CRISPR/Cas-MEDIATED GENE EDITING IN TREE CROPS: AN UPDATED REVIEW

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Perennial tree crops are foundational to global agriculture, forestry and natural ecosystems, yet they pose considerable challenges for genetic improvement due to long generation cycles, high levels of heterozygosity and recalcitrant tissue culture systems. The advent of CRISPR/Cas based genome editing technologies has revolutionized functional genomics and crop improvement by enabling precise, efficient and heritable genetic modifications. This review provides a comprehensive overview of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems' evolution, classification and molecular mechanisms, illustrates modified versions of Cas9 and next-generation tools such as base editing and prime editing. The application of these tools in tree species including *Populus* (poplar), *Malus domestica* (apple), *Citrus* spp., *Gossypium* (cotton), *Actinidia* (kiwifruit), *Hevea brasiliensis* (rubber), *Elaeis guineensis* (oil palm) and *Theobroma cacao* (cocoa) is discussed, with a particular emphasis on trait improvement related to biotic and abiotic stress resistance, flowering time modulation, fruit quality and yield enhancement. Among these, *Populus* has emerged as the most extensively edited tree genus due to its well-annotated genome, fast growth, amenability to transformation and status as a model woody plant. Notable advances include gene function characterization, lignin biosynthesis modification and stress-resilience enhancement. The review also explores novel genome editing strategies like DNA-free editing that mitigate transgenic concerns and facilitate regulatory approval. Despite significant progress, challenges remain regarding efficient regeneration systems, delivery methods, off-target risk and varying international regulatory frameworks. Overall, CRISPR/Cas technologies hold transformative potential for accelerating genetic improvement and advancing functional genomics in perennial tree crops, supporting more resilient, productive and sustainable forestry and horticulture practices worldwide.

Keywords: Cas-CLOVER, Cas9 and Cas12a (Cpf1), CRISPR, Gene editing, Tree crop improvement

INTRODUCTION

The substantial contribution of tree crops to the economy of many countries on different continents needs no emphasis. It is well known that from the beginning of human life, trees provided two essentials resources: food and oxygen. Other products like timber, wood, fuel, medicine, tools and shelter are gifted by trees. Along with evolution, as human needs increased, trees offered more benefits and their role expanded to satisfy the needs of the modern world. In the present era of climate change, trees moderate the effects of the sun, rain and wind and support the above and below-ground ecosystems. A major portion of cultivated crops that are exploited by human beings for their livelihoods are woody trees. Many of these crops are

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