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Applications of New Breeding Techniques in Fruit Trees

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Abstract
There is ongoing demand on the fruit business to develop superior varieties due to climate change and the quick adaptation of invasive pathogens. In order to fulfill the demands of an expanding global population, new breeding techniques have arisen as a possible alternative. Their goal is to speed the development of better-adapted cultivars. CRISPR/Cas genome editing, cisgenesis, and accelerated breeding have shown promise in improving crop traits and have been used to a number of plant species. The successful use of these technologies in fruit trees to enhance quality attributes, provide pathogen resistance, and tolerate abiotic stress is the main topic of this review. We also go over the enhancement and expansion of CRISPR/Cas genome editing techniques used on fruit trees, including site-specific recombination systems, multiplexing, and CRISPR/Cas-mediated base editing. In order to obtain exogenous fruit tree species devoid of DNA, advances in protoplast regeneration and distribution methods are discussed. These methods include the use of nanoparticles and virally produced replicons. There is also discussion of the regulatory environment and the general social acceptability of cisgenesis and CRISPR/Cas genome editing. This review offers a comprehensive summary of the range of applications available for improving fruit crops, along with an analysis of the issues that still need to be addressed for future optimization and the possible introduction of novel breeding methods.

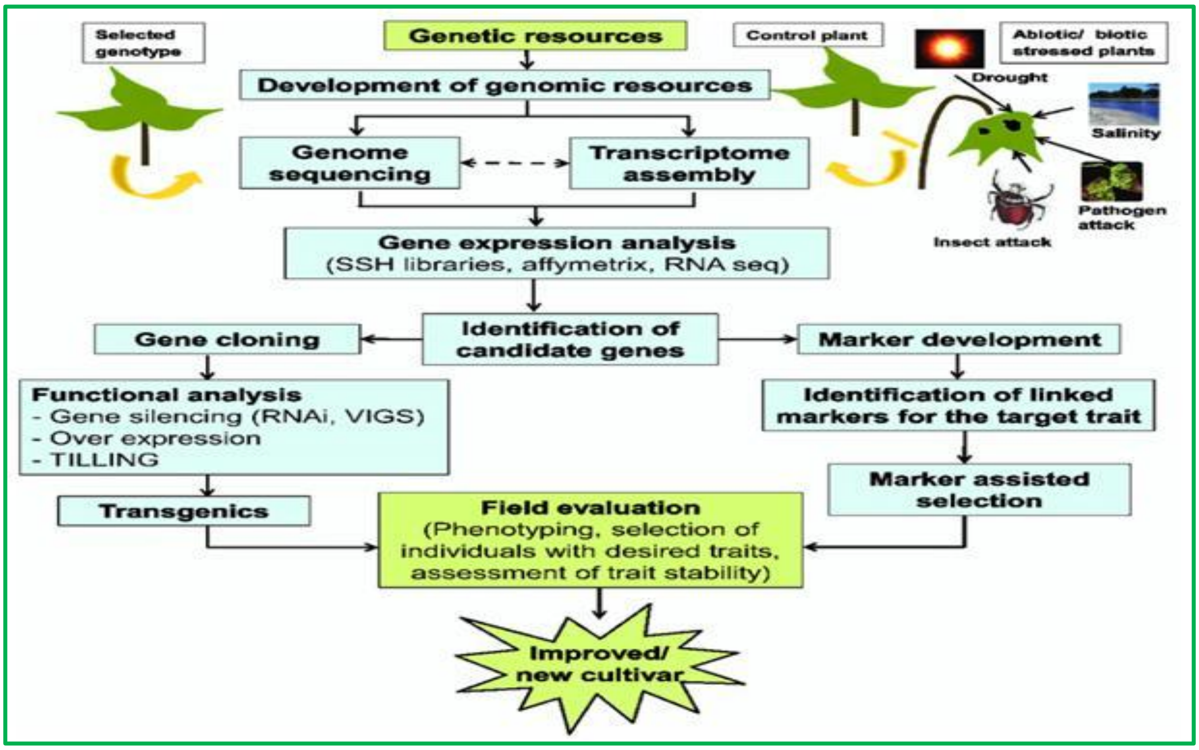
Introduction
Fruit trees are important for the world's food security as well as from an ecological and economic standpoint. Fruit tree breeding has historically been a time-consuming and difficult process in which desired qualities like disease resistance, increased yield, and fruit quality are selected for. But thanks to recent developments in biotechnology, fruit tree breeding is undergoing a revolution thanks to novel breeding techniques (NBTs), which provide quicker and more accurate ways to create new kinds. Now let's see how these innovative methods are being used in the field of fruit tree breeding.

1. Genome editing techniques: CRISPR-Cas9: This versatile method has shown great promise for precisely modifying the genomes of a variety of organisms, including fruit trees. This method makes it possible to precisely alter particular genes linked to desired characteristics like fruit quality and disease resistance. Breeders can hasten the creation of better fruit tree kinds by adding or modifying advantageous genetic variants. Applications: To improve features like disease resistance, fruit texture, and shelf life, CRISPR-Cas9 has been effectively used on fruit plants such as apples, oranges, and grapes. By using CRISPR, for instance, scientists have been able to make apples resistant to common illnesses like apple scab, which eliminates the need for chemical pesticides.
2. Methods of Mutagenesis: Specific Mutagenesis: Targeted mutagenesis techniques enable precise modifications in specific genes, in contrast to standard mutagenesis, which causes

random mutations across the genome. Breeders can more effectively introduce or disrupt particular features using this method, producing speedier and more predictable results. Applications: To produce unique genetic variations, targeted mutagenesis techniques such as ZFNs (Zinc Finger Nucleases) and TALENs (Transcription Activator-Like Effector Nucleases) have been used in fruit tree breeding. Researchers want to create fruit types with better consumer preferences and longer shelf lives, therefore they are focusing on genes related to fruit ripening, size, and flavor.

3. Genomic Selection: Unlike traditional phenotypic selection, genomic selection (GS) predicts an individual's genetic merit based on its entire genome. Breeders can more precisely and effectively select the most promising individuals for breeding programs by evaluating enormous quantities of genetic and phenotypic data. Applications: GS makes it possible to choose the best individuals early on in fruit tree breeding, which drastically cuts down on the time and money needed for conventional breeding techniques. Breeders can quickly generate new kinds with enhanced qualities like disease resistance and fruit quality by using genomic data to find genetic markers linked to significant attributes.
4. Clonal Propagation and Hybridization: Hybridization: Breeders can combine desirable qualities from multiple parent kinds through hybridization, which is still a vital strategy in fruit tree development. Better hybrids can be produced faster thanks to improvements in molecular techniques like marker-assisted selection (MAS), which allows breeders to more accurately detect and choose desirable features.

   Clonal Propagation: Elite fruit tree types with desirable qualities can be quickly multiplied by clonal propagation techniques including tissue culture and grafting. These methods guarantee consistency in fruit quality and performance in various conditions by preserving genetic homogeneity.



Adherence to regulations and wider social acceptance: The swift integration of cisgenesis and gene editing methods, including CRISPR/Cas and TALEN, in research and breeding has led to discussions on the legality of genetic modifications that do not include the introduction of DNA from unrelated species in numerous jurisdictions. The primary question was and remains whether genetic modifications, which can also come from natural mutations or conventional breeding, should really be subject to the legal evaluation and labeling regulations set forth for transgenic genetically modified organisms.

The community of organic farmers, GMO-critical civil society organizations, and GM-free food producers—the latter of whom just formed their own European representation, the European Non-GMO Food and Feed Sector, ENGA—are the main sources of opposition. The latter two groups in particular see genome editing as a threat to their business model since it prevents them from using analytical techniques to ensure that their products are free of chemicals derived from genome-edited plants.

Consumers' and the public's general perceptions do not appear to have changed.Although some publications noted changes, Spök et al. (2022) undertook a more thorough analysis of the available data and came to the conclusion that respondents are still largely against genetically modified organisms (GMOs) and, to a lesser extent, against genome edited species.

However, acceptance is far higher when advantages are involved, albeit this varies based on how important the benefits are to customers, farmers, or the environment.The importance of sustainability advantages appears to have grown over the past few years. One explanation for this could be that people are becoming more conscious of the various sustainability issues facing the agro-food system and are realizing how urgent it is to take action.

Breeding more quickly: Fruit trees, including citrus, apple, and peach trees, have lengthy flowering seasons. Conventional cross-breeding usually takes a minimum of twelve years or longer to produce new kinds. The lengthy juvenile phase and, thus, the breeding cycle of perennial trees are mostly to blame for this extended breeding time.For instance, the juvenile stage of peaches lasts three years or more, that of grapevines is two to five years, that of apples is much longer at six to twelve years, and that of citrus is between five and ten years.Climate change and invading infections are putting increasing strain on the fruit sector, necessitating the creation of more sustainable and better-adapted varieties. But the current demand for new types is greater than what traditional breeding can produce at the current rate. Through genetic engineering, recent developments in biotechnology and genomic research present the possibility of accelerating cultivar development in a number of fruit crops.For instance, expression of floral activators or repressors can be used to specify dormancy requirements and floral transition in key fruit tree species.In addition to cutting the breeding cycle short, accelerated breeding technology makes it possible to create advanced selections devoid of transgenes. There are currently no particular legal restrictions for plants produced using this method. Nonetheless, given that the final elite clones contain no transgenes, it is possible that their commercial distribution will be deregulated in areas where the use of other NBTs is deregulated.

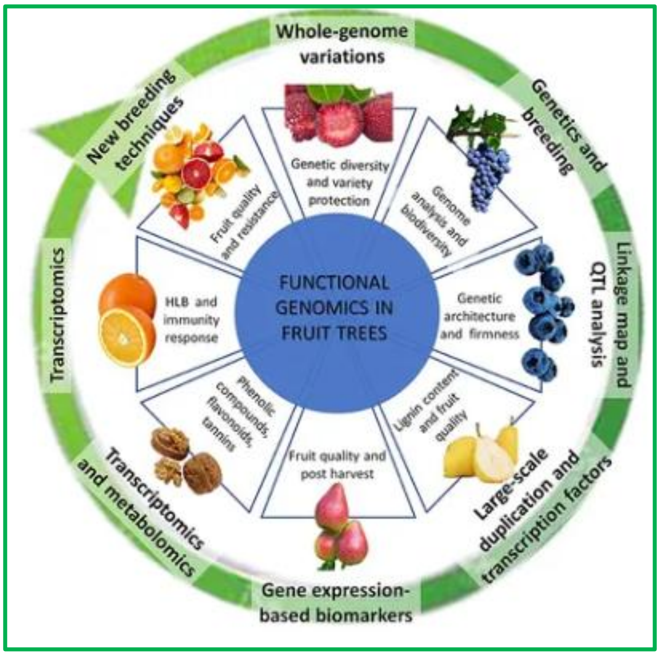
The processes of cisgenesis and intragenesis: The genetic modification of plants by the use of genes from a cross-compatible species or the recipient species is known as cisgenesis. Cisgenesis is a fast and non-crossing method of introducing features from wild ancestors, such as disease resistance. Together with its introns, flanking native promoter, and terminator in a normal sense orientation, the inserted gene is regarded as an additional gene copy to the current genome and a natural variation.It has been discovered that the public accepts cisgenic crops more than traditional transgenic genetically modified crops, genome-edited crops, or crops treated with synthetic or natural insecticides.

Contrary to cisgenesis, intragenesis implies the creation of a novel gene combination through the in vitro assembly of naturally occurring elements, such as promoter and terminatator regions, as well as P-DNA borders—which are borders resembling the left and right T-DNA borders—isolated from the sexually compatible DNA pool.

Transformation of protoplasts: Notwithstanding recent advancements, CRISPR/Cas9 application in fruit tree species still needs to get over technological obstacles and comply with the legislative framework currently in place regarding GMO regulations. Determining a GMO-free method for delivering Cas9/gRNA to plant cells is essential.CRISPR/Cas9 RNPs or plasmid DNA can be directly delivered to protoplasts in a number of fruit trees, including citrus, apple, grapevine, chestnut, and banana plants, via PEG, electroporation, or particle bombardment. The effectiveness of successful editing is largely dependent on the success of protoplast regeneration, which is still difficult or impossible in a number of fruit species, in addition to the editing process and the cellular uptake of RNPs. Studies on such fruit species frequently only show the RNP-mediated transgene-free gene-editing method, rather than successful plant regeneration.

Adherence to regulations and wider social acceptance: The swift integration of cisgenesis and gene editing methods, including CRISPR/Cas and TALEN, in research and breeding has led to discussions on the legality of genetic modifications that do not include the introduction of DNA from unrelated species in numerous jurisdictions. The primary concern has been and continues to be whether genetic alterations—which can also come from spontaneous mutations or conventional breeding—should really be subject to the legal evaluation and labeling regulations established for transgenic genetically modified organisms.Major GM crop cultivators like the United States, Canada, Brazil, and Argentina were among the first to either exempt plants with SDN1, SDN2, and cisgenic genetic modifications from the scope of their GMO legislation or establish a fast-track to market for them, treating them the same as plants derived from conventional breeding, paving the way for the first commercial products.Similar regulations were implemented or declared in other nations, such as China and India, where the adoption of GM crops has been proceeding more slowly. GMO-critical civil society organizations, the organic agricultural community, and GM-free food producers—the latter of whom have formed its own European representative, the European Non-GMO Food and Feed Sector, ENGA—are putting up the biggest resistance.

Transformation mediated by Agrobacterium: While transformation mediated by Agrobacterium yields. Despite being a GMO, it is nevertheless widely utilized and useful for functional studies on genetics. Gene function characterization can be utilized as a proof of concept to choose particular targets to focus breeding, which might be the foundation for the use of further DNA-free methods. Technical aspects. The following will examine developments and applications meant to mitigate biotic and abiotic stress:



Technological advancements: Establishing and/or refining the methodology is a major focus of papers on CRISPR/Casmediated genome editing in fruit trees.Typically, genes that are easily scorable are targeted for this aim. The most commonly targeted gene is the one that codes for phytoene desaturase

(PDS), which causes the albino phenotype, a distinctive decoloration.
Biotic stresses: Gaining resilience to biotic stressors has been the main focus of most CRISPR/Cas research projects in fruit trees.
Abiotic conditions: One of the main issues raised by climate change is addressed in a recent study on grapevine by Clemens et al. (2022): water pressure.By employing CRISPR/Cas9 to alter the grapevine gene Epidermal Patterning Factor Like 9 (EPFL9), which causes stomata formation in vascular plants, lower stomata density was achieved in the edited plants, highlighting the function of EPFL9 in a perennial fruit species. It's interesting to note that the altered lines' inherent water-use efficiency increased, suggesting that adjusting stomatal density may have benefits for future climate change.

Additional uses: Genome editing has been used to study additional features besides biotic and abiotic stressors. For instance, Iocco-Corena et al. (2021) used CRISPR/Cas9-mediated genome editing in grapevine to investigate genes related to branching, such as CCD8, and floral morphology, such as VviPLATZ1 (Ren et al. 2020).A single nucleotide polymorphism in the protospacer adjacent motif sequence that only causes cleavage in bulk cocoa DNA was used to distinguish between fine and bulk cocoa in a reported novel application of CRISPR/Cas9. This system produced a quantitation method that could be used in routine admixture detection applications.

Prospects for the Future

Incorporating non-biological traits (NBTs) into fruit tree breeding has great potential to tackle worldwide issues including climate change, food security, and sustainable agriculture. Regulating structures, public acceptance, and ethical issues related to the usage of these technologies must all be taken into account, though. To fully utilize NBTs and ensure their ethical and responsible use in fruit tree breeding, cooperation between researchers, breeders, legislators, and other stakeholders is essential.

Conclusion

In conclusion, there are previously unheard-of chances to generate improved varieties with improved features more quickly thanks to the use of innovative breeding procedures in fruit trees. Through the integration of advanced biotechnologies with conventional breeding methods, it is possible to produce fruit tree kinds that are resilient and productive enough to fulfill the needs of a fast changing agricultural environment.

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