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Review

Modern Technologies Provide New Opportunities for Somatic Hybridization in the Breeding of Woody Plants

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Abstract: Advances in cell fusion technology have propelled breeding into the realm of somatic hybridization, enabling the transfer of genetic material independent of sexual reproduction. This has facilitated genome recombination both within and between species. Despite its use in plant breeding for over fifty years, somatic hybridization has been limited by cumbersome procedures, such as protoplast isolation, hybridized-cell selection and cultivation, and regeneration, particularly in woody perennial species that are difficult to regenerate. This review summarizes the development of somatic hybridization, explores the challenges and solutions associated with cell fusion technology in woody perennials, and outlines the process of protoplast regeneration. Recent advancements in genome editing and plant cell regeneration present new opportunities for applying somatic hybridization in breeding. We offer a perspective on integrating these emerging technologies to enhance somatic hybridization in woody perennial plants.

Keywords: somatic hybridization; cell fusion; breeding; regeneration; protoplast; woody plants

Citation: Liu, S.; Li, X.; Zhu, J.; Jin, Y.; Xia, C.; Zheng, B.; Silvestri, C.; Cui, F. Modern Technologies Provide New Opportunities for Somatic Hybridization in the Breeding of Woody Plants. *Plants* **2024**, *13*, 2539. https://doi.org/10.3390/plants13182539

Academic Editors: Monika Tuleja and Hasan Mehraj

Received: 19 June 2024
Revised: 25 August 2024
Accepted: 3 September 2024
Published: 10 September 2024

1. Introduction

Breeding technologies have evolved from selective breeding and hybridization to contemporary gene-editing approaches [1]. Hybridization, the practice of combining genetic material from different organisms through sexual reproduction, gained scientific prominence following the rediscovery of Mendel's laws of inheritance in the early 20th century [2]. Traditional hybridization is mostly used in sexually compatible species with relatively short vegetative periods. This constraint restricts the gene pool to those species that can interbreed, thereby limiting genetic diversity and potential improvements. To overcome these limitations, distant hybridization techniques have been developed. Distant hybridization involves crosses between species, genera, or even higher taxonomic ranks, aiming to introduce new genetic material into breeding programs [3,4]. Efforts have also been made to fix hybrid vigor [5,6]. Despite its potential, distant hybridization faces challenges such as hybrid lethality, which is mitigated by embryo rescue techniques that involve in vitro culture to prevent embryo degeneration, though success has been limited to a few species [7].

In the 1960s, the advent of cell and protoplast fusion techniques marked a significant advancement in plant breeding. These methods, collectively known as somatic hybridization, circumvent the limitations of sexual reproduction by allowing for the fusion of cells from different species or genera [8,9]. This approach has proven particularly valuable for woody perennials, which typically have long vegetative periods that make traditional hybridization time-consuming and inefficient. Somatic hybridization provides a means to combine genomes and plastids from different species, offering a potential solution to the limitations of traditional hybridization [10,11].

One prerequisite for successful cell fusion is reducing the negative charge on the phosphate groups of cell membranes. Various strategies have been employed for this purpose, including the use of calcium ions (Ca^{2+}), high pH conditions, sodium nitrate ($NaNO_3$), polyvinyl alcohol (PVA), and polyethylene glycol (PEG) [12,13]. Techniques such as electrofusion, chemical fusion, and photofusion have been developed to enhance the efficiency and stability of fusion bodies. Among these, PEG-mediated electrofusion has become the most widely used method due to its effectiveness [14,15]. Later, the use of lower PEG concentrations (40% for chemical fusion and 20% for electro-chemical fusion) in combination with direct current pulses has further refined the technique, integrating the advantages of both chemical and electrofusion methods [16].

Somatic hybridization was initially regarded as a highly promising technology due to its potential to combine not only the nuclear genomes but also the plastids of different species [8,16,17]. However, although great efforts have been made to create new species via somatic hybridization, successful combinations are still rare [18]. The primary challenge lies in the low frequency of hybrid regeneration, which may be attributed to issues such as plastid–nucleus incompatibility, inefficient regeneration methods, or developmental lethality [18–20]. Even when hybrids are successfully regenerated, they often exhibit sterility and fail to produce viable seeds [19]. This limitation has led to a cyclical pattern of optimism and setbacks for somatic hybridization over the past fifty years [20]. In crops propagated via seeds, embryo failure poses a significant challenge. However, the fact that commercially important woody plants are typically propagated asexually (e.g., through cuttings and micropropagation) offers a potential advantage, as it could circumvent the issue of hybrid sterility. The lack of efficient cell regeneration systems remains a major hurdle for woody plants. Recent advances in cell proliferation and organogenesis research provide new opportunities to optimize regeneration protocols for recalcitrant species, offering promising prospects for the application of somatic hybridization in woody plants.

This review aims to provide a comprehensive evaluation of the progress made in the somatic hybridization of woody plants, highlighting both the limitations and advantages of this breeding approach. We will explore the procedures for regenerating somatic hybrids and discuss how modern breeding technologies can be integrated into somatic hybridization to broaden its application in woody species.

2. The Application of Somatic Hybridization in Woody Plants

Woody species, particularly trees, present a unique challenge for breeding due to their long vegetative periods. Traditional breeding methods are often time-consuming and labor-intensive, making somatic hybridization an attractive alternative. This technique facilitates protoplast fusion under in vitro conditions, allowing for the creation of hybrid plants that might not be achievable through sexual reproduction alone [8,9]. Somatic hybridization is particularly valuable for breeding species that are sexually incompatible, asexually propagated, or sterile. By bypassing the sexual phase, somatic hybridization can significantly reduce breeding cycles and introduce new genetic variations into the breeding pool [21]. Unlike traditional hybridization, which typically produces nuclear hybrids while preserving non-inheritable organelle genes, protoplast fusion allows for the transfer and recombination of organelles. This capability promotes plastid evolution, potentially enhancing traits such as product quality, stress resistance, and growth rates [22–24]. Thus, somatic hybridization has advantages in promoting plastid evolution and creating desirable plastids. As a result, somatic hybridization has been used to improve various characteristics in plants, including yield, disease resistance, and environmental adaptability [25–27].

Citrus species are among the most notable examples of the successful application of somatic hybridization in woody plants. Citrus plants have well-established regeneration methods, making them suitable candidates for somatic hybridization experiments [28,29]. Since the successful regeneration of the first intergeneric Citrus somatic hybrid in 1985, nearly 250 cases of citrus somatic hybrids have been cultivated worldwide, with over 40 cases reported from China alone [30,31]. The ‘leaf protoplasts + embryogenic callus

protoplasts' model has proven effective in Citrus cell fusion, facilitating the selection and regeneration of fused bodies. This model has also played a key role in improving Citrus resilience and fruit quality. Both symmetrical and asymmetrical somatic hybridizations have been employed to produce stress-resistant cultivars, seedless fruits, and disease-resistant varieties [26,32,33]. For example, symmetrical somatic hybridization between Citrus cultivar species and *Poncirus trifoliata* has been conducted to produce stress-resistant cultivars or ideal rootstocks [33–36]. Generally, symmetrical somatic hybridization has been widely used for rootstock breeding, as undesirable traits can be incorporated into the fused product. However, with asymmetric hybridization, the situation can be very different. Asymmetrical somatic hybridization has been conducted in Citrus to obtain male-sterile trees, seedless fruits, and disease-resistant cultivars [37–39]. Many somatic hybrid Citrus cultivars have been commercialized. Typical cases that have been published are summarized in Table 1.

Beyond Citrus, efforts to apply somatic hybridization to other woody species have yielded some success. Early cases included cherry, pear, persimmon, Passiflora species, and apple [40–44]. More recent progress has been made. Hybrid cells have been achieved in species such as mango, mulberry, jujube, and rose [22,45–48]. Despite these advances, the regeneration of fused cells—the final step of somatic hybridization, which determines the success of somatic hybridization breeding—remains a critical challenge. For instance, mulberry hybrid cells were successfully obtained in the 1980s but failed to develop into plants [47]. Woody species' failure to regenerate could explain why cell fusion technology has mainly succeeded in herbaceous species, as these have more sophisticated regeneration systems [49,50]. Recent achievements in protoplast culturing have been reported in species like grape [51], litchi [52], olive [53], camellia [54], Jasminum [55], pecan [56], apricot [57], poplar [58], and various gymnosperms [59–61], providing very good preconditions for the application of somatic hybridization. However, issues such as the instability of fusion bodies and abnormalities in embryo development continue to pose challenges [62]. Establishing robust regeneration systems is crucial for advancing somatic hybridization in woody species.

Table 1. Protocol for woody species with successful protoplast fusion.

Category	Latin Name	Improvement in Agronomical Characteristics	References
Pear/cherry	*Pyrus communis* var. *pyraster* L. × *Prunus avium* × *pseudocerasus*	Enhanced traits such as chromosome number, morphological characteristics, and leaf isozyme profiles	[40]
Persimmon	*Diospyros glandulosa* × *D. kaki*	Resolved the hybridization barrier in *D. kaki*	[41]
Mango	*Mangifera indica* L.	Hybrids between cultivars	[46]
Passiflora	*Passiflora edulis* f. *flavicarpa* Degener. × *P. cincarnata* L.		[42]
	Passiflora edulis f. *flavicarpa* Degener. × *P. amethystina* Mikan		[43]
Citrus	*Citrus reshni* Hort. Ex Tan × winter Haven citrumelo *Citrus reshni Hort.* Ex Tan × *citrange* *Citurs sinensis* L. Osbeck × (C. × *paradisi* × *Poncirus. trifoliata*)	Promote high fruit quality and good yields	[35]
	Citrus australasica F. Muell × *Citrus sinensis* *Citrus australasica* F. Muell × *Citrus 'Ju You'*	Perceive tolerance to Huanglongbing (HLB)	[33]
	Citrus japonica Thunb × *Citrus paradisi Macfad*	Enhancing resistance to Citrus canker	[39]
	Citrus deliciosa Ten × *Poncirus trifoliata* (L.) Raf	Improving natural chilling and light stress tolerances	[63]
	Citrus latipes × W. Murcott	Improving resistance to greening disease and reducing seedlessness	[64]

Table 1. *Cont.*

Category	Latin Name	Improvement in Agronomical Characteristics	References
	Citrus reticulata × *Citrus paradisi*	Improving fruit characteristics	[65]
	Citrus unshiu Marc. × *Citru sinensis* L. Osb.	CMS	[66]
	Citrus sinensis L. Osbeck × *Citrus limon* L. Burm	Improving fruit oil quality	[67]
	Citrus sinensi s × [*Citrus reticulata* × (*Citrus paradisi* × *Citrus reticulata*)]	Improving citrus scion	[68]
	Citrus sinensis L. × *Poncirus trifoliata* L.	Addressing the threats posed by abiotic constraints	[69]
	Citrus unshiu Marc. × [*Citrus reticulata Blanco* × (*Citrus reticulata Blanco* × *Citrus paradisi Macf*)]	CMS	[70]
	Citrus sinensis × *Citrusdeliciosa*	Improving the tolerance to *Xanthomonas axonopodis* pv. *citri* and *Xylella fastidiosa*	[71]
	Citrus medica L. × *C. limon* (L.) Burm. F.	Improving acidity	[72]
	Citrus unshiu × *Citrus grandis*	CMS	[37]
	Citrus limonia L. Osbeck × *Citrus aurantium* L.	Improving blight and CTV resistance	[73]

3. Protoplast Regeneration: A Key Step in Somatic Hybridization

Protoplast regeneration is a critical step in somatic hybridization and often follows methods developed for herbaceous plants. This process typically involves the use of growth regulators such as auxins and cytokinins to stimulate genome reprogramming and promote cell differentiation [74]. However, most regeneration methods were initially developed for tissue or organ propagation rather than single-cell regeneration. Effective regeneration from protoplasts requires the development of cellular conditions similar to zygotic embryos, a process known as de novo organogenesis or somatic embryogenesis [75]. Over recent decades, many culture recipes have been invented to cultivate corresponding plant species [76,77]. More than 46 woody genotypes have been regenerated from protoplasts in the last century, representing 32 species, 18 genera, and 12 families [78]. The factors influencing protoplast regeneration will be discussed below.

The isolation of protoplasts is a crucial factor for successful regeneration [79,80]. This process involves the degradation of the cell wall using enzymes and maintaining protoplast turgor with osmotic regulators [81]. Protoplasts can be isolated from leaves, cotyledons, roots, petioles, hypocotyls, petals, calli, and suspension cultures. Regardless of the tissue type, the condition of the plant materials is fundamental for subsequent regeneration. Generally, juvenile tissues are considered more amenable to regeneration [79,82]. Factors such as plant growth conditions, tissue pretreatment, and enzyme and buffer composition significantly impact protoplast yield and viability [83]. Tissue pretreatments such as physical disruption (e.g., slicing), vacuum infiltration of the enzyme solution, or pre-plasmolysis treatment will significantly improve protoplast yield (revised in [84]). Multiple cell wall-degrading enzymes (cellulases, beta-glucanases, xylanases, protopectinases, polygalacturonases, pectin lyases, and pectinesterases) have been used, and a mixture of Cellulase R-10, Macerozyme R-10, and Pectolyase Y-23 is currently the most-used enzyme recipe [84,85]. The buffer solution for enzymolysis usually contains osmolytes (KCl, $CaCl_2$, mannitol, sorbitol, or salts), pH buffer (MES, ethanesulfonic acid), reducing agent (β-mercaptoethanol), and enzyme-protecting agents (bovine serum albumin, BSA). Fine-tuning the buffer recipes is necessary for optimal enzyme activity. Additionally, temperature is a factor impacting enzyme activity [84,85].

The digestion period varies between species and enzyme concentrations. Typically, periods ranging from 1 to 18 h have been reported in the literature, with 2 and 4 h being the most common [86]. Both the concentration of enzymes and the duration of digestion have significant impacts on protoplast regeneration. When all conditions are optimized, cultivation in the dark has been reported to further improve protoplast regeneration

ability [83]. After optimized digestion, protoplasts need to be purified to remove cell wall debris and undigested materials. This process eliminates negative effects on cell division and development [61].

The culture medium used for protoplast regeneration is also a key factor. A suitable culture medium will enhance regeneration frequency. Commercial media, such as WPM, MS, Gamborg B5, KM, Y3, and Nitsch, are usually initially tested for the species used in somatic hybridization. Then, the recipes are optimized according to the specific nutrient requirements of each species [87]. The carbon supplements also vary between species. Usually, 1–3% sucrose is used [79]. Osmotic pressure, provided by chemicals like mannitol, sorbitol, and sucrose, is essential for hybrid cell regeneration [49]. Mannitol is the most common. However, some species prefer myo-inositol or other osmotic agents [88]. During the cultivation of hybrid cells, the cell wall will re-form. Thus, osmolarity needs to be gradually decreased. Growth regulators are essential for plant regeneration. To establish a regeneration system, multiple types of hormones will be screened, including cytokinins and auxins [84]. Typically, once the types of plant-responsive cytokinins and auxins are determined, the concentrations of these two hormones need to be finely adjusted to optimize the efficiency of regeneration. Additionally, gibberellic acid is necessary for the regeneration of some cultivated calli [89], and the ratio between auxin and abscisic acid is especially important for the regeneration of gymnosperms [90].

Even under optimized conditions, many woody plants are still recalcitrant to regeneration. Thus, multiple supplements have been added to promote regeneration. Reactive oxygen species (ROS), phenolic compounds, and ethylene are considered the major negative effectors of plant regeneration [91]. Therefore, antioxidants (ascorbic acid, citric acid, reduced glutathione, and L-cysteine), phenolic absorption materials (such as polyvinylpyrrolidone and activated charcoal), and ethylene inhibitors (silver nitrate) are often used to mitigate these effects [92–94].

The initial phase of protoplast regeneration involves culturing a single fused cell into a multicellular cluster. In somatic hybridization breeding, it is crucial to develop the callus from a single hybrid cell to achieve a homozygous line, making it essential to prevent contact between fused cells. To minimize cell aggregation, fused cells are typically cultured on semi-solid media at a low density rather than in liquid media [84,95,96]. These cells are then microscopically screened within a few days of cultivation to identify desirable hybrid candidates. Research into poplars has shown that stress-free conditions along with contact with a solid surface, promote the successful development of protoplasts into multicellular structures [80]. Overall, regeneration is a pivotal process in somatic hybridization. Citrus species have produced the most cultivars through somatic hybrid breeding (Table 1), owing much of their success to highly efficient regeneration systems [29,97]. Therefore, media with precisely optimized hormone levels must be meticulously prepared for each stage of protoplast regeneration.

4. Limitations of Somatic Hybridization in Woody Plants

The process of somatic hybridization in plants encompasses three primary stages: protoplast isolation, fusion, and the propagation/regeneration of hybrids. Correspondingly, the limitations associated with somatic hybridization breeding can be delineated into three categories: the genotype-dependent nature of protoplast isolation and hybrid regeneration, the occurrence of sterile hybrids, and the low survival rates observed in hybrid progeny [81,98–100]. While protocols for isolating protoplasts from woody plants mirror those used for herbaceous species, the unique characteristics of woody plant cell walls and protoplasts render cell wall degradation and protoplast isolation less efficient and less well established [101,102]. Factors such as tissue type, enzymatic composition, and solution concentrations play pivotal roles; however, protoplast yield is also highly contingent on plant genotypes [100,103]. For instance, successful protoplast isolation has eluded varieties like *Rosa indica*, *R. multiflora*, and *R. corymbifera* cultivars ‘Laxa’ and ‘Elina’, even after more than four decades since the advent of cell fusion techniques [104].

Another significant hurdle in the somatic hybridization of woody plants is the generally low survival and regeneration rates of hybrids. Many woody species lack established in vitro cultivation methodologies [105], and there exists substantial genetic variability among tree and shrub species concerning cell differentiation and regeneration [105–107]. Only a select few species, such as those within the *Citrus* and *Vaccinium* genera, possess well developed regeneration protocols [29,108], thereby limiting the applicability of somatic hybridization. Even in species with mature and stable cell fusion technologies, like Citrus, success rates remain modest. For example, Xiao et al. generated over 100 embryoids via electrofusion in Citrus but successfully developed and transplanted only 12 plants [66]. The polyploid nature of hybrids contributes to low survival rates; in persimmon, both octoploid and hexaploid lines were produced through cell fusion, yet only some octoploid lines matured into healthy plants [41]. Sterility is a common issue in symmetric protoplast fusion in woody plants, where quantitative data are scarce. In herbaceous plants, for example, one-third of the hybrid progeny resulting from the fusion between *Brassica napus* cv. Zhongshuang4 and its wild relative *Sinapis arvensis* were sterile [109]. The underlying causes of sterility in heterozygous hybrids remain inadequately understood. Hybrids derived from parent plants with unequal ploidy levels are typically sterile [110], and asymmetric protoplast fusion can result in incompatible plastids within hybrid cells, also leading to sterility [107]. Currently, effective solutions to address sterility are lacking.

Nevertheless, woody plants possess an inherent ability for asexual propagation both in natural settings and cultivation environments. Consequently, seed production in somatic hybrids of woody plants is less critical than in annual species. Thus, the regeneration of hybrid cells could serve as the culminating step in the somatic hybridization breeding of woody plants. Despite this, protoplast regeneration remains challenging for most woody species. Establishing a regeneration system for recalcitrant species using traditional methodologies is not only exceedingly laborious but also fraught with difficulty, thereby impeding the broader application of somatic hybridization in woody plants.

5. Current Plant Regeneration Methods and Their Potential for Tree Breeding with Somatic Hybridization

Currently, multiple novel approaches are under investigation to enhance plant regeneration, with some demonstrating genotype-independent effects [111]. Plant regeneration, which involves extensive genome reprogramming, can be stimulated by various pioneer genes. Optimized protocols (such as specific culture media and appropriate hormone concentrations) combined with the introduction of regeneration-promoting genes have significantly improved protoplast regeneration, even in species that are typically difficult to regenerate [111]. It has been observed that hormones commonly used in tissue culture, such as auxin and cytokinin, enhance plant regeneration by modulating the expression of key genes like *WUSCHEL* (*WUS*) and *BABY BOOM* (*BBM*) [112]. Expression of these genes in somatic cells, either individually or in combination, is often sufficient to initiate whole plant regeneration or shoot formation [113]. Additionally, genes associated with auxin/cytokinin biosynthesis, injury responses, and cell-fate determination, such as IPTs, glutamate receptor-like proteins, STMs, and WOXs, have also been successfully employed to promote plant regeneration [114]. However, the constitutive overexpression of these genes often disrupts normal plant development and fertility. To address this, three strategies have been developed: the use of inducible promoters to control gene expression, the Cre/loxP system for cassette removal post-regeneration, and non-integrating methods [115]. Inducible promoters have been particularly successful in inducing regeneration across multiple woody species [116]. While Agrobacterium-mediated transformation has been the primary method, protoplast-mediated transformation offers a promising alternative for similar regenerative outcomes.

Recent research has identified genes with minimal impact on overall plant development, such as *WOX5*, *GRF4*, and *GIF1*, which show greater potential in promoting regeneration. The GRF complexes have been applied in various crops with promising

results [113,117,118]. In Citrus, two negative regulators of somatic embryogenesis were identified, and the knockdown of these regulators significantly enhanced somatic embryogenesis [119]. As RNA interference (RNAi) can be delivered via viruses without altering the plant genome [120], these negative regulators hold potential for advancing hybrid cell regeneration. Ongoing research continues to explore growth-regulating genes in shrub and tree species, with new genes being identified that help overcome regeneration barriers, thereby advancing somatic hybridization as a viable breeding method.

The CRISPR-Cas system, derived from a bacterial immune mechanism, has been widely adopted in gene editing for plant breeding due to its ability to make precise DNA cuts guided by designed RNA sequences [121–123]. While it is theoretically feasible to use CRISPR-Cas9 to knock out genes that inhibit protoplast regeneration, the permanent loss of these genes could adversely affect normal plant growth. To mitigate this, the CRISPR-Cas9 system has been adapted into an RNA-guided platform for sequence-specific gene regulation. In this modified version, Cas9 is rendered catalytically inactive but retains its ability to bind specific DNA sequences, allowing it to repress or activate nearby gene expression when coupled with a strong activator [124]. The CRISPR-Combo system, which integrates CRISPR-Cas9 gene editing with MCP SunTag activator-mediated gene activation, allows for simultaneous editing and activation of regeneration-promoting genes [125,126]. This technology has been applied in poplar and offers the advantage of flexibility and multiplexing, enabling the testing of various combinations of endogenous morphogenic transcription factors (MTFs) [125,127]. When combined with the inducible LexA-VP16-ER system, CRISPR-Combo can be activated at specific times to enhance regeneration [128]. Consequently, CRISPR technology not only facilitates precise genome editing but also supports the editing of genes to enhance the stability and developmental potential of cell fusion bodies [129]. The theoretical framework for single-cell regeneration is now well established [130,131], and these advancements hold promise for accelerating the development of regeneration systems in tree and shrub species, thereby expanding the potential applications of somatic hybridization in these plants.

6. Integrating Precise Gene Editing with Protoplast Fusion: A Promising Breeding Strategy

Gene editing provides a precise approach to plant breeding [132,133], while cell fusion allows for the recombination of entire chromosomes between cells. The integration of these two methods holds great potential for advancing breeding strategies, though it requires the development of robust gene transformation and efficient regeneration systems. However, gene transformation is highly genotype-dependent, with significant variability in transformation efficiency, even among plants of the same species [51,134]. For instance, in commercial crops where regeneration systems may be well established for one cultivar, other cultivars can still prove resistant to Agrobacterium-mediated gene transformation [111].

Somatic hybridization, which involves protoplasts, offers an alternative by facilitating Agrobacterium-independent transformation through direct plastid transformation. This method has already yielded DNA-free, edited plants in several woody species via protoplast transformation [51,84,135]. This approach allows for the seamless integration of transgene-free gene editing with somatic hybridization. Alternatively, cell fusion can act as an intermediary step. For example, gene-editing cassettes can first be introduced into varieties amenable to gene transformation, and then the edited cells can be fused with those from recalcitrant varieties (Figure 1). This strategy is particularly advantageous for species with long vegetative phases, such as shrubs and trees, where introducing cassettes through sexual crosses is time-intensive. In addition to gene editing, cassettes containing regeneration-promoting genes can also be applied using this strategy.

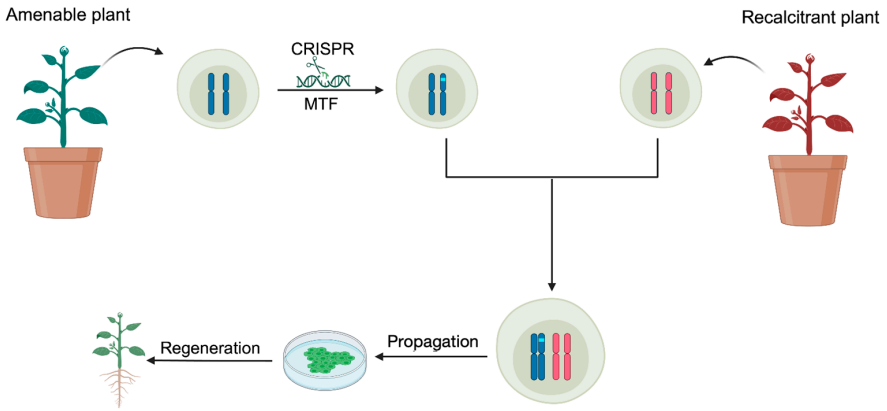

Figure 1. Strategies for application of current breeding biotechnologies in gene transformation-recalcitrant plants via somatic hybridization. The plant that is amenable to gene transformation serves as an intermediate to incorporate gene cassettes. Somatic hybridization combines the two genomes with the gene cassettes. Gene cassettes play roles such as gene editing and regeneration promotion in the hybrids.

Recent advancements in CRISPR technology, along with morphogenic transcription factor (MTF)-assisted transformation methods, show significant potential in overcoming genotype dependency [113]. By introducing CRISPR-associated regulators or MTFs into transformation-amenable varieties and subsequently fusing them with recalcitrant ones, regeneration can be promoted, enhancing the overall efficiency of breeding programs.

Somatic hybridization is recognized as a biosafe breeding method. Integrating modern techniques, such as gene editing and somatic hybridization, offers a promising approach for breeding woody plants. However, this integration raises concerns regarding the use of genetically modified organisms (GMOs) and CRISPR technology in somatic hybrid plants. Biosafety issues surrounding transgenic plants may present barriers to the adoption of somatic hybrids that incorporate gene transformation [136]. The CRISPR-Cas system, however, enables gene editing without the integration of foreign DNA into the plant genome [137]. Specifically, transgene-free strategies, including the transient expression of CRISPR vectors or the use of Cas protein-guide RNA complexes, can alleviate concerns related to GMOs [51,84,135]. Despite these advancements, the issue of off-target effects in CRISPR-Cas systems necessitates the further refinement of CRISPR technology [138].

In conclusion, somatic hybridization is a well-established breeding method that has been enhanced by recent technological advancements. While current technologies that reduce genotype dependency in regeneration and transformation have not yet been fully integrated into somatic hybridization, they hold significant promise for their application in modern breeding. Somatic hybridization, with its ability to combine genetic material across species and genera, remains an irreplaceable breeding method. This review highlights the development of cell fusion techniques in breeding and underscores their potential when combined with contemporary breeding technologies.

Author Contributions: S.L. and X.L. wrote the first draft with contributions from J.Z., Y.J. and C.X. B.Z., C.S. and F.C. revised and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Natural Science Foundation of China (Grant No. 32371831), the Natural Science Foundation of Zhejiang Province (Grant No. LY22C160005), and the Opening Project of Zhejiang Provincial Key Laboratory of Forest Aromatic Plants-Based Healthcare Functions (2022E10008).

Acknowledgments: We thank Cuihuan Li, Ping Yang, and Haibao Ji for their excellent laboratory management.

Conflicts of Interest: The authors declare no conflicts of interest.

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