

## Viewpoints

# Social and biological innovations are essential to deliver transformative forest biotechnologies

### Summary

Forests make immense contributions to societies in the form of ecological services and sustainable industrial products. However, they face major challenges to their viability and economic use due to climate change and growing biotic and economic threats, for which recombinant DNA (rDNA) technology can sometimes provide solutions. But the application of rDNA technologies to forest trees faces major social and biological obstacles that make its societal acceptance a 'wicked' problem without straightforward solutions. We discuss the nature of these problems, and the social and biological innovations that we consider essential for progress. As case studies of biological challenges, we focus on studies of modifications in wood chemistry and transformation efficiency. We call for major innovations in regulations, and the dissolution of method-based market barriers, that together could lead to greater research investments, enable wide use of field studies, and facilitate the integration of rDNA-modified trees into conventional breeding programs. Without near-term adoption of such innovations, rDNA-based solutions will be largely unavailable to help forests adapt to the growing stresses from climate change and the proliferation of forest pests, nor will they be available to provide economic and environmental benefits from expanded use of wood and related bioproducts as part of an expanding bioeconomy.

With the recent launch of the new section of *New Phytologist* on 'transformative plant biotechnology (Halpin *et al.*, 2023)', it is an apt time to consider what is needed for transformative leaps that can enhance forest tree biotechnology. By biotechnology, we mean modification by recombinant DNA (rDNA) in the form of gene-editing (i.e. targeted modification of native DNA) or gene insertion, not applications such as genomic selection that can accelerate conventional breeding. We are both biotechnologists who have conducted basic and applied research on genomic and molecular biology of trees, including field trials of prototype-modified trees in the United States and Europe (e.g. reviewed in Pilate *et al.*, 2002; Strauss *et al.*, 2016; Chanoca *et al.*, 2019; De Meester *et al.*, 2022b). We have experience in research, translation,

and interaction with legal and market systems, as well as engagement with the general public.

We believe there are urgent needs for both social and biological innovations if biotechnology is to have a significant and beneficial impact on the productivity and quality of tree plantations or other types of forests. First, we need to create a regulatory and market environment that is conducive to innovation, rather than one that poses tremendous impediments; the latter is the rule throughout most of the world today. This is especially critical in the context of today's reality of rapid climate change, where genetic improvements of all sorts must occur as rapidly as possible if they are to help the ongoing adaptation of tree plantations and other forests to changing climates and pathogen pressures (Jacobs *et al.*, 2023). Second, there is a critical need for accelerated research, both fundamental studies such as the analysis of basic processes underlying plant regeneration and wood development, and technology advancements to make genetic modification more efficient and reliable in multiple tree species and genotypes. Third, it is essential that rDNA methods are well-integrated with conventional and genomic breeding, though many technical and social obstacles make this difficult. This would include extensive field research, as most results from laboratory or glasshouse experiments have limited relevance to field environments due to the very different stresses and other aspects of tree ecophysiology in the field vs indoor conditions (Viswanath *et al.*, 2012; Khaipho-Burch *et al.*, 2023). Field research is also important for public education about the true benefits and risks of modified trees. Finally, there are a number of larger contextual issues related to rDNA-modified forests, including questions of how to evaluate risks from rDNA technology vs that from other forms of breeding and management in a climate change world, and when and where rDNA methods should be applied. We believe that the primary uses will be in intensively managed, short-rotation clonal plantations, for which wood products have distinct industrial uses, but that there are also important opportunities to help with the protection or restoration of wild or feral forests. We will discuss each of these areas, giving examples from our own research and experience.

### Social innovation

Perhaps the most impactful social constraint is the implicit or explicit legal presumption of guilt due to the use of rDNA methods, with only minor exceptions for gene-edited plants that are similar or identical to those that could be produced from conventional breeding (Strauss *et al.*, 2010, 2022). By contrast, conventional breeding methods – whether they involve species transfer among continents, production of novel hybrids, clonal deployment, or radiation/chemical mutagenesis – have no such presumption of guilt. Some exceptions exist in the form of strict regulations on some exotic species that are known to be highly invasive and

damaging. This dichotomy is true essentially everywhere, including in the United States, where it is inherent to both the US Department of Agriculture (USDA) and Environmental Protection Agency (EPA) regulatory systems. However, the recently enacted USDA SECURE regulatory system is a modest exception; though regulation is triggered by the use of rDNA methods, regulatory decisions are intended to focus exclusively on whether there is a plausible risk that an rDNA-modified plant will cause or exacerbate a pest problem. It also has a means for rapid deregulation decisions, and exemptions for simple types of gene-edited plants. We regard this system, with its trait focus and options for exemptions and rapid decisions, as a first step in the right direction (Goraloglia *et al.*, 2021). There are now also regulatory exemptions for simple gene-edits in the regulatory systems of many other countries around the globe (Smyth, 2019; Turnbull *et al.*, 2021).

We are also encouraged by the mostly growing acceptance of rDNA-based methods by the public. The ‘eco’vandalism of recent decades against rDNA trees, whether due to concerns about violation of trees that are perceived as ‘natural’ or concerns about corporate control of forests, appears to have abated internationally. In addition, the sensational and highly derogatory headlines about rDNA crops, particularly in Europe, have become extremely rare. Although concerns about the safety of ‘GMO food’ have risen in the United States in the last decade and are shared by approximately half of the public, many view GMOs as a ‘necessary evil’ given their perceived value for helping to ‘feed the world’ amidst growth in population and standards-of-living (Funk, 2020). Likewise, social media messages have become distinctly less negative in recent years (Evanega *et al.*, 2022). Unfortunately, scientifically false statements, mostly negative ones, are still very common in mainstream print and online media, with a rate of 9% and considerably higher in Africa (*c.* 20%) during 2019–2021 (Lynas *et al.*, 2022).

On the other hand, the US EPA continues to increase its regulatory coverage in what seems far beyond its original mandate to protect the public and environment from pesticide harms; it now covers growth-improved plants and pest-resistant plants that do not involve pesticide use at all, but engage natural resistance mechanisms. This is a very effective deterrent for the use of pest-resistant trees, and indeed even the blight-resistant American chestnut, which involves no novel pesticides, has been held up in regulatory limbo there for years. The US EPA’s ‘exemption’ for gene-editing is also very limited, still requiring safety data and proof that the edit is already present in a sexually compatible relative (Stokstad, 2023). It appears that congressional action will be needed if this regulatory overreach is to be reversed. A similar sentiment was reflected in a recent US Senate report; it highlighted the need for regulatory discipline if biotechnology innovations are to flourish (National Security Commission on Emerging Biotechnology, 2023).

In contrast to most of the regulatory systems world-wide, for decades, scientists have called for a science-based regulatory system that focuses on the comparative risk associated with the presence vs non-presence of the trait, not with the method employed, and that would allow a broad array of rDNA-modified varieties to be planted and commercialized unless there are tangible, major risks associated with the novel trait (e.g. National Academies of Sciences

Engineering and Medicine, 2016). Such risks could include novel disease resistance mechanisms that might substantially impact wild or feral populations, or the generation of herbicide-resistant plants that will compromise plantation or weed management. A system focused on trait-based risk would also not preclude all gene flow during field research and breeding, thus allowing rDNA-modified plants to be readily deployed alongside conventionally bred plants during field trials unless they pose a tangible risk at the relevant rates of gene flow (normally very low in research/breeding trials) compared with potential benefits; this is critical for the expanded use of field trials as discussed below.

We note that a change to a trait-based system would not imply that all gene flow is risk-free, only that society will tolerate low levels and their impacts, just as it does for conventional breeding and agricultural production of the large majority of crop species, in exchange for the benefit of accelerated innovation to help cope with climate change and social stresses on food and fiber production systems. Of course, regulations that define what ‘tangible risk at the relevant rates of gene flow compared to potential benefits’ would look like is complex and beyond the scope of this paper to explore in depth; but, we imagine a system similar to the Canadian rDNA regulatory system (Lassoued *et al.*, 2020) in that it would apply to both rDNA and conventional breeding. The new USDA SECURE system, which tries to define ‘plausible plant pest risk’, faces similar challenges in definition (no surprise given biological and ecological complexity and diversity). A biological framework for such a system, which might be based on changes in native genes and their expression as familiar vs modifications that impart truly novel and exogenous trait mechanisms (e.g. new pest resistance toxins), and whether the trait is a domesticating one or likely to exacerbate weediness, was described more than two decades ago (Strauss, 2003). Due to extensive global trade in food and forest products, such a change to a trait-based system would ultimately need to occur at an international level, presumably through modifications to the Cartagena Protocol. Thus, it will require strong international leadership at the highest political levels.

In addition to regulatory considerations, the market also provides major constraints to rDNA innovation. Most importantly, the now widespread forest certification systems impose a complete ban on the use of rDNA-modified trees in production forests (Strauss *et al.*, 2019). This ban has been long-standing, and recent efforts by the Forest Stewardship Council (FSC) to lift the ban, or to at least allow joint research projects with developers of rDNA-modified trees, have failed due to its controversial nature within the FSC. This ban has discouraged investment in biotechnology throughout most of the globe – as our personal experience working with forestry companies has clearly shown. Regulatory revisions notwithstanding, until such bans are broadly and permanently lifted, most companies will continue to be unwilling to undertake significant investments in rDNA biotechnology.

## Science and technology innovation

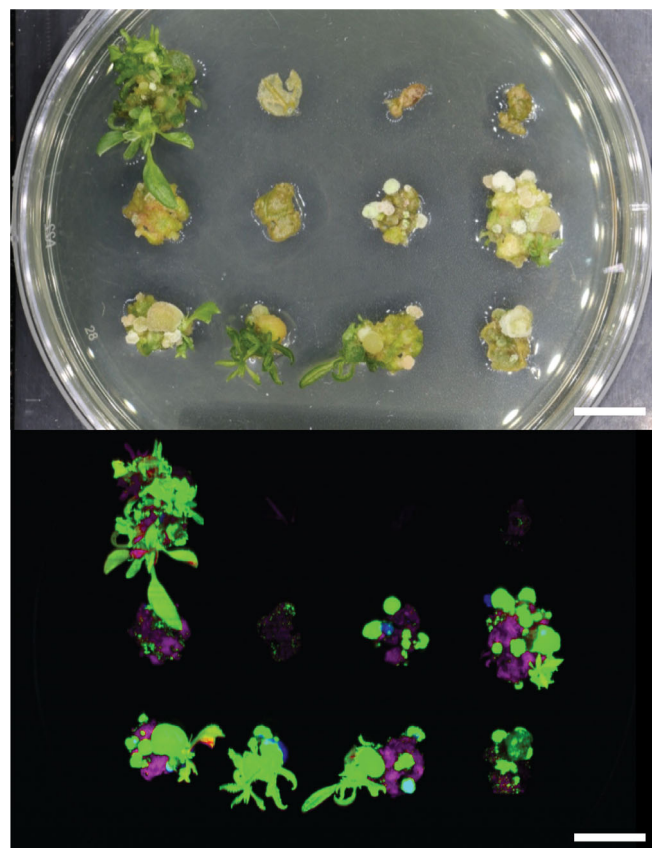
Informed by genomic databases and a growing understanding of the genes and pathways that control plant physiology and growth, the opportunities and the efficiency of trait modification have

expanded rapidly. Innovations such as basic gene transfer and regeneration methods, which were first developed in model plants (Barton *et al.*, 1983; De Block *et al.*, 1984) and then applied to crop plants, found their way into forest trees very soon after, first in the model tree poplar (Fillatti *et al.*, 1987) and then in horticultural tree crops, such as apple (James *et al.*, 1989), followed by the more difficult to transform conifers (Ellis *et al.*, 1989). Gene silencing (or RNA interference (RNAi)) was first observed in the model plant petunia (Napoli *et al.*, 1990), and once its mechanism was revealed and its application was optimized, it was widely applied to modify a diversity of traits in forest trees, from flowering (Rottmann *et al.*, 2000) to wood chemistry (Van Doorselaere *et al.*, 1995). Gene-editing methods using for instance zinc fingers were rapidly applied to forest trees (Lu *et al.*, 2016), and soon replaced by CRISPR, which has ever since been widely used to study tree physiology, flowering, and wood chemistry (Fan *et al.*, 2015; Zhou *et al.*, 2015; Elorriaga *et al.*, 2018; Sulis *et al.*, 2023). It is clear that the power and precision of rDNA methods will continue to grow, providing a further motivation for breeders to start taking advantage of its capabilities. We describe two areas of economic importance, in which rDNA innovations have been growing, as examples.

### Regeneration and transformation

Large improvements in transformation efficiency are essential for biotechnology to address the wide diversity of germplasm in tree breeding programs. In crop plants, genetic transformation methods have taken great steps forward in the last decade, largely the result of the use of developmental or morphogenic regulator (*DEV*) genes such as *WUSCHEL* and *BABYBOOM*. The benefits have been the greatest in monocots (cereal crops), which, combined with decades of research to develop embryogenic propagation systems, have led to transformation protocols that work in a wide variety of species and explant types (Gordon-Kamm *et al.*, 2019; Wang *et al.*, 2023). Similar progress is now being made for dicot species, including poplar, though these appear to often require different types of *DEV* genes or delivery systems that fit with their biology. For example, Ryan (2022) reported that the success of using the recently popular GRF-GIF types of *DEV* genes in poplar and eucalypts was highly genotype- and construct-dependent, requiring substantial gene customization and refinement of transformation protocols for successful use. Many woody species, both dicots and most notably conifers, also have distinct reproductive biology and/or regeneration systems compared with commonly studied, seed-propagated annual plants. In addition, the epigenetically 'mature' clonal tissues usually employed have distinct and highly variable levels of totipotency, which appear to affect their potential for embryogenesis or organogenesis. As discussed earlier (Strauss *et al.*, 2022), transformation and gene-editing systems for forest trees will require substantial further innovation and refinement for *DEV* genes to help mitigate the transformation bottleneck.

The very limited budgets for research in forest tree regeneration and transformation have prompted most researchers to simply try the genes and *Agrobacterium* strains available from agricultural crops – rather than to seek to understand the fundamental causes of



**Fig. 1** New phenomic systems enable more precise and rapid means for scientific research and technology development. Here, new imaging and machine vision methods facilitated genome wide association studies (GWAS) of transformation. A hyperspectral imaging system, combined with machine learning, was used to quantify transformation rate as part of a GWAS of its biological basis in *Populus trichocarpa* (Nagle *et al.*, 2024a). Brightfield image above, and false color image below showing GFP-expressing tissues in green, and non-transgenic, chlorophyll-containing tissues in magenta. Bars: 1 cm.

recalcitrance to standard transformation methods. However, it is likely that significant inroads could be made by taking a fresh look at the distinct biology of trees and their development first. For example, recent genome wide association studies (GWAS) facilitated by new phenomic methods (Fig. 1) have shown that there are numerous genes involved with amenability to regeneration or transformation, and very few of these appear to be identical to those that have been effective in aiding transformation in crops (Nagle *et al.*, 2023, 2024a, b). In addition, the massive immune response, extreme genetic diversity, and strong epigenetic/ontogenetic variation discussed above warrant investigation into their biological mechanisms and variability. Finally, some of the basic technologies required for advanced rDNA-based modification, such as that of gene excision, appear to work very poorly or not at all in trees, likely a reflection of the extensive DNA methylation that accompanies recombinase induction (Liu *et al.*, 2021). The desire to remove any transformation-promoting genes from the genome to avoid pleiotropy, or to remove gene-editing reagents to ease regulatory acceptance and reduce off-target mutagenesis, will require smarter excision systems as well as much improved transformation



technologies. There are also promising editing systems that rely solely on transient expression or viral inoculation (e.g. Huang *et al.*, 2023; Sun *et al.*, 2024), thus not requiring gene excision. However, transient systems may be very difficult to apply in species that are already highly recalcitrant to transformation. Although wide host range viral systems that can carry out transient editing, such as tomato spotted wilt virus, are known (Liu *et al.*, 2023), to our knowledge, their infectiousness, potential for systemic spread, and rate of editing have not been established for any forest trees.

## Wood chemistry and structure

One of the biggest markets for wood is the pulp and paper industry. Wood is largely composed of cellulose, hemicellulose, and lignin, but the lignin fraction needs to be extracted from wood chips in order to obtain high-quality pulp. The extraction of lignin is costly, as it requires the use of harsh chemicals at high temperature. Reducing lignin content or altering its chemical composition in trees would increase pulp yield and, thus, reduce the area of planted trees needed to supply pulp mills. A developing market for wood is in the biorefinery sector, in which lignocellulosic material is enzymatically converted to fermentable sugars to feed the bioeconomy. Also here, lignin is a main hurdle that limits the efficiency of biomass processing – although extracted lignin can also be an energy source or a feedstock for the synthesis of chemicals and materials (Brienza *et al.*, 2024). According to the Food and Agriculture Organization (FAO), the consumption of primary processed wood products is predicted to grow by 37% by 2050 in a business-as-usual scenario and will increase even more in a bioeconomy scenario (FAO, 2022). Clearly, the expected increase in wood consumption will put further pressure on the available space for plantation forestry, justifying rDNA-based efforts to improve the efficiency of wood productivity and processing.

Glasshouse and short-term confined field trials have demonstrated that strong improvements in wood processing efficiency can be achieved using rDNA technologies (reviewed by Chanoca *et al.*, 2019; De Meester *et al.*, 2022b; Li *et al.*, 2024; Zhu & Li, 2024). For example, downregulation of *CSE* in poplar by RNAi reduced lignin content by 25% and increased saccharification by 80–90% in glasshouse-grown trees (Saleme *et al.*, 2017), and 8-month-old, field-grown poplar CRISPR-edited in *CSE* showed a reduced lignin content by 29%, resulting in a 25% increase in saccharification efficiency (Jang *et al.*, 2021). Although these trees could provide obvious environmental benefits, including less chemical use and more pulp per acre of planted forest, the wood products derived from such trees cannot receive an FSC or other forest certification label simply because of the rDNA method used to engineer them, as discussed above (Strauss *et al.*, 2019).

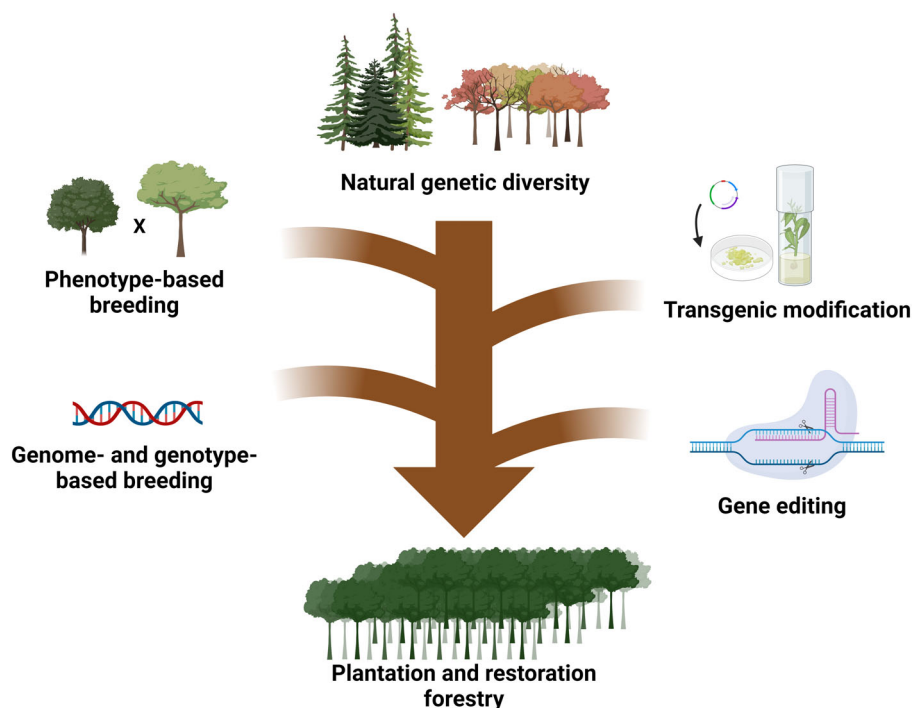
Lignin has many important physiological functions for sustaining upward growth, stem rigidity, and water conduction under tension in the xylem. Thus, strong modification in lignin content may lead to compromised tree health, especially under stressful environmental conditions, limiting the extent to which wood chemistry and physical properties can be productively modified (De Meester *et al.*, 2022b). One means to reduce or eliminate the negative impact of lignin modification is to target lignin

modification to specific tissues and cell types. For example, targeted downregulation of lignin biosynthesis in xylem fibers, while keeping vessel lignification intact, largely alleviates the negative effect on tree health while wood processing efficiency is still significantly improved (Gui *et al.*, 2020; De Meester *et al.*, 2021). Using gene-editing, a variety of strong and weak alleles can be created, allowing further ‘fine-tuning’ of lignin modification. Indeed, while poplar trees with a bi-allelic (full) knockout of the lignin biosynthesis gene *CCR2* could barely survive, CRISPR-based engineering of one knockout *CCR2* allele in combination with a leaky *CCR2* allele resulted in healthy trees with a 25–41% improved wood processing efficiency (De Meester *et al.*, 2020). A recent development is the engineering of lignin types that are entirely new by directing the biosynthesis of alternative lignin monomers in wood cells. These new monomers will be incorporated into the lignin polymer, facilitating biomass deconstruction (Wilkerson *et al.*, 2014; Mottiar *et al.*, 2016; Sibout *et al.*, 2016; Oyarce *et al.*, 2019; Hoengenaert *et al.*, 2022). Such new lignin structures may also improve the valorization of the lignin fraction itself, making lignin easier to fractionate into basic building blocks for the chemical industry (Van den Bosch *et al.*, 2015; de Vries *et al.*, 2021). Multiplex gene-editing or stacking of genes involving wood quality traits (e.g. lignin and wood density) with genes known to improve biomass yield (e.g. for pest resistance (Klocko *et al.*, 2014) or nitrogen metabolism (Jing *et al.*, 2004)), possibly combined with sterility genes to help in the management of gene flow (Strauss *et al.*, 2017), may help to promote a more sustainable wood products industry (Chang *et al.*, 2018; Sulis *et al.*, 2023).

## Integration of breeding and rDNA methods

Genetic improvements to adapt trees to new environments or to improve economically important traits depend on the presence of genetic variation in the breeding germplasm (Fig. 2). Forest trees are in the earliest stages of domestication and harbor a great deal of genetic and phenotypic diversity that can be exploited by conventional breeding. For example, in wild *Populus trichocarpa*, lignin content varies from 15.5% to 27.9%, indicating that major gains in wood processing efficiency can be made by conventional breeding alone (Studer *et al.*, 2011). In addition, rare defective alleles for lignin biosynthesis genes (and probably many other traits) are naturally present in the wild germplasm (MacKay *et al.*, 1997; Marroni *et al.*, 2011; Yamamoto *et al.*, 2020), and they can have a large effect on the trait if made homozygous (Vanholme *et al.*, 2013). However, forest tree breeding takes many decades, inbreeding is difficult (dioecious species such as poplar are mostly impossible to self-pollinate), and new sexual crosses to integrate such rare alleles into the genome of elite clones will break up the clone’s genetic constitution, thus requiring extra generations of breeding (Vanholme *et al.*, 2013).

In contrast to conventional breeding, rDNA methods such as RNAi or gene-editing are not limited to standing genetic variation; they make it possible to create or insert alleles as homozygous or dominant forms in any elite line without shuffling their genotypes as in conventional breeding. Moreover, they can be introduced in



**Fig. 2** Overview of methods for genetic improvement of forest trees. To accelerate genetic improvement, conventional and new breeding methods need to be fully integrated, as suggested by the central arrow. Genetic improvement starts by exploiting phenotypic diversity from natural trees and provenances by conventional breeding (sexual crossing followed by phenotypic selection). Current advances in genome sequencing and genotyping allow conventional breeders to accelerate the use of natural genetic diversity using strategies such as genomic selection. A variety of rDNA tools, including transformation (constitutive or tissue-specific overexpression or downregulation using transgenes) and gene-editing (to genetically modify native genes by insertion, deletion, translocation, or inversion), can insert or create desirable alleles for inclusion in breeding populations to modify high-value traits. Figure created with [BioRender.com](https://biorender.com).

multiple parental lines of the breeding germplasm provided that broadly effective transformation technologies are available, as discussed above. rDNA technologies also allow weak alleles to be generated (i.e. not just knockouts or strong overexpression), and these can be readily edited at multiple loci, giving a wide variation of effects that would be sorted out during field studies as part of conventional breeding. Thus, rDNA modification should not be regarded as an alternative to conventional breeding, but rather as a co-developed, complementary tool for generating diversity that is directed at specific traits of interest.

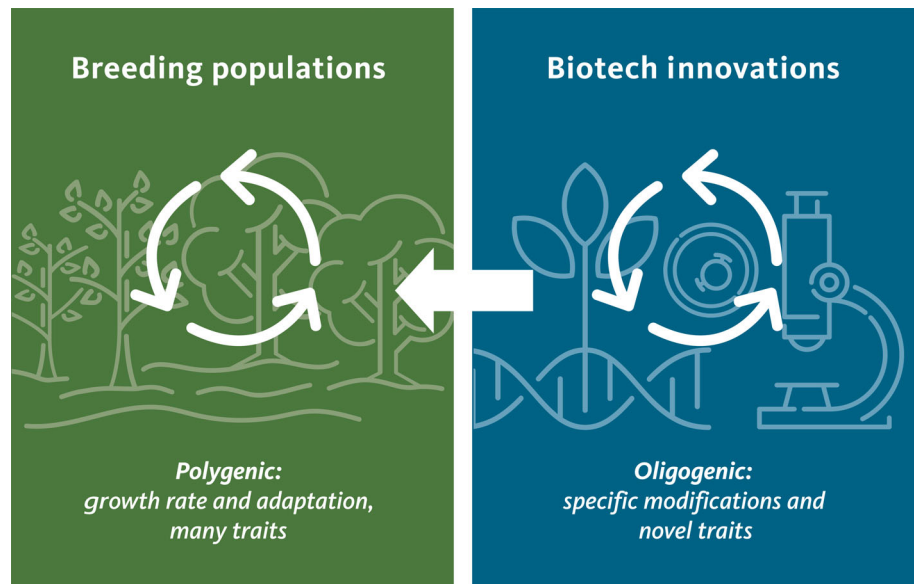
Genetic variation in most traits of economic importance, including yield, crown form, and overall stress tolerance, is controlled by hundreds to thousands of genes. For such traits, conventional breeding, in some cases aided by genomic selection, is the method of choice to improve these highly polygenic traits (Fig. 3). rDNA methods, which require knowledge of specific genes and their roles in the control of valuable traits, are currently best suited for physiologically simpler, mostly qualitative traits such as changes to plant chemistry, sexual fertility, and resistance to selected pests and pathogens that are controlled by a few genes (Strauss *et al.*, 2022). However, successful engineering of ecophysiological complex traits, such as abiotic stress tolerance or yield, has been demonstrated numerous times in the glasshouse and field, and achieved to a limited extent in commercial agricultural crops such as wheat and maize (Simmons *et al.*, 2021; Gupta, 2024). With the advent of multiplex editing and advanced phenomic methods, the division between traits suitable for rDNA-based modification and conventional breeding may weaken over time.

With the growing demand for wood, and the increasing pressure on forests due to climate change and rapidly changing pest populations, it is especially critical that rDNA technologies are

integrated in conventional breeding programs in a manner that does not slow conventional breeding. Most important is the early evaluation of rDNA-modified trees in the field (Fig. 4), preferably at several locations and over several years that approach a harvest cycle, and that the trait is introduced in multiple genotypes of the target commercial species. Currently, most rDNA-based research in forest trees is conducted in laboratories and glasshouses but is rarely further translated through field trials. Contained, indoor studies work well for fundamental research directed toward understanding biological processes and gene function; a well-controlled environment enhances the capacity for the detection of biological effects from a specific genetic modification. However, trees grown in the field experience daily and seasonal differences in weather conditions, including exposure to periods of drought, rain, frost, high UV radiation, wind, and pathogens. Furthermore, they undergo the seasonal cycles of growth and dormancy that have major effects on wood structure and stress tolerance, and also undergo a transition from juvenility to maturity, only starting to flower after several years. This maturation also affects the structure of wood and the expression of most other traits, including tolerance to stresses and pathogens. It is therefore no surprise that the phenotypes of field-grown trees, including their basic chemical composition (Viswanath *et al.*, 2012), are very different from those of glasshouse-grown trees, and that phenotypic differences observed for rDNA-modified trees grown in greenhouse conditions are often not maintained in the field (e.g. Lu *et al.*, 2015; Van Acker *et al.*, 2017; De Meester *et al.*, 2022a, b; Derba-Maceluch *et al.*, 2023). Indeed, conventional breeding, including the statistical model development needed for genomic selection, relies almost exclusively on field results for the same reasons.

Under current regulations and research programs, rDNA-based modifications are usually made in a single model genotype that is

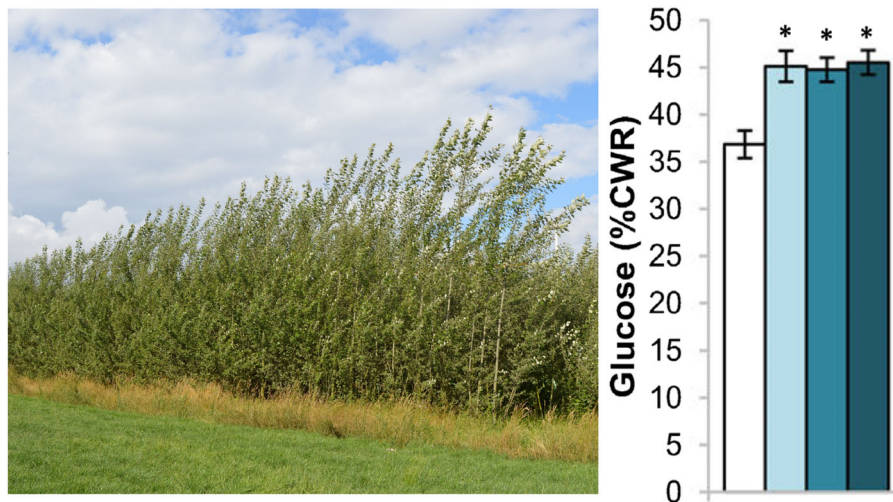
**Fig. 3** Integration of rDNA-based modification and conventional breeding. rDNA-based modification is today focused on traits under single gene or oligogenic control and provides qualitative trait modifications (blue box, circular arrows). Conventional and genomic breeding focuses on complex traits that are under polygenic control, resulting in quantitative trait modification (green box, circular arrows). Both forms need to be efficiently integrated so as not to slow conventional breeding progress. The horizontal arrow represents rDNA trees that enter the breeding program.



easily transformed; thus, rDNA-modified trees are evaluated completely independent of breeding populations. For integration into breeding, the rDNA-based modifications would first need to be recreated in appropriate germplasm and then re-evaluated in field studies in parallel (i.e. as breeding continues to improve germplasm). If the transformation of relevant germplasm and field testing could be readily undertaken as part of initial rDNA concept testing, the real-world value and pleiotropy from rDNA-based modifications could be evaluated much more rapidly (Khaipho-Burch *et al.*, 2023). Successful rDNA-based modifications could then be ready for inclusion in breeding programs soon after, and further tested as part of its crossing and field evaluations.

Early testing is also important because these novel rDNA-based modifications require scrutiny; most have not previously been subjected to multi-generational natural selection as have the highly polymorphic alleles in wild gene pools (Burdon & Walter, 2001).

The few early field studies that have been conducted indeed support their value for rapid evaluation of useful rDNA-based traits. For example, when a variety of RNAi constructs and insertion events were explored in the field with the intent of modifying the xylan content of poplar wood to aid saccharification, several constructs and events were identified that led to favorable results without compromising productivity, whereas others did not result in any improvements (Derba-Maceluch *et al.*, 2023). These



**Fig. 4** Field studies of rDNA trees are essential for the integration of rDNA and breeding. Shown is a field trial and saccharification analysis of wild-type (WT) and transgenic *Populus tremula* × *P. alba* with altered lignin composition. Left, trees grown for 2 yr after coppicing. Right, wood from three transgenic lines (blue bars) was found to be easier to deconstruct with cell wall-degrading enzymes as compared to that from WT trees (white bar). Shown is the amount of glucose released as percentage of cell wall residue (CWR). Bars represent means ± SD. Asterisks show that all transgenic lines were significantly different from the WT ( $P < 0.05$  for a two-way ANOVA with Dunnett's *post hoc* test).  $n = 18$  for WT and all three transgenic lines. Data are from De Meester *et al.* (2022a).



events could not have been predicted from laboratory studies alone. In a field experiment with poplars intended to identify transgenes that could elevate productivity by modification of active gibberellin levels, only one of several constructs was found to be successful, and would be a logical candidate for further testing and breeding (Lu *et al.*, 2015). Two field trials with transgenic poplar downregulated for *CAD* and with altered lignin demonstrated that the level of *CAD* downregulation is important to balance wood modification with normal growth; modest downregulation resulted in improved wood quality and normal growth, whereas strong downregulation resulted in a strong yield penalty (Pilate *et al.*, 2002; De Meester *et al.*, 2022a). The latter trees had shown no-yield penalty when grown in a well-controlled glasshouse, it was only observed in the field (Van Acker *et al.*, 2017). A similar phenomenon was seen with poplars downregulated for *CCR2* (Leplé *et al.*, 2007; Van Acker *et al.*, 2014).

## Putting opportunities and risks in context

### Plantation and 'natural forest' biotechnology

Although intensive plantation forestry is likely to be the main focus for rDNA innovations, wild and feral 'natural' trees (including those in forests affected by humans in the past or present) continue to be subject to devastating pathogens and insects for which, in some cases, there is limited innate resistance. This, coupled with the slowness of traditional tree breeding, especially when resistant germplasm is rare, creates a demand for new solutions, potentially including that of rDNA technologies. The most famous case is that of the American chestnut, which was devastated throughout its natural range by an exotic Asian fungal pathogen unintentionally imported to the United States via shipments of plant materials. Despite recent setbacks (Grandoni, 2023) and continuing regulatory obstacles, a transgenic, fully, or partially blight-resistant American chestnut appears likely to be the first rDNA-modified tree used for wild plantings (Newhouse & Powell, 2021). rDNA-based solutions for ecosystem repair beyond the chestnut have been very few, and we know of none that are in advanced field research, which is, to our opinion, due to several factors, including the following: (1) the extensive genetic diversity in forest tree germplasm, which therefore usually includes resistant germplasm, (2) the challenges and costs of transformation technology for most species, (3) the lack of in-depth knowledge of resistance mechanisms and the genes that are needed to support gene-editing or RNAi approaches, and (4) most importantly, the social obstacles in the form of regulatory and market blockages, as discussed above, which severely impede investments in research that could lead to rDNA-based solutions.

### Field trials as communication tools

In addition to their essential role in rDNA trait evaluation, field trials are also excellent means for communicating with the public about the science surrounding rDNA-modified trees. Seeing trees in the field – where they generally look like, well, trees! – provides a stark contrast to the caricatured, demonizing picture often painted

by activists. Unfortunately, regulations that require strict laboratory and glasshouse containment are the rule throughout most of the world – and make casual viewing of rDNA trees problematic. In some regions, companies are able to establish useful field trials; however, they are not usually open to the public due to proprietary and 'eco' vandalism concerns. Public–private partnerships are likely to be important to enable wider use of field trials for the goals of trusted public education and dialog (May *et al.*, 2024).

### Activist concerns about rDNA-modified trees

As forest tree biotechnology causes skepticism among some sectors of the public and the scientific community, it is important to recognize that – similar to tree breeding and other forestry research (White *et al.*, 2007) – assessment of its value and impact will occur incrementally over many years and locations as part of the common forestry practice of 'adaptive management' (e.g. Bolte *et al.*, 2009); the large majority of forest lands and species will see no significant applications of rDNA technologies at all for the foreseeable future. This is a result of the realities of scale-up and replacement of other types of trees and crops as they reach harvest age; the technical and social constraints discussed above; and of the fact that rDNA methods are today mainly suitable for a few intensively grown and clonal plantation species, such as eucalypts and poplars, and a very short list of wild species that are under serious biotic or abiotic threats, such as the American chestnut as discussed above. The realm for gene-editing in forest trees is likewise expected to be very limited for many years, as discussed in depth by Strauss *et al.* (2022). Thus, risks of rDNA use in forest systems will be highly focused, and there will be literally decades for impacts to be assessed and changes in technology or use made if needed. In addition, any risks from rDNA modification will occur along with the growing use and risks from a variety of novel breeding and planting approaches, including assisted migration and species replacement (Gömöry *et al.*, 2020), that are being forced by increasing climate impacts and pest stresses. The risks and benefits of rDNA-modified trees, as well as the risks from their non-use, must be considered in proportion to these very large new risks.

Unlike some activists that regularly emphasize 'genetic contamination' of forests by rDNA trees (e.g. Smolker & Petermann, 2019), we do not consider this, in general, to be a major biological issue. This does not mean that it would not be wise to use genetic containment means (sterility) at scale when socially or ecologically prudent, such as to avoid the spread of herbicide resistance where it would compromise weed control, avoid the spread of an unsafe protein into a food-producing forest tree such as chestnut, or to avoid further spread from exotic forest species that are known to be invasive (Strauss *et al.*, 2017). However, the use of rDNA-based methods *per se* does not, in our view, constitute a sufficient risk to require strict containment, and particularly not at the level of field research and breeding. When rDNA-modified trees could promote environmental values such as stress or disease resistance and thus are potentially critical to sustaining forests, we believe that the risks from some low-level spread into wild or feral trees must be considered acceptable. Indeed, during conventional breeding and ornamental horticulture, genes are continuously

**Box 1** Key requirements for rDNA-based innovations to enhance forest productivity and adaptation

- (1) Removal of strong regulatory and marketplace impediments to field research and commercial activities;
- (2) Expanded investment in basic and applied research, including transformation and gene-editing technology;
- (3) Early integration with breeding programs, including publicly visible and scientifically vetted field studies.

'leaked' from test plantations, generally without significant impact. Of course, until laws and market standards change, the spread of recombinant genes or propagules will present serious legal or market risks and thus severely constrain research.

## Conclusions

Forests, both planted and natural, are immense sources of ecological services and sustainable industrial products throughout much of the world. Plantations, the main focus of efforts to employ rDNA technologies, are superior to other forest communities when wood production is the primary goal (Hua *et al.*, 2022), and thus can relieve pressure on native forests for intensive harvesting.

For rDNA biotechnology to help increase forest productivity and adaptation to ongoing rapid climate change, however, it must have a similar freedom to innovate and be deployed to that of conventional breeding. Climate change is well known to existentially threaten forests nearly everywhere, and research in crops suggests that rDNA biotechnology may help in adaptation (Anderson *et al.*, 2020). We are unable to conceive of any of the often-stated risks from biotechnology that are on a par with the risks of doing little to nothing due to the current obstructive social environment. We therefore firmly believe that nothing short of a radical change in regulations, market restrictions, and research investment is warranted (Box 1).

There remain substantial gaps in the technical capacity for efficient use of rDNA technologies in forest trees. This primarily results from the very limited investment from either private or public sources in technology and variety development, including necessary field evaluations, a result that we believe is caused by the daunting social constraints discussed above. Thus, we must have social innovation first if we are to make substantial scientific and technical progress. We therefore should not wait for technology to mature to convince society of the case for strong investments and regulatory relief.

Finally, although acceptance of rDNA technologies in food and forest systems is clearly a 'wicked' problem (Durant & Legge Jr, 2006), we believe that substantial social innovations to regulations and market restrictions are not impossible to achieve. Some of these have in fact occurred in recent years, such as those embodied in the USDA Secure system (Hoffman, 2021) and the new UK Genetic Technology (Precision Breeding) Bill (Caccamo, 2023). Relaxations of regulation are occurring through these new policies, and throughout the world for basic gene-editing, and

even the EU is seriously considering revision of its GMO regulatory policies to exempt some categories of gene-edited and cisgenic plants (Nature Plants, 2023). However, we believe that regulatory relief only for simple forms of gene-editing will be woefully insufficient to meet most of the major threats that forests face; a much fuller set of rDNA biotechnologies will need to be included. For example, transgenes or edits that enhance weed management, pest control, pollution reduction, and wood quality have already been demonstrated to provide substantial benefits in pilot field studies (discussed in Strauss *et al.*, 2022). We will need these technologies, closely integrated with conventional breeding in all its forms, to have a chance of significantly mitigating the effects of climate stress on forest health and survival. We also will need to act expeditiously, as the extraordinary pace of climate change in recent years has shown us that time is not on our side. As has been said many times and in many ways (Council for Agricultural Science and Technology, 2013), we believe that today's precaution with regard to rDNA technologies is not to be found in the lengthy delays typically associated with the Precautionary Principle, but in extensive innovation and field research that can provide new options for production and adaptation.

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## Competing interests

None declared.


## Author contributions

WB and SHS both conceived and wrote the article. WB and SHS contributed equally to this work.

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