



CRISPR/Cas9: A Revolution in Genome Engineering for Horticultural Crop Improvement

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Horticultural crops, integral to food and nutritional security, face increasing threats from pests, diseases, and climatic stress. CRISPR/Cas9 has emerged as a transformative genome editing tool for precise and efficient modification of plant genomes. This article explores CRISPR/Cas9-mediated advances in horticultural crop improvement, highlighting its applications in vegetable, fruit, and ornamental crops, as well as in metabolic engineering and disease resistance. With its high specificity, ease of use, and capability for multiplexed gene targeting, CRISPR/Cas9 is rapidly becoming an indispensable tool for horticultural breeding and research.

Keywords: CRISPR/Cas9, genome editing, horticultural crops, vegetables, fruits, disease resistance, metabolic engineering

Introduction

Horticulture encompasses diverse crops like vegetables, fruits, and ornamental plants which are vital for nutrition and economy. However, they are increasingly affected by both biotic and abiotic stress factors. Traditional breeding methods are limited by genetic variability, long breeding cycles, and crossing barriers between species. The CRISPR/Cas9 system provides a rapid and accurate method for introducing or removing genetic traits, circumventing the need for lengthy traditional methods. Its adoption in horticulture holds promise for accelerating genetic gains, enhancing stress tolerance, improving nutritional content, and developing disease-resistant varieties (Ashraf, 2010; Tester & Langridge, 2010).

Genome Editing by Sequence-Specific Nucleases

Genome editing tools like ZFNs, TALENs, and CRISPR/Cas9 enable precise manipulation of DNA sequences. Among these, CRISPR/Cas9 stands out for its simplicity, efficiency, and programmability. It utilizes a guide RNA (gRNA) to direct the Cas9 nuclease to a specific DNA sequence, causing a double-stranded break. This break is repaired by the cell using either non-homologous end joining (NHEJ), which introduces insertions or deletions, or homologous recombination (HR) for more precise edits (Jinek et al., 2012). Tools like CRISPRdirect and GT-scan facilitate selection of unique target sites to minimize off-target effects (Naito et al., 2015).

CRISPR/Cas9 in Vegetable Crops

Vegetable crops, particularly tomato, have served as a model for CRISPR/Cas9 applications. Multiple genes influencing growth, development, and resistance have been targeted. For instance, ARGONAUTE7 (SIAGO7) regulates leaf development (Brooks et al., 2014), while ANT1 influences anthocyanin biosynthesis (Cermak et al., 2015). Other genes like SIHAA9 (Ueta et al., 2017) and SIAGL6 (Klap et al., 2017) were edited to induce parthenocarpy.

Disease resistance was achieved by targeting genes such as SIMlo1 for powdery mildew resistance (Nekrasov et al., 2017). Additionally, crops like lettuce and Brassica oleracea have been edited for functional genomics (Woo et al., 2015; Lawrenson et al., 2015).

CRISPR/Cas9 in Fruit Crops

Despite their importance, fruit crops face challenges like long juvenile phases and limited transformation protocols. Nonetheless, successful edits have been reported in citrus, grape, and watermelon. The CsLOB1 gene in citrus was modified to confer resistance to citrus canker (Jia et al., 2017; Peng et al., 2017). In grape, CRISPR was used to target Phytoene desaturase (VvPDS) for carotenoid biosynthesis studies (Nakajima et al., 2017). The development of databases like Grape-CRISPR aids in identifying specific CRISPR target sites (Wang Y. et al., 2016).

CRISPR/Cas9 in Other Horticultural Crops

Applications of CRISPR/Cas9 extend to ornamental and medicinal crops. In Chrysanthemum morifolium, fluorescence genes were targeted to evaluate editing efficiency (Kishi-Kaboshi et al., 2017). Lotus japonicus was used to study nitrogen fixation through editing of SYMRK and LjLb genes (Wang L. et al., 2016). In Salvia miltiorrhiza, editing of SmCPS1 demonstrated control over tanshinone biosynthesis, a valuable medicinal compound (Li et al., 2017). These examples showcase CRISPR/Cas9's potential for enhancing crop quality and therapeutic value.

Future of Genome Editing in Horticultural Crops

CRISPR/Cas9 is poised to revolutionize the horticultural industry by enabling traits such as improved shelf life, enhanced flavor, nutrient enrichment, and resistance to diseases and environmental stress. It also allows for the removal of anti-nutritional compounds and unwanted traits. Genome editing may soon enable development of “stay sweet” peas by targeting the SBE1 gene (Bhattacharyya et al., 1990) or bitterness-free cucumbers by editing cucurbitacin biosynthesis pathways (Shang et al., 2014). Although regulatory and public acceptance issues persist, the benefits of CRISPR/Cas9-driven crop improvement are substantial and expanding.

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