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REVIEW

Making headway toward enduring changes: perspectives on breeding tree crops through genome editing

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Abstract

Tree crops are explored for food sources and raw materials for industrial sectors. However, breeding tree species to meet current economic demands amid forecasts of agricultural resource scarcity and climate instability is a challenging task. This is especially true due to their long juvenile phase, often difficulties related to reproductive biology, and scarcity of genetic resources, which largely delays phenotyping and selection of segregating populations. On the other hand, genome sequence and transcriptomic data are becoming increasingly available for perennial crops, along with optimized protocols for genetic transformation and in vitro regeneration. Due to the development of these fields altogether and the advances in gene editing technologies, it is now possible to glimpse the design of tree crops with optimized traits for cultivation. We review the status of genome projects and the application of CRISPR-Cas-based systems in tree crops, alongside an exploration of gene editing technologies to develop perennial crop ideotypes. Herein, we seek to raise attention to the capabilities and potential of crop designing applied to tree species and to the opportunity that we have, as a society, to create stepwise strategies to tailor the breeding of perennial crops in the context of the current environmental challenges and increasing population demands.

Keywords Breeding · CRISPR-Cas9 · Genome · Gene editing · Molecular design

Introduction

The organization and persistence of human society depend essentially on crop cultivation, which is the basis of food supply as well as many raw materials for industrial uses. Among the crops we currently cultivate around the globe, many are arboreal plants used to obtain a diverse range of products, such as fruits, nuts, oils, syrup, rubber, and lignocellulose-based products such as timber, fiber, charcoal, and cork. As perennial crops, their cultivation requires a long-term commitment of resources by the farmer, which cannot be easily changed according to market fluctuations. Beyond the intrinsic difficulties related to tending to a plant to harvest its commercial products, many tree species have been domesticated only recently and still display many non-fixed traits, particularly clonally propagated species (Spengler, 2019). For most tree crops, compared to annual crops, breeding is still in its infancy due mostly to the time required to surpass the juvenile phase of a tree (some requiring several years and even decades) (Neale et al. 2017) and the decision of defining varieties as clonally propagated genotypes selected from segregating populations.

Our ability to cultivate crops to provide resources to meet human needs is fundamental for the continued development of agriculture. Increasing yields and quality of primary products have been, to a large extent, the result of scientifically informed breeding, which was a pillar of the first Green Revolution. Since then, technological leaps have led to transformational improvements in our ability to modify plant traits

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through genetic manipulation. The recent CRISPR-based gene-editing technology and the advancements in plant molecular genetics and genomics are leading us toward the fourth generation of crop improvement, marked by the precise and fast breeding of traits informed by gene function and an enhanced ability to quickly tailor crops to specific demands of farmers and consumers while maintaining the sustainability of agricultural systems (Fernie and Yan 2019).

At this point, gene editing has already been applied to some arboreal plants, e.g., poplar (*Populus tomentosa*) (Fan et al. 2015), coffee (*Coffea canephora*) (Breitler et al. 2018), apple (*Malus domestica*) (Charrier et al. 2019), and pomelo (*Citrus maxima*) (Jia and Wang 2020). However, the challenges of carrying out progeny studies, genome projects, or even manipulating plants in vitro can hamper the broader application of this technology to tree species. Still, in envisioning the potential and expanding the capacity of CRISPR-based genome editing, as demonstrated by recent advances in base editing (Grünewald et al. 2020) and the development of prime editing (Anzalone et al. 2019; Lin et al. 2020), many possibilities remain to be explored for refining pipelines of tree genetic improvement, including the domestication of species that are not conventionally cultivated for commercial purposes.

When producers (in small farms or big companies) decide to cultivate tree crops, they expect the plants to commercially produce for many years or even decades to offset the initial investment and opportunity costs. Therefore, tree breeding programs must consider limited farm inputs and climate resilience for sustainable production. The use of gene editing to either directly solve breeding constraints or identify the causal genes of important traits will assist in the establishment of feasible roadmaps for cultivar improvement. Alongside the focus on elite varieties of established tree crops, a more diversified panel of species can now be achieved through modern domestication via gene editing of potential wild crops to innovate by increasing cultivation diversity and creating new commercial niches for primary products.

The examples are many and widespread across the globe of native species awaiting domestication or which are not explored at its fullest despite their great potential, such as pawpaw (*Asimina triloba*, Annonaceae) and black walnut (*Juglans nigra*, Juglandaceae) from the eastern USA; many Myrtaceae species native to South America, such as gabiroba and cambuci (*Campomanesia* spp.), savanna pear, uvaia, cagaita, and the Brazilian cherry or grumixama (all *Eugenia* spp.); red bush apple or bemburrtyak (*Syzygium suborbiculare*, Myrtaceae) from Australia; Malabar plum or jambolao (*Syzygium cumini*, Myrtaceae) and emblic (*Phyllanthus emblica*, Phyllanthaceae) from the Indian subcontinent; jackfruit (*Artocarpus heterophyllus*, Moraceae), star fruit (*Averrhoa carambola*, Oxalidaceae), the Sapindaceae rambutan (*Nephelium lappaceum*) and lychee (*Litchi chinensis*) from Asia; marula (*Sclerocarya birrea*, Anacardiaceae) and mongongo nut (*Schinziophyton rautanenii*, Euphorbiaceae) from Africa, to name a few species of edible fruits. Even though some of these species are commercially produced, cultivation could be facilitated mainly by taming several traits (e.g., plant size and architecture, removing toxic metabolites, fruit shape, uniform ripening, and improved post-harvest qualities).

Despite the initial hurdles of awaiting years for commercial production to start atop breeding difficulties, tree crops fit perfectly into the perspective of investing in perennial plants to achieve agriculture sustainability because they do not require annual soil disturbances, need much less energy to cultivate than annual crops, have a much lower carbon footprint, and contributing to carbon sequestration and storage (Crews et al. 2018; DeHaan et al. 2020). Therefore, investing in improving cultivated species and expanding tree crop diversity in agriculture is imperative to meet the current environmental needs while keeping up with the demands of an increasing population in the twenty-first century. Here we explore this scenario in light of plant genomics and gene editing technology capabilities.

The challenges of breeding of perennials: genomics and genetic modification of tree crops

Tree crops are defined mainly by three characteristics: an elongated, erect stem (often leading to a large plant) with a woody structure (lignified stem), and a perennial growth habit that produce food or industrial raw material (Neale et al. 2017). Humans cultivate a very long connection with trees by consuming their fruits and nuts already a significant dietary portion of hominids and hunter-gatherers. Even though this relationship existed for many millennia, the process of tree domestication differed from that of annual crops, mainly because the selection of genetic variants was followed by clonal (asexual) propagation in many cases, ultimately leading to the modern cultivated varieties without fixed mutations (Spengler 2019). This means that, due to the high level of heterozygosity in their genomes (often due to obligatory cross-pollination in allogamous species), the progeny from an ideotype tree may not exhibit desired phenotypes because the traits are segregating wildly.

Most commercial tree improvement programs seek to mine the natural genetic variation in wild populations for desirable traits. The main aim is to maximize genetic gain per time unit related to yield and sustainability with the highest economic efficiency. To this end, breeding programs go through a cyclical selection process from a genetic base (e.g., natural populations and germplasm banks) to establish

a breeding population. Often, breeders cast a wide net to establish their base population from a broad set of provenances, such as landraces, hybrids, and exotic varieties. They continue to infuse their base population with diverse genotypes at each generation, aiming to introgress favorable or new traits and reduce inbreeding depression to select the "best" genotypes within a given cycle. The criteria for "best" genotypes are defined by the breeding goals and targeted hardiness zones (edaphoclimatic conditions).

Establishing the genetic merit (breeding value) of a given individual may take several years for tree species, given their lengthy sexual reproduction cycle and poor juvenile-mature correlation for most traits of commercial importance. The breeding cycle length is determined by the duration of the plant's juvenile phase, which corresponds to the time required for the first flowering to occur and can take from three years (e.g., stone fruit and citrus) to up to more than a decade (e.g., persimmon, avocado, and olive) when planted from seeds (Nocker and Gardiner 2014; Moreno-Alías et al. 2010). This extended juvenile phase of arboreal plants compared to annual crops also hampers the development of high-resolution genetic maps, which are needed for establishing the position of markers on linkage groups or chromosomes.

Plenty of genomic resources for tree crops have been developed. The first tree to have its genome sequenced and assembled to the chromosome level was *Populus trichocarpa* (poplar, Tuskan et al. 2006), which was published just 6 years after the publication of the first angiosperm genome (*Arabidopsis thaliana*, Kaul et al. 2000). We have summarized 46 arboreal genome publications since poplar, among which the most recurring genus is *Citrus* (Table 1), and some recent examples comprise the oil-tea tree (*Camellia chekiangoleosa*, Shen et al. 2022), the tropical fruit tree, lychee (*Litchi chinensis*, HU et al. 2022) and a wild, caffeine-free coffee species (*Coffea humblotiana*, Raharimalala et al. 2021).

Genomic information on tree crops allows for advances in understanding plant evolution (Wu et al. 2014; Rendón-Anaya et al. 2019; Liu et al. 2020b), the dynamics of gene families (Pinto et al. 2019; Zhang et al. 2021b), genome-wide exploration, association studies (Pinto et al. 2021; Fahrenkrog et al. 2017; Zhang et al. 2021a), and population genomics (Wang et al. 2020; Chhetri et al. 2019). Also, the sequencing and analysis of the genetic pools of crop relatives, called pan-genomics (Gao et al. 2019; Alonge et al. 2020; Jayakodi et al. 2020; Tao et al. 2021), is shedding new light on understanding the influence of structural variants on traits and advancing gene discovery. Among tree crops, such resource is available for poplar and apple (Pinosio et al. 2016; Sun et al. 2020), and opportunities are wide open for other species.

Using genomic information with recombinant DNA technologies allied to tissue culture will pave the way to innovative genetic engineering in tree crops. Efficient in vitro regeneration and genetic transformation protocols are established for many tree crops with sequenced genomes. However, due to technical difficulties of the genetic transformation and regeneration process, besides the nontechnical aspects related to regulatory agencies and consumer acceptance, trees make less than 3% of the total approved transgenic crops worldwide (ISAAA 2022). The recent technological expansion of genetic breeding provided by cheaper genome sequencing and efficient gene editing (Jinek et al. 2012; Nadakuduti and Enciso-Rodríguez 2021) is helping to balance the cost–benefit for tree biotechnology and genetic research and leading scientists and companies to embrace gene editing to create new tree varieties. We propose that the road be open for de novo domestication of wild trees (Zsögön et al. 2018; Gasparini et al. 2021) with commercial potential or improve traits of semi-domesticated arboreal species (Petersen et al. 2012; Sganzerla et al. 2021).

A brief analysis of the expanding potential of CRISPR-based gene editing for crop genetic improvement

The trajectory of crop improvement is divided into four generations: (i) crop domestication and breeding via phenotype-based selection performed by independent farmers (starting circa 10,000 BCE); (ii) scientific breeding by mating designs, hybrid breeding, statistical analyses, and the use of synthetic fertilizers and pesticides, marked by the high yielding dwarf plants that culminated in the first Green Revolution (1950–1960); (iii) biotechnology through tissue culture, transgenics (genetically-modified organisms, GMO), and marker-assisted breeding (second Green Revolution: 1980–2000s) mainly focused on farming and retailer traits, such as the long shelf life products (e.g., *Flavr Savr* tomato), resistance to insects (Bt crops), the herbicide glyphosate (roundup ready crops), and the papaya ringspot virus (e.g., the rainbow hybrid), in addition to the high-beta-carotene rice (golden rice); (iv) which led to the current stage of plant breeding, with the advent and ample adoption of genomics, precision breeding (including molecular assisted-breeding), mining of big data, gene editing, and the rise of artificial intelligence (cf. Cheng et al. 2021), and often referred to as the third Green Revolution.

The current stage of crop improvement is marked by efforts to increase the sustainability of agricultural systems imposed by social and environmental pressures (Fernie and Yan 2019), facilitate farming and trading, as well as improve consumer-oriented traits such as food nutrition (e.g., high-lycopene and high-anthocyanin tomatoes, optimal lipid composition in canola), size and shape (watermelon and pepper), aspect (e.g., the frivolous

Table 1 Cultivated tree species with sequenced genomes

Tree crop species	Common name	Genome size	# Protein-coding transcripts	#Public RNA-seq samples*	Reference genome publication
Actinidia chinensis	Kiwifruit	653 Mb	40.464	322	Huang et al. 2013 (draft); Pilkington et al. 2018; Wu et al. 2019
Actinidia eriantha	Kiwifruit	690.4 Mb	42.988	25	Tang et al. 2019
Annona muricata	Soursop	639.6 Mb	23.375	19	Strijk et al. 2021
Azadirachta indica	Neem tree	N/A	N/A	26	Krishnan et al. 2016
Camellia chekiangoleosa	Oil-tea tree	2.73 Gb	64.608	56	Shen et al. 2022
Camellia sinensis	Tea plant	3.02 Gb	36.951	2496	Xia et al. 2017
Carica papaya	Papaya	135 Mb	27.793	229	Ming et al. 2008 (draft)
Cinnamomum kanehirae	Stout camphor tree	730.7 Mb	27.899	8	Chaw et al. 2019
Citrus clementina	Clementine	301.4 Mb	33.929	59	Wu et al. 2014
Citrus grandis	Pomelo	380 Mb	42.886	323	Wang et al. 2017
Citrus ichangensis	Ichand papeda	391 Mb	43.103	6	Wang et al. 2017 (draft)
Citrus medica	Citron	407 Mb	47.506	33	Wang et al. 2017 (draft)
Citrus reticulata	Mandarin orange	370 Mb	42.653	166	Wang et al. 2018a, b (draft)
Citrus sinensis	Sweet orange	319 Mb	46.147	836	Xu et al. 2013 (draft); Wu et al. 2014
Coffea canephora	Robusta coffee	580 Mb	25.574	287	Denoeud et al. 2014
Coffea humblotiana	Wild coffee	420.72 Mb	32.874	4	Raharimalala et al. 2021
Corymbia citriodora	Eucalypt	408 Mb	35.632	5	Healey et al. 2021
Elaeis guineensis	Oil palm	1.8 Gb	26.059	485	Singh et al. 2013
Eucalyptus grandis	Eucalyptus	691 Mb	46.28	255	Myburg et al. 2014
Ficus carica	Common fig	248 Mb	36.138	121	Mori et al. 2017
Ficus erecta	Wild fig	331.6 Mb	51.806	0	Shirasawa et al. 2020
Handroanthus guayacan	Guayacan	339.77 Mb	70,146 (contigs)	0	Burley et al. 2021
Hevea brasiliensis	Rubber tree	1.46 Gb	43.792	390	Tang et al. 2016
Jacaranda copaia	Parapará	616.19 Mb	53,041 (contigs)	0	Burley et al. 2021
Jacaranda mimosifolia	Jacaranda	707.32 Mb	30.507	4	Wang et al. 2021
Juglans regia	Walnut	606 Mb	32.496	351	Martinez-García et al. 2016
Litchi chinensis	Lychee	470 Mb	31.896	344	Hu et al. 2022
Lycium barbarum	Wolfberry	1.67 Gb	33.581	75	Cao et al. 2021
Malus domestica	Apple tree	688 Mb	45.166	2055	Velasco et al. 2010; Daccord et al. 2017
Mangifera indica	Mango tree	392.9 Mb	41.251	222	Wang et al. 2020
Melaleuca alternifolia	Tea tree	326 Mb	37.266	18	Voelker et al. 2021 (draft)
Morus alba	Mulberry	346.4 Mb	N/A	287	Jiao et al. 2020
Olea europaea	Olive tree	1.14 Gb	50.684	460	Cruz et al. 2016
Persea americana	Avocado	N/A	24.616	181	Rendón-Anaya et al. 2019
Pinus taeda	Pine	22 Gb	N/A	207	Zimin et al. 2014; Zimin et al. 2017
Populus trichocarpa	Poplar	392.2 Mb	52.400	1637	Tuskan et al. 2006
Prunus avium	Sweet cherry	272.4 Mb	43.349	393	Shirasawa et al. 2017
Prunus persica	Peach	227.4 Mb	47.089	1267	Verde et al. 2013
Psidium guajava	Guava	443.8 Mb	25.601	51	Feng et al. 2021
Punica granatum	Pomegranate	274 Mb	30.903	243	Qin et al. 2017; Yuan et al. 2018
Pyrus betuleafolia	Wild pear	532.7 Mb	59.552	30	Dong et al. 2020
Pyrus bretschneideri	Pear	512 Mb	42.812	269	Wu et al. 2013
Pyrus communis	European pear	577 Mb	43.419	222	Chagné et al. 2014
Tectona grandis	Teak	338 Mb	46.826	15	Zhao et al. 2019
Theobroma cacao	Cocoa tree	346 Mb	39.991	196	Argout et al. 2011
Vitis vinifera	Grapevine	487 Mb	55.564	6352	Jaillon et al. 2007
Ziziphus jujuba	Jujube	437.65 Mb	32.808	304	Liu et al. 2014

* Public data (fastq files) available in NCBI-SRA, accessed on May 24th, 2022

non-browning *Arctic* apple), and scent and aroma (e.g., tomatoes and ornamental roses). Often, traits of interest are genetically complex due to being multigenic, which require specific allelic sets to produce the desired phenotype—this is especially the case of drought, temperature and salt tolerances, and nutrient uptake and use efficiency.

The advent of the CRISPR-based gene editing technology occurred after a demonstration that the bacteria immunity system could be harnessed as a molecular tool to perform programmed genetic changes in higher organisms less than a decade ago (Jinek et al. 2012—for a comprehensive historical review, cf. Lander 2016). Since its adaptation as a molecular tool, the key ability of the CRISPR system to be explored and used is the programmable DNA double-strand break (DSB), which is catalyzed by the nuclease Cas9 [and other type-2 Cas nucleases, such as Cpf1 (Zetsche et al. 2015, 2017)], guided by a programmable single-stranded guide RNA (sgRNA or just guide RNA), designed to be complementary to a targeted genome sequence (Jinek et al. 2012). DSB is repaired by the innate DNA repair machinery of the cell through two different pathways, the non-homologous end joining (NHEJ) and homology-directed repair (HDR) (Jiang and Doudna 2017). The error-prone NHEJ is the most used option in somatic plant cells (Rozov et al. 2019). Due to the repeated action of the Cas nuclease on the targeted site, the region tends to be misrepaired, leading to a permanent mutation.

The disruption in the genetic code caused by the DSB misrepair can be targeted to virtually any segment of an organism's genome by designing a complementary sgRNA. When targeting an open reading frame (ORF) of a gene, it can potentially disrupt the protein function; in the case of a UTR-ORF (uORF), it may affect the translation of a gene downstream (principal ORF), and when the mutation is programmed to a promoter region, it can influence its transcriptional activity (Fig. 1). Despite being less common, somatic plant cells can also use the HDR pathway in response to DSB. Incorporating a selected DNA fragment into a desired genomic position is possible by delivering a donor DNA template and the CRISPR-Cas reagents (i.e., Cas enzyme and sgRNA). Although this approach is technically challenging, significant progress has been made in developing this knock-in strategy in plants (e.g., rice: Lu et al. 2020; Dong et al. 2020).

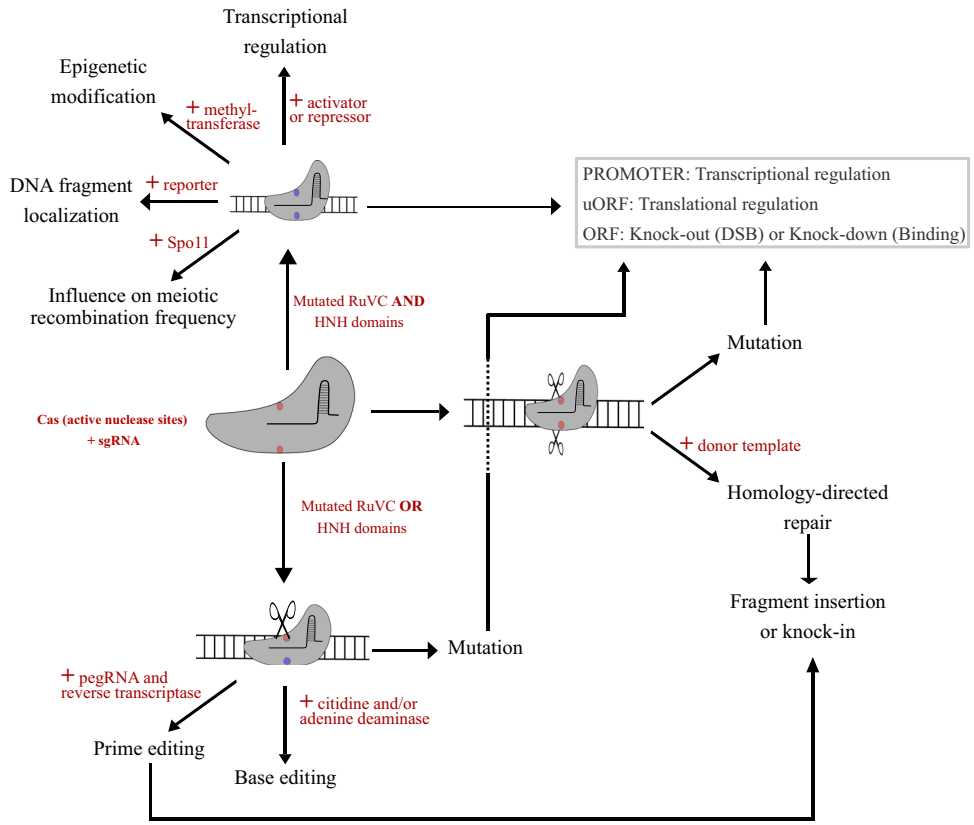

Fig. 1 The versatility of CRISPR systems. Cas proteins complexed with sgRNAs promote DSB, which can either generate mutations through the endogenous nonhomologous end joining (NHEJ) pathway or induce gene insertions through the homology-directed repair (HDR) pathway when a template is available. An enzymatically dead protein (dCas9) can be harnessed to assist with varied functions via protein fusions, such as transcriptional regulation, epigenetic modification, DNA fragment localization, and enhanced meiotic recombination. A Cas9 in which only one DNase domain is inactivated functions as a nickase (nCas9) by catalyzing one-strand DNA breaks and is used for base editing and prime editing. Mutations targeted to promoter regions, open reading frames (ORFs), or untranslated ORFs (uORFs) can induce transcriptional changes, knock out or knockdown of gene functions, and translational rates, respectively

Beyond the outstanding breeding potential of the original DSB-inducing CRISPR-Cas system, manipulating the catalytic Cas9 DNase domains (RuvC and HNH) made it possible to induce single-strand breaks (nicking) on the DNA by deactivating either of the domains (nickase: nCas9) or turning the nuclease into a programmable binding protein by eliminating both DNase activities (dead-Cas9: dCas9) (Qi et al. 2013; Ran et al. 2013) (Fig. 1). Fusions of nCas9 and dCas9 with other proteins, such as transcriptional activator or repressors, cytidine or adenine deaminases, methyltransferases, reporter proteins, and meiosis-specific endonucleases (e.g., Spo11), creates powerful tools to manipulate transcription, induce specific single-base substitutions (C → T and A → G), epigenetic modifications (DNA methylation and histone modifications), visualize DNA fragments on the chromosome context, and alter recombination rates during meiosis (Larson et al. 2013; Paixão et al. 2019; Qin et al. 2020; Hilton et al. 2015; Dreissig et al. 2017; Sarno et al. 2017) (Fig. 1). Moreover, different versions of Cas proteins can be exploited for the versatility of PAM sites (the DNA recognition motif for the Cas protein, such as 5′-NGG for Cas9 or 5′TTTV for Cpf1/Cas12a) and substrate affinity (e.g., the RNA editing capability of Cas13), making the CRISPR system a very versatile molecular tool for genetic engineering (Manghwar et al. 2019).

Recent breakthroughs revolving around the CRISPR-Cas system include a method to induce small programmable insertions on specific genomic locations, named prime editing (Anzalone et al. 2019), which has already been tested on wheat and rice (Lin et al. 2020). Prime editing is based on a nickase (nCas9) fused to a reverse transcriptase (RT) guided by a prime editing-gRNA (pegRNA) that guides the complex to the targeted genomic region and contains the sequence of interest to be incorporated into the DNA by the RT action after nCas9 performs the nicking. Furthermore, recent developments extend the toolkit of gene editing in plants, such as the C:G to G:C editing system (Chen et al. 2021), the compact CRISPR-Casϕ system (Pausch et al. 2020), and the development of near-PAMless CRISPR-based genome editors (Walton et al. 2020). In addition to the examples mentioned above of CRISPR-Cas gene editing approaches applied to crops, we refer the reader to recent reviews to deepen the comprehension of this technology (Wada et al. 2020; McCarty et al. 2020; Mishra et al. 2020; Moradpour and Abdulah 2020; Anzalone et al. 2020).

CRISPR technology is constantly being adapted and generating new tools, reflecting its remarkable adaptability and potential for molecular biology and plant breeding. Noteworthy systems include CRISPR-associated transposases (CAST), which could be harnessed on eukaryotes to drive DNA insertions guided by long RNAs efficiently, thus surpassing an existing bottleneck on genome editing, i.e., the controlled insertion of specific sequences to a specific location of the genome (Klompe et al. 2019; Strecker et al. 2019; Saito et al. 2021). The application of the CRISPR technology in plants is a multidisciplinary effort that requires advances in tissue culture (e.g., in vitro regeneration rates in recalcitrant species, especially trees; and the efficient protoplast manipulation protocols for allogamous or polyploid species), genetic transformation, genome sequencing technologies and bioinformatics, and vector manipulation based on efficient cloning techniques, such as Gibson assembly, type-II restriction enzyme-based cloning (golden gate and modular cloning), BP/LR recombination (gateway), ligation-independent cloning (LIC), or gene synthesis (e.g., Čermák et al. 2017; Hahn et al. 2020).

The expanding capacity of gene editing makes crop designing possible and will facilitate the domestication and breeding of arboreal species. Figure 2 shows some gene-editing approaches for performing targeted genetic modifications that could be applied to the molecular breeding of trees.

Fig. 2 CRISPR applicability for generating stable gene-edited tree crops. **A** Editing of genes already functionally characterized in tree species. **B** Orthologs genes identified in tree genomes and gene editing. **C** Genes or QTLs associated with traits of agricultural importance with potential for gene editing. **D** Editing anti-crossover factor genes to increase meiotic recombination. The resulting decrease in linkage drag can lead to increased genetic diversity in population studies. **E** Editing domestication traits in wild species to create novel, resilient crops through de novo domestication. **F** Generating transgene-free edited plants via the introduction of pre-assembled Cas9 and sgRNA into protoplasts. **G** Genome editing protocols that do not rely on tissue culture have been proposed by using RNA virus-based vectors with mobile sgRNAs or manipulating genes that regulate plant meristem identity maintenance via infecting growing plants ex vitro

CRISPR-based approaches applied to tree crops

Even though the technology is relatively recent, gene editing has already become feasible in tree crops (Table 2; Suppl. Table S1). The first CRISPR-edited arboreal plant was the Chinese white poplar (*Populus tomentosa*, Salicaceae), which genome was manipulated to knock out the *PtoPDS* gene, resulting in photobleached, albino-like plants as a proof-of-concept for gene editing (Fan et al. 2015). The same strategy was later applied to other trees for protocol development: citrus (*Citrus trifoliate* × *C. sinensis*, Rutaceae), apple tree (*Malus* × *domestica* and *Malus prunifolia* × *M. pumila*, Rosaceae), grapevine (*Vitis vinifera*, Vitaceae), coffee (*Coffea canephora*, Rubiaceae), kiwifruit (*Actinidia* spp. Actinidiaceae), and banana (*Musa spp.*) (Zhang et al. 2017; Charrier et al. 2019; Osakabe et al. 2018; Nakajima et al. 2017; Breitler et al. 2018; Wang et al. 2018a, 2018b; Ntui et al. 2020).

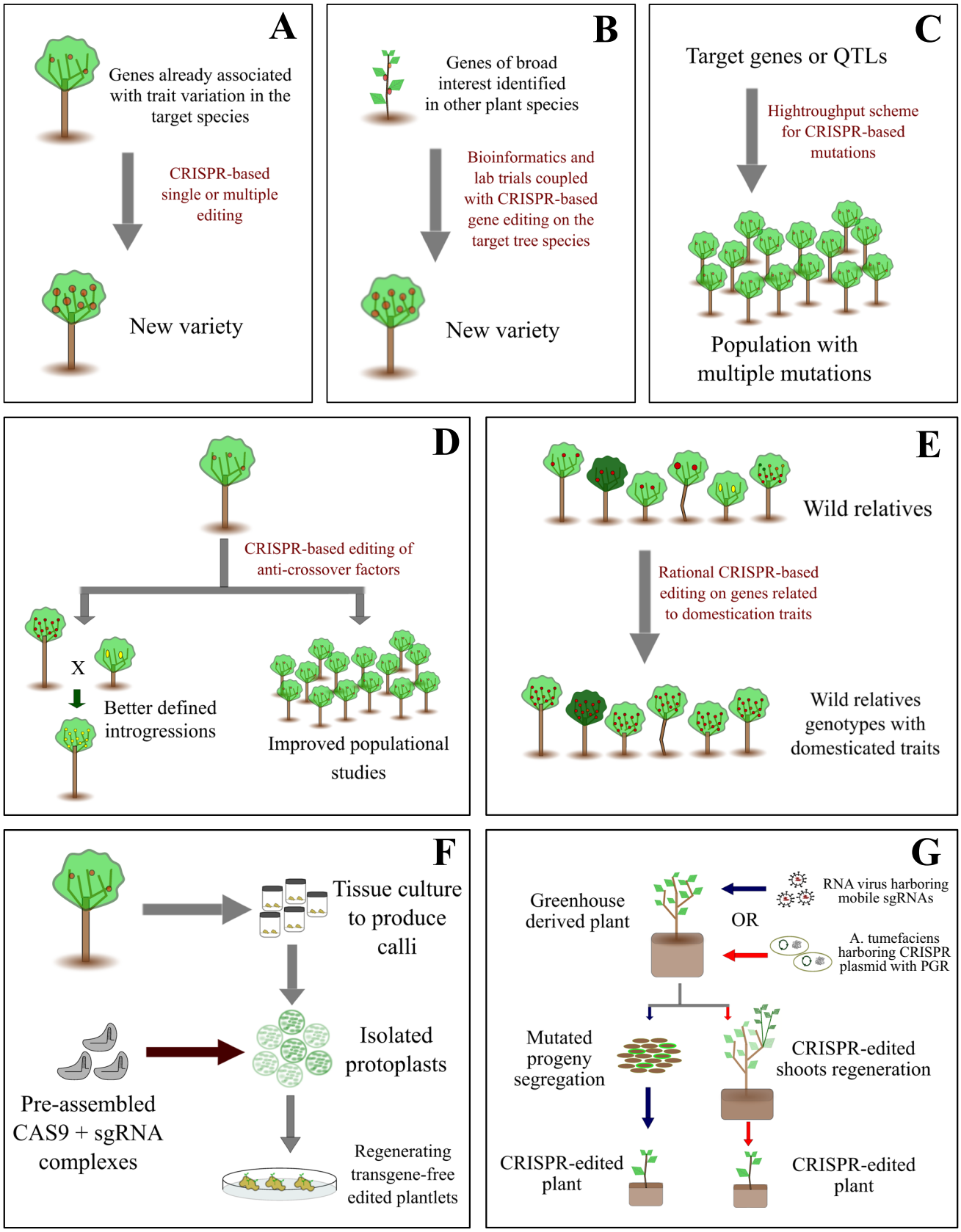
A
Genes already associated with trait variation in the target species
CRISPR-based single or multiple editing
New variety
B
Genes of broad interest identified in other plant species
Bioinformatics and lab trials coupled with CRISPR-based gene editing on the target tree species
New variety
C
Target genes or QTLs
Hightroughput scheme for CRISPR-based mutations
Population with multiple mutations
D
CRISPR-based editing of anti-crossover factors
X
Better defined introgressions
Improved populational studies
E
Wild relatives
Rational CRISPR-based editing on genes related to domestication traits
Wild relatives genotypes with domesticated traits
F
Tissue culture to produce calli
Isolated protoplasts
Pre-assembled CAS9 + sgRNA complexes
Regenerating transgene-free edited plantlets
G
Greenhouse derived plant
RNA virus harboring mobile sgRNAs
OR
A. tumefaciens harboring CRISPR plasmid with PGR
Mutated progeny segregation
CRISPR-edited shoots regeneration
CRISPR-edited plant
CRISPR-edited plant

Table 2 Tree species with genomes manipulated via gene editing

Tree crop species	Target	Regeneration pathway	Mutant phenotype	References
Actinidia spp	*AcPDS*	shoot organogenesis	albino-like shoots	Wang et al. 2018a, b
Citrus maxima	*LOB1* promoter	shoot organogenesis	canker-resistant citrus plants	Jia et al. 2020
Citrus maxima	*LOB1 promoter*	shoot organogenesis	canker resistant mutants	Jia et al. 2022
Citrus paradisi	*CsLOB1*	shoot organogenesis	canker-resistant citrus plants	Jia et al. 2017
Citrus paradisi	*CsLOB1* promoter	shoot organogenesis	canker infection alleviation	Jia et al. 2019
Citrus sinensis	*CsLOB1* promoter	shoot organogenesis	alleviate symptoms against XccΔpthA4:dCsLOB1.3 (artificial dTALE for CsLOB activation)	Jia et al. 2016
Citrus sinensis	*CsLOB1* promoter	shoot organogenesis	enhanced canker resistance on plants	Peng et al. 2017
Citrus sinensis	*CsWRKY22*	shoot organogenesis	decreased susceptibility to citrus canker	Wang et al. 2019
Citrus sinensis × *Poncirus trifoliata*	*CsTI1/CsTI2*	shoot organogenesis	Increased shoot branching by conversion from thorns	Zhang et al. 2020
Citrus sinensis × *Poncirus trifoliata*	*CsCEN*	shoot organogenesis	Modified shoot architecture—axillary meristems converted to thorns	Zhang et al. 2021a, b
Citrus trifoliata × *C. sinensis*	*CsPDS*	shoot organogenesis	albino-like plants	Zhang et al. 2017
Coffea canephora	*CcPDS*	indirect somatic embryogenesis	Chlorosis and lanceolate leaves	Breitler et al. 2018
Eucalyptus grandis × *E. urophyla*	*ELFY*	shoot organogenesis	dysregulation on flowering-related gene's expression; underdeveloped or absent floral organs	Elorriaga et al. 2021
Fortunell hindsii	*FhCCD4/FhPDS*	shoot organogenesis	albino-like plants	Zhu et al. 2019
M. prunifolia × *M. pumila*	*PDS*	Shoot organogenesis	Albino-like plantlets	Nishitami et al. 2016
Malus prunifolia × *M. pumila*	*PDS*	Shoot organogenesis	Albino-like shoots	Osakabe et al. 2018
Malus sieversi	*mdm-miR171i/MsPDS*	Shoot organogenesis	Increased drought tolerance/ albino-like plantlets	Wang et al. 2020
Malus × *domestica*	*MdDIPM4*	Shoot organogenesis	Reduced susceptibility	Pompili et al. 2020
Malus × *domestica*	*MdPDS/MdTFL1*	Shoot organogenesis	Albino-like plantlets	Charrier et al. 2019
Musa spp.	*MaPDS*	Indirect somatic embryogenesis	Albino-like plantlets	Ntui et al. 2020
P. × *canescens*	*TAC1*	Shoot organogenesis	Plants with upright oriented leaves	Fladung 2021
Parasponia andersonii	*PanHK4/PanEIN2/ PanNSP1/PanNSP2*	Indirect shoot organogenesis	Effects on non-symbiotic hormonal aspects; Absence of nodule formation; Impaired nodule formation	Zeijl et al. 2018
Parasponia andersonii	*PanNIN/PanNF-YA1*	Indirect shoot organogenesis	Absence of root nodule formation	Bu et al. 2020
Populus alba	*PalWRKY77*	Indirect shoot organogenesis	Enhanced salt stress tolerance	Jiang et al. 2021
Populus alba × *P. glandulosa*	*PdNF-YB21*	Shoot organogenesis	reduced root growth and drought resistance	Zhou et al. 2020
Populus alba × *P. glandulosa*	*CSE1/2*	Indirect shoot organogenesis	Plants with reduced lignin content and increased saccharification efficiency	Jang et al. 2021

Table 2 (continued)

Tree crop species	Target	Regeneration pathway	Mutant phenotype	References
Populus alba×*P. glandulosa*	*PagPDS*	Indirect shoot organogenesis	Albino-like mutants (high editing rate—75%)	An et al. 2021
Populus tomentosa	*PtoPDS*	Indirect shoot organogenesis	Albino-like shoots	Fan et al. 2015
Populus tomentosa	*PtSGT1/2*	Shoot organogenesis	Mutants with altered sugars profile and xylem cellular structure	Xue et al. 2021
Populus tremula×*P. alba*	*4CLI/PII*	Indirect shoot organogenesis	Decreased lignin accrual and altered monolignol composition of biallelic mutants	Li et al. 2021
Populus tremula×*P. alba*	*CSE1/2*	Shoot organogenesis	cse1cse2 double mutant plants with reduced lignin content and growth penalty	de Vries et al. 2021
Populus tremula×*P. tremuloides*	*PLFY/PAG*	Shoot organogenesis	No phenotype evaluated	Elorriaga et al. 2018
Populus tremula×*tremuloides and Populus tremula*×*alba*	*PopSAP*	Not mentioned	Sterile mutants with potential growth penalties	Azeez and Busov 2021
Populus trichocarpa	*PtrCesAs*	Shoot organogenesis	Mutants with impaired cellulose synthesis and specific fibers content	Xu et al. 2021
Populus trichocarpa	PtrAREB1-2/PtrADA2b-3/PtrGCN5-1	Indirect shoot organogenesis	High drought sensitiveness	Li et al. 2019
Populus trichocarpa	*PtrLBD39*	Shoot organogenesis	Plants with decreased tension wood formation and cellulose content; increased lignin content	Yu et al. 2022
Populus×*canescens*	*BRANCHED-1/2*	Shoot organogenesis	Enhanced bud outgrowth	Muhr et al. 2018
Populus nigra×*(P. deltoides*×*P. nigra)*	*PdGNC*	Indirect shoot organogenesis	Severe retarded growth and enhanced secondary xylem development	An et al. 2020
Pyrus communis	*PcPDS/PcALS*	Shoot organogenesis	Chlorsulfuron resistant lines/albino-like plants	Malabarba et al. 2020
Pyrus communis	*PcTFL1*	Shoot organogenesis	vegetative growth cessation	Charrier et al. 2019
V. vinifera cv. Chasselas×*V. berlandieri*	*UFGT/CBF4*	Indirect embryogenesis	Mutants with higher expression of the target genes	Ren et al. 2022
Vitis vinifera	*idNDH*	Indirect somatic embryogenesis	Not detected	Ren et al. 2016
Vitis vinifera	*VvWRKY52*	Indirect somatic embryogenesis	Increased resistance to *Botrytis cinerea*	Wang et al. 2018a, b
Vitis vinifera	*VvPDS*	Indirect somatic embryogenesis	Albino-like plantlets	Nakajima et al. 2017
Vitis vinifera	*TAS4/MybA7*	Indirect somatic embryogenesis	Not detected	Sunitha and Rock 2020
Vitis vinifera	*TMT1/2/PDS*	Indirect somatic embryogenesis	Albino-like plantlets/reduced sugar levels	Ren et al. 2021
Vitis vinifera	*VvCCD7/VvCCD8*	Indirect somatic embryogenesis	Increased shoot branching	Ren et al. 2020
Vitis vinifera	*VvPR4b*	Indirect somatic embryogenesis	Increased susceptibility to *P. viticola*	Li et al. 2020
Vitis vinifera	*idNDH*	Indirect somatic embryogenesis	Not mentioned	Osakabe et al. 2018
Vitis vinifera	*VviPLATZ1*	Indirect somatic embryogenesis	Flowers with reflex stamens	Iocco-Corena et al. 2021

Aiming to optimize gene editing capabilities in plants, studies are being conducted to explore the influence of technical aspects related to the CRISPR system. In *V. vinifera*, VvU3 and VvU6 promoters (native from *V. vinifera*) proved to be worth integrating a CRISPR-based editing strategy, as an increase of more than threefold sgRNA expression was achieved when regulated by these promoters compared to AtU6 (from *A. thaliana* and commonly applied for many crops gene editing strategies). The higher sgRNA expression resulted in a higher mutation rate (e.g., 23.53% for the AtU6-based vector versus 43.24% for VvU6.2) (Ren et al. 2021). Similarly, the effect of using different promoters was tested in poplar, in which expression of Cas9 driven by the MAS promoter (from *Mannopine synthase*) led to higher mutation rates compared to the conventional double CaMV35S promoter (75% for MAS against 67.5% for 2xCaMV35S: An et al. 2021).

Base editing enables the exploration of already characterized single nucleotide variations to directly modify traits of a variety without the need for time-consuming crossing cycles. This is especially interesting and was already applied to tree crops, such as pear (*Pyrus communis*, Malabarba et al. 2020), apple (*Malus domestica*, Malabarba et al. 2020), and poplar (*Populus alba* × *P. glandulosa*, Li et al. 2021). For pear and apple, researchers explored the cytidine deaminase fused to a nickase (SpnCas9-PmCDA1-UGI) to induce base edits on the *PDS* and *ALS* genes as a proof-of-concept of the system feasibility, and they successfully obtained edited lines for both targets. Similarly, a broad study was performed to optimize the base editing strategy in *P. alba* × *P. glandulosa*, in which two cytosine base editor complexes were compared (PmCDA1-BE3 and A3A/Y130F-BE3) was tested alongside an adenine base editor (ABEmax_V1/V2). High rates (> 90%) of base editing were obtained for all tested systems in at least one of the targets of the study, even though the activity window varied significantly between the two cytosine editors.

Although some of its potentials has been well demonstrated, gene editing is still a relatively new technology that has yet to reveal its full potential in tree crops. Genetic transformation and in vitro regeneration processes remain challenging and time-consuming for many species. As a result, only a few reports have been published demonstrating the feasibility of gene editing in trees, in contrast to the many publications applying CRISPR systems to modify agricultural traits in annual crops. Still, an expanding number of papers describing mutations induced by CRISPR aim to rationally modify traits of interest in trees. One such example comprises efforts to cope with canker disease in citrus by mutating the coding region and the promoter of *CsLOB1*, which led to the development of resistant plants (Jia et al. 2017; Jia and Wang 2020; Jia et al. 2022). Also related to pathogen resistance, a *WRKY* transcription factor (*VvWRKY52*) was targeted in grape (*V. vinifera*). Its knockout resulted in a cv. Thompson seedless mutant lines with the reduced spreading of lesions caused by the inoculation of *Botrytis cinerea* (Wang et al. 2018a).

Recent works also demonstrate the capacity to tackle morphological traits in tree crops. In citrus (*Citrus sinensis* × *Poncirus trifoliata*), scientists identified the molecular basis of thorn development regulation and manipulated plant architecture by converting thorns into branches through the disruption of *TI1* and *TI2* transcription factors (Zhang et al. 2020). Moreover, the simultaneous knockout in the poplar of two paralogs orthologous to the rice *TAC1* (Tiller Angle Control 1) led to the development of plants with upright-oriented leaves. This novel phenotype can be harnessed for commercial plantations to increase the density of individuals planted per area, increasing the overall yield (Fladung 2021). Since poplar is cultivated for wood and has potential for biofuel production, initial studies are also being conducted to investigate the genetic basis of lignin formation and the possibilities of manipulating its content, with recent examples of gene editing aimed to alter cell wall composition for increased saccharification efficiency (Jiang et al. 2021; Li et al. 2021).

Although not considered a crop due to lack of commercial exploration, it is worth highlighting gene-editing work conducted on the tropical tree *Parasponia andersonii* (also called *Trema andersonii*, Cannabaceae). This is a non-leguminous, nitrogen-fixing species with a recently developed genetic transformation protocol that is relatively fast and efficient for the application of CRISPR strategies (Zeijl et al. 2018). The main studies published so far regards the investigation of the genetic basis of nodule formation for symbiotic nitrogen fixation, which has been poorly explored in trees (Zeijl et al. 2018; Bu et al. 2020).

Potential gene targets in arboreal species for improving cultivation and sustainability

Traits of interest for tree crop cultivation are, in many cases, like annual crops (e.g., fruit size, root depth, and leaf shape), and the genetic basis governing these aspects are also most often conserved in different species. Thus, one approach to identifying genes for manipulating trees for a given trait is to use the knowledge gained from model species and annual crops. For that, in addition to sequence similarity, synteny and gene expression profiles should be added to improve the process of identifying functional orthologs.

For many fruit trees, larger fruit is a desirable trait. Scientists may be able to take advantage of studies in tomato (*Solanum lycopersicum*, Solanaceae), which is a model for fresh fruit (berry) development. For example, the AP2/ERF transcription factor *excessive number of floral organs* (*ENO*)

◂directly inhibits the expression of *WUSCHEL* (*WUS*, a controller of meristem size) in the floral meristem, and its loss-of-function resulted in the development of a larger fruit (Yuste-Lisbona et al. 2020). If this gene is functionally conserved and its loss of function leads to little pleiotropic effects, it could be a good target for gene editing in fruit trees, including semi-domesticated species. Likewise, a well-conserved regulator of shoot apical meristem (SAM) development is the peptide signal *CLV3*, which mutations in either its coding region or promoter have been shown to lead to fruit increase in tomatoes (Zsögön et al. 2018; Li et al. 2018).

Another critical aspect in many crop species is photoperiod sensitivity, which is related to meristem determination and often dictates cultivation latitudes. In tomatoes, loss-of-function of the flowering repressor *SELF PRUNING 5G* (*SP5G*) gene, a member of the *CETS* family, promotes early, photoperiod-independent flowering and, consequently, an earlier harvest (Soyk et al. 2017; Zsögön et al. 2018; Li et al. 2018). Functional studies of the *CETS* family may lead to the regulation of flowering induction in arboreal plants by revealing a roadmap to manipulating juvenile phase length, thus accelerating breeding and speeding up commercial production. The simultaneous mutations in *SP5G*, *SP* (another *CETS* gene), and *SlER* (a leucine-rich receptor kinase) led to a very compact tomato plant (Kwon et al. 2020), which is another highly desired trait for many tree crops.

Growing tall is a common trait of trees and encompasses a significant advantage in the race for light. However, in an agricultural setting, crops can be neatly arranged and managed to minimize competition for light. Therefore, growing too tall can often lower profits due to the challenges of harvesting the commercial product. A strategy exploring dwarfism-inducer mutations could be interesting when height is a logistical problem. Fortunately, there are some examples of using CRISPR-based gene editing to achieve this goal in plants. In rice, directed mutations on the gene *SD1* (*Semi-Dwarf1*) led to shorter plants (at least 30% of height reduction, depending on nitrogen supply) without significantly affecting yield in elite landraces (Hu et al. 2019). Similarly, targeting *ERECTA* homologs in cucurbits (*Cucumis melo*: melon; *Cucurbita moschata*: squash; and *Cucumis sativus*: cucumber) efficiently decreased stem length (Xin et al. 2022).

Although fruit weight and yield are critical, agricultural systems must also prepare for increased resource limitations (energy, fertilizers, and freshwater) and climatic instabilities (heat, rainfall patterns, and wind). Critical traits for this scenario include increased tolerance to drought, heat, and salinity, improved phosphate and nitrogen uptake and use, and increased resistance to pests and diseases. To support gene editing strategies focused on such traits, we compiled a list of target genes whose losses of function led to increased fitness in agricultural systems under environmental pressures (Suppl. Table S2). We also refer the reader to recent reviews on annual crops (Gonçalves et al. 2020; Janni et al. 2020; Huisman and Geurts 2020).

A recent report describes the influence of a B-type cyclin (*NtCycB2*) on glandular trichome formation of tobacco (Wang et al. 2022). Its knock-out by gene editing enhanced trichome formation and more compound exudation, resulting in stronger aphid resistance, a compelling achievement for pest management. Thus, potential functional orthologs of this gene could be targets for pest resistance in tree crops (Therezan et al. 2021).

Works describing induced mutations affecting abiotic stress tolerance have been recently published. The loss of function of *Aluminium-activated Malate Transporter* (*ALMT* and *SlALMT15*), which affects stomatal density in tomato leaves, enhanced tolerance to drought without penalties to the net photosynthetic rate in tomatoes (Ye et al. 2021). By the same token, the *OsTT2* loss of function led to greater wax retention and increased heat tolerance (Kan et al. 2022). Moreover, two recent pieces exploring nutrient uptake using CRISPR-induced mutations led to increased uptake of iron (*AtERF96* knockout) and phosphate (*SlHA8* knockout) in Arabidopsis and tomato, respectively (Liu et al. 2020a; Yao et al. 2022).

Genome editing strategies for tree breeding to take off

Even though gene editing applied to tree species is challenging, partly because of the lack of knowledge on the genetic basis of many traits, some promising innovations involving CRISPR systems can facilitate the pipeline, such as the high-throughput mutagenesis recently applied to maize (*Zea mays*, Poaceae) (Liu 2020). This approach involved the application of multiplexed CRISPR-Cas9 on a batch pipeline with pooled transformation and barcoded deep sequencing, resulting in 118 mutated genes (412 different mutations) in the population. The same strategy could be applied to trees with sequenced genomes and efficient genetic transformation protocols (e.g., citrus and poplar) to identify causal genes for traits of interest without time-costly crossing (as in conventional functional genetics studies) or random mutation experiments.

Indeed, even though the candidate gene approach is interesting for unveiling causal genes coordinating traits of interest, it does not replace population segregation analyses. Such studies can reveal the genetic bases of novel traits without any previous knowledge of the genomic location of the variant. Fortunately for tree geneticists, some recent results demonstrate the capability of targeted mutagenesis for accelerated breeding. The analysis of an EMS-derived mutant collection showed that a disruption

on helicases *RECQ4* and *FANCM*, which are anti-crossover factors, resulted in over two-fold increase of meiosis recombination in three distantly related species: rice (*Oryza sativa*, Poaceae), tomato (*Solanum lycopersicum*, Solanaceae), and pea (*Pisum sativum*, Fabaceae) (Mieulet et al. 2018). The manipulation of crossover factors can increase recombination frequencies and faster develop high-definition genetic maps. In addition to accelerating the construction of genetic maps, the controlled recombination with directed nucleases promises a paradigm shift for breeding by increasing population diversity, fine-tuning introgressions during pre-breeding, and obtaining favorable haplotypes for breeding (Taagen et al. 2020).

The increase of genetic recombination frequencies is interesting for breeding strategies involving introgressions from related species to elite genotypes since higher recombination rates increase the chances of isolating the desired gene from nearby genetic elements, thus decreasing the linkage drag that could affect genetic gains. Indeed, the use of CRISPR-Cas9 to disrupt *RECQ4* was successfully applied to tomatoes for this purpose. Interspecific tomato hybrids (*S. lycopersicum* × *S. pimpinellifolium*) with the loss-of-function for *recq4* showed higher recombination frequencies than the wild type (Maagd et al. 2020). This approach paves the way for applications in the breeding of arboreal species, in which performing successive crossing cycles to attenuate linkage drag is difficult, costly, and time-consuming.

It is helpful to bear in mind that, while gene editing can be useful to create novel, improved genotypes, the presence of transgenic CRISPR cassettes can affect marketability, for example, by GMO labeling in some countries or refusal by a significant portion of consumers. Nonetheless, while gene-edited crop regulation is politically fluid and highly variable from country to country, the resulting variety does not necessarily contain a transgene—thus, technically being not a GMO (Metje-Sprink et al. 2020).

Segregating the transgene out of a genome requires at least one backcrossing cycle, representing many years for a tree. It can also be impractical for elite varieties that are not inbred lines, as with most tree crops. A promising alternative for such cases is the transgene-free strategy uses ribonucleoprotein (RNP) complexes. It requires the purified nuclease to be pre-assembled in vitro with a designed sgRNA. Both Cas9 and Cas12a (Cpf1) were successfully tested for this strategy. The Cas-sgRNA complex can be delivered into protoplasts via PEG-mediated osmotic shock (Woo et al. 2015; Kim et al. 2017; Brandt et al. 2020) or directly into explant somatic cells via shotgun (Liang et al. 2018; Dong et al. 2021). This method has been reported for apple, grapevine, and rubber tree (*Hevea brasiliensis*, Euphorbiaceae) (Malnoy et al. 2016; Osakabe et al. 2018; Fan et al. 2020). Delivery of plasmids containing Cas9 and sgRNAs directly to protoplasts by inducing the transient expression and selecting transgene-free events is also a possibility (Lin et al. 2018), albeit not reported on arboreal species yet.

The most considerable caveat of these transgene-free delivery methods is the need for efficient tissue culture and in vitro regeneration protocols, which have not been optimized for most tree species. Therefore, the ideal scenario for the broad application of CRISPR technology on tree species is a transgene- and tissue-culture-free gene-editing method. Two recent reports show that gene-edited plants can be obtained without tissue culture (Ellison et al. 2020; Maher et al. 2020). One approach infects ex vitro a transgenic plant (*Nicotiana tabacum*, Solanaceae) expressing the Cas9 protein with a tobacco rattle virus (TRV)-based vector containing sgRNAs cleverly fused with a translocable mRNA (*FT* gene product). *This in planta transformation* resulted in 60 to 100% of edited progeny when a single genomic site was targeted and up to 30% of edited progeny when three sites were targeted (Ellison et al. 2020). The second approach induced de novo development of plant meristems in Cas9-expressing plants by concomitantly expressing developmental regulators related to meristem identity maintenance (*WUS2*, *IPT*, *STM*, and *BBM*) and gene-editing reagents (sgRNAs targeting the *PDS* gene). Shoots regenerated from the de novo-induced meristems with fixed mutations, and some of them were transgene-free (Maher et al. 2020). These approaches highlight the potential of enabling gene editing tree species by circumventing transformation and in vitro regeneration.

In addition to editing the genome of elite varieties of cultivated species, we should also better explore the existing genetic diversity of plants by using non-domesticated species. By studying domestication traits and uncovering their genetic bases, we will be able to convert wild plants into novel domesticated crops by tackling those domestication traits yet preserving critical adaptive traits, such as strong levels of resistance to abiotic and biotic stresses. This concept has been proposed as de novo domestication (Zsögön et al. 2017; Gasparini et al. 2021; Curtin et al. 2022; Zsögön et al. 2022) and has been shown to be feasible for wild tomatoes (Zsögön et al. 2018; Li et al. 2018), as well as the semi-domesticated groundcherry (*Physalis pruinosa*, Solanaceae) (Lemmon et al. 2018). As the genetic bases of domestication traits become more understood and biotechnological tools are developed for perennial species, domesticating new plant species for agricultural purposes will become increasingly feasible, including arboreal species.

Alongside the technology development and the conceptualization of disruptive projects and perspectives on gene editing in tree species, improvements in legislation aspects around the globe must be performed to update the laws in the face of these new techniques. Some regulatory agencies (i.e., the Forest Stewardship Council (FSC), which certifies

cellulosic products from responsibly grown forest crops around the globe) struggle to incorporate the new paradigm that arises with the advancement of genome editing technologies, like the fact that a crop can bear editions in its DNA at a specified target site without carrying any transgenes. Members of the scientific community are participating in the discussion regarding these matters (Strauss et al. 2019; Harfouche et al. 2021), and this is primordial to avoid misconceptions regarding genetic manipulation of crops and demonstrate the consequent possibilities of advancing scientific knowledge and increase the sustainability of modern agriculture responsibly.

Concluding remarks

The cultivation of arboreal species is challenging due to the long juvenile phase and the time required until harvest. New breeding strategies are being developed to cope with these drawbacks, and substantial advancements in the molecular design of tree crops are underway. The application of CRISPR-based gene editing technologies expands the horizon of genetic improvement programs and, given its versatility, along with the development of new cloning techniques, DNA sequencing technology, plant transformation platforms, molecular biology, and plant genetics, will enable advancing agriculture to the point of tailoring crops and domesticate de novo wild species to meet society needs. This is urgent, albeit particularly challenging, for the tree species we cultivate as crops since these plantations must be resilient to all agricultural threats that are forecast for the near future. Ergo, now is the time to plan for the coming decades, and the potential of gene editing for tree breeding awaits to be fully explored.

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Declaration

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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