DEVELOPMENT CRISPR/CAS9-MEDIATED RESISTANCE IN SUNFLOWER AGAINST *O. CUMANA*

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ABSTRACT

Sesquiterpene lactones (STL) are a group of natural compounds found in various plant species, including sunflowers (Helianthus spp.), and they have been studied for their potential role in allelopathy and defense against pests and pathogens. Allelopathy refers to the ability of certain plants to release chemicals that affect the growth and development of neighboring plants and organisms. In the context of sunflower broomrape (Orobanche cumana) and sunflowers, STLs have been of particular interest due to their potential role in inhibiting the growth of the broomrape parasite. Some STLs have been shown to possess allelopathic properties, which means they can influence the germination and growth of other plants, potentially including parasitic plants like sunflower broomrape. Research has suggested that certain STLs found in sunflowers may exhibit inhibitory effects on the germination and growth of sunflower broomrape seeds. These compounds could potentially be released from the sunflower roots and into the soil, creating a hostile environment for the parasite. However, the effectiveness of sesquiterpene lactones in controlling sunflower broomrape is still an active area of research, and their practical application as a management strategy requires further investigation. In recent years, secretion of Sesquiterpene Lactones (STLs) from sunflower roots has been found to trigger the germination of broomrape seeds. The genes encoding the enzymes (*HaGAS, HaGAO, HaG8H, HaCOS*) functional in STL biosynthesis in sunflower have been well characterized. CRISPR-Cas9 is a powerful genetic editing tool that has been used to modify specific genes in various organisms, including plants, for a range of purposes, including crop improvement and pest resistance. In the light of all these information, genes of the enzymes that catalyze the production STLS was aimed to knockout with CRISPR/Cas9 technique in the study. It has been hypothesized that mutant sunflower lines developed in this way will have full resistance to broomrape. The sequences of four genes (HaGAS, HaGAO, HaG8H, HaCOS) encoding the enzymes functional in STL biosynthesis were retrieved from the database and processed with CRISPR-P 2.0 software to find out the best guide RNAs (gRNAs) that can target exon parts of the genes. By this way, four best gRNAs (one gRNA for each gene) were selected for simultaneous targeting of the first exon of the genes. All gRNAs were then transferred into a Cas9 containing agrobacterium plasmid (pHSE401) by using golden gate cloning. gRNA/Cas9 containing vectors were then inserted into agrobacterium rhizogenes and positive colonies were verified with colony PCR. The seed, cotyledon and hypocotyl explant of the sunflower genotype (NS3) was then treated with A. rihizoneges to insert of the gRNA/Cas9 into explants and root formation. Rooted mutants' explants were then put into broomrape seed containing tissue culture media. The results indicated that 79% of the mutant roots have high resistance to broomrape. After DNA isolation, the target genes were amplified with PCR and sequenced to see the CRISPR-mediated mutation in the genes. among the broomrape resistant rooted explants 83% of them were recorded to carry mutation in the gene of interest. This is the first study developing broomrape resistant sunflower genotypes by using CRISPR genome editing system. Optimization of CRISPR mediated gene transfer and regeneration protocol will fasten and made important contribution to sunflower breeding. Genome editing-based strategies used to enhance crop resistance to parasitic weeds and its prospective applications will be discussed in the congress.

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Key words: CRISPR, Sunflower, Sesquiterpene Lactones, broomrape, *O. cumana*, resistance