data of various lignin biosynthesis mutants in *P. trichocarpa* into a mathematical model to predict the wood properties of poplar upon the engineering of one or multiple lignin pathway genes [87]. The creation of multiple allelic variants in combinations of these genes by multiplex genome editing enables the iterative testing of this mathematical model and has the potential of engineering wood quality traits in poplar beyond what is achievable using single gene modification. Field evaluations will be essential given that most of the reverse genetics studies have been performed in greenhouses, and given the large differences in phenotypes between greenhouse- and field-grown trees [88].

An alternative source of target genes for (multiplex) genome engineering comes from the identification of quantitative traits loci (QTLs) through genome-wide association studies (GWAS) [89]. QTL effects are often caused by single nucleotide polymorphisms (SNPs) that affect gene expression levels or protein activities within a wild population. However, the contribution of individual SNPs to the overall phenotype is often limited, and highly depends on the genotype and the environment [90]. Regardless of their small effects, a GWAS does identify genes that play a causal role in establishing the trait [91-94]. Through the use of multiplex genome editing in elite germplasm, a large number of novel allelic variants at multiple QTLs can be generated, as exemplified in a model maize variety for yield genes [95]. The subsequent phenotypic screening of the obtained gene-edited population in the field will allow identifying trees with novel allelic combinations that might outperform the parental clone (Figure 1). As such, multiplex editing might become a powerful tool to accelerate future tree breeding programs.

Concluding remarks and future perspectives

In this review we highlighted three focus points that could stimulate the integration of gene editing with conventional breeding to accelerate the domestication of wood quality. Reverse genetic studies and GWAS will aid in selecting suitable targets for (multiplex) engineering. As forest trees are characterized by high levels of heterozygosity, sequence information of both haplotypes will be required to design effective gRNAs. Nowadays this can be achieved via whole-genome sequencing or RNA-seq of tissues known to express the gene(s) of interest. Once sequence information is at hand, the development of advanced gene-editing tools in trees, such as base and PE, with potentially alleviated PAM restrictions, will facilitate the editing of one or both alleles in these highly heterozygous genotypes. In addition, efforts should focus on the development of protocols that allow the efficient transformation and regeneration of elite genotypes. Transformation technologies which avoid the integration of Cas-encoding DNA into

the host genome might alleviate regulatory concerns on the unconfined field release of CRISPR-engineered trees. In the European Union, gene-edited plants are still considered as genetically modified organisms (GMO) and must comply with the GMO directive 2001/18/EC. From a scientific point of view, there are no arguments to distinguish gene-edited plants containing small deletions or insertions from plants obtained via conventional breeding or classical mutagenesis. Biosafety regulations should therefore be focused on the product and evaluate its risks/benefits, irrespective of the breeding method used [96, 97]. In the face of climate emergency and the exploitation pressure on our native forests, the integration of gene-editing and conventional breeding to speed up wood domestication should not further be delayed, and biosafety regulations should be based on science, not politics.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

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Highlighted references

Of outstanding interest

**An et al., Efficient Genome Editing in *Populus* Using CRISPR/Cas12a. *Frontiers in Plant Science* (2020)

The authors evaluate a CRISPR/Cas12a system, encompassing three different Cas12a nucleases with different PAM requirements, in the model tree *Populus alba × Populus glandulosa*. Using *PHYTOENE DESATURASE 8* (*PDS8*) as target, they identified AsCas12a as the most efficient genome editor out of the three tested nucleases, which resulted in editing efficiencies of up to 70%.

**Ma et al., Highly efficient DNA-free plant genome editing using virally delivered CRISPR–Cas9. *Nature Plants.* (2020)

The authors report the use of a negative-strand RNA virus for the DNA-free delivery of CRISPR/Cas9 cassettes in tobacco to achieve high-frequency editing. Over 90% of the regenerated plants contained mutations at the expected target site.

**Pan et al., Boosting plant genome editing with a versatile CRISPR-Combo system. *Nature Plants*, (2022)

The CRISPR-Combo system allows the simultaneous activation and editing, including base editing, of target genes using optimized gRNAs. Using their Combo system, the authors accelerate the regeneration of gene-edited poplar trees by activating the morphogenic regulator-encoding gene *WUSCHEL,* which resulted in a doubling of the rooting rate and halved the number of days to root.

Of special interest

* Anzalone et al., Programmable deletion, replacement, integration and inversion of large DNA sequences with twin prime editing. *Nat Biotechnol* (2022)

The paper describes an advancement of the prime editing system in mammalian cells, the so-called twin-PE. Using the prime editor protein and two distinct pegRNAs, the authors were able to replace an endogenous DNA sequence, situated between two PAM recognition sites, by a DNA sequence of choice.

* Li et al., Highly efficient C-to-T and A-to-G base editing in a *Populus* hybrid. *Plant Biotechnol J* (2021)

The authors demonstrate the applicability of ABE and CBE in *P. tremula* x *P. alba*, by targeting *4-COUMARATE: CoA LIGASE1* (*4CL1*), and *PII.* Their highest editing efficiencies (78.9%-100%) were achieved by employing the *AtU3* promoter, whereas the editing efficiencies (0%-50.0%) achieved by the *AtU6* promoter were considerably lower, which highlights the importance of the correct choice of polymerase III promoters for base editing strategies in poplar.

* De Meester et al., Tailoring poplar lignin without yield penalty by combining a null and haploinsufficient CINNAMOYL-CoA REDUCTASE2 allele. *Nature Communications* (2020)

This paper describes the identification and characterization of a weak *CCR2* allele in *P. tremula x P. alba*. First, the authors demonstrated that biallelic knockout mutations in the lignin biosynthesis gene *CCR2* result in drastic growth defects, while monoallelic knockout mutations yield plants that are indistinguishable from the wild type. The authors describe a gene-edited line that combines a null *CCR2* allele with a haploinsufficient *CCR2* allele, yielding normal growth and improved biomass properties.

* Wang et al., Improving wood properties for wood utilization through multi-omics integration in lignin biosynthesis. *Nat Commun* **9,** 1579 (2018).

In this study, a multi-omics quantitative integrative analysis of lignin biosynthesis is performed. The authors perturbed 21 lignin pathway genes in *P. trichocarpa* and integrated transcriptomic, proteomic, fluxomic and phenomic data into a predictive model. Their analysis estimates how changing expression of (multiple) pathway genes affects protein abundances, metabolic fluxes, metabolite concentrations and a variety of wood properties, including lignin content and composition, tree growth, wood density and strength, and saccharification efficiency. The predictive model could be tested by multiplex gene editing.

* Walton et al., Unconstrained genome targeting with near-PAMless engineered CRISPR-Cas9 variants. *Science*, 368:290-296 (2020)

The paper describes two newly engineered *Streptococcus pyogenes* Cas9 variants (SpCas9), with altered or nearly abolished PAM requirements. The first reported variant, namely SpG, recognizes an expanded set of NGN PAMs whereas the further evolved second variant, called SpRY, has nearly abolished PAM requirements, by recognizing NRN and to a lesser extent NYN PAMs. The authors not only tested the nuclease activities in human cells, but furthermore demonstrated the applicability of their variants in base editing.

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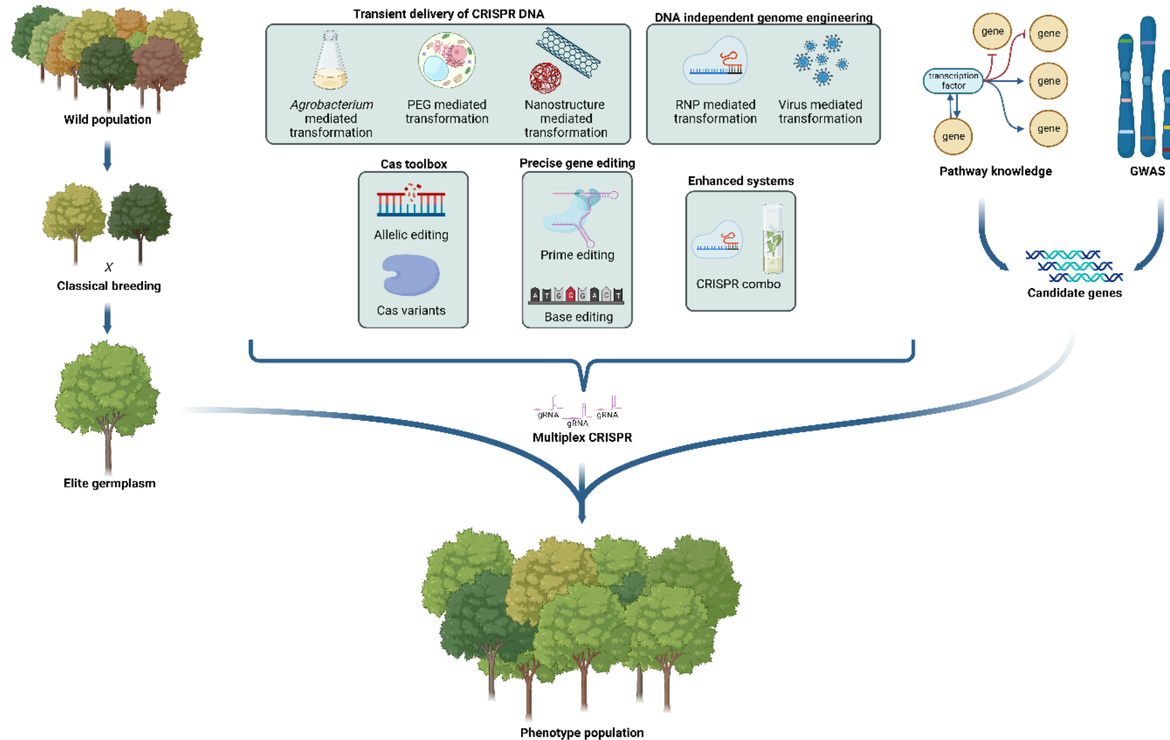

Figure 1. Accelerating the genetic improvement of forest trees through genome editing

The genetic variability that is naturally present within forest trees represents an important resource for conventional breeding. Using selective crossings and phenotypic selection of progenies, breeders combine existing allelic variation to generate elite germplasm with favorable biomass traits and tolerance to biotic and abiotic stress. The use of new breeding techniques (i.e., base editing, prime editing, or editing using Cas variants with relaxed PAM requirements), in combination with multiplexed editing approaches, will allow to create combinations of novel alleles at multiple target loci that were prioritized based on prior reverse genetics or GWAS research. To anticipate unconfined field release and commercialization, gene editing methods that avoid the integration of Cas-encoding DNA into the host genome are needed. Phenotyping and genotyping of the obtained gene-edited population in the field allow the identification of genetically improved trees that outperform their parental clone. Figure 1 was created with BioRender.com.