World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:16, No:01, 2022

CRISPR/Cas9 Based Gene Stacking in Plants for Virus Resistance Using Site-Specific Recombinases

Authors : Sabin Aslam, Sultan Habibullah Khan, James G. Thomson, Abhaya M. Dandekar

Abstract : Losses due to viral diseases are posing a serious threat to crop production. A quick breakdown of resistance to viruses like Cotton Leaf Curl Virus (CLCuV) demands the application of a proficient technology to engineer durable resistance. Gene stacking has recently emerged as a potential approach for integrating multiple genes in crop plants. In the present study, recombinase technology has been used for site-specific gene stacking. A target vector (pG-Rec) was designed for engineering a predetermined specific site in the plant genome whereby genes can be stacked repeatedly. Using Agrobacterium-mediated transformation, the pG-Rec was transformed into Coker-312 along with Nicotiana tabacum L. cv. Xanthi and Nicotiana benthamiana. The transgene analysis of target lines was conducted through junction PCR. The transgene positive target lines were used for further transformations to site-specifically stack two genes of interest using Bxb1 and PhiC31 recombinases. In the first instance, Cas9 driven by multiplex gRNAs (for Rep gene of CLCuV) was site-specifically integrated into the target lines and determined by the junction PCR and real-time PCR. The resulting plants were subsequently used to stack the second gene of interest (AVP3 gene from Arabidopsis for enhancing cotton plant growth). The addition of the genes is simultaneously achieved with the removal of marker genes for recycling with the next round of gene stacking. Consequently, transgenic marker-free plants were produced with two genes stacked at the specific site. These transgenic plants can be potential germplasm to introduce resistance against various strains of cotton leaf curl virus (CLCuV) and abiotic stresses. The results of the research demonstrate gene stacking in crop plants, a technology that can be used to introduce multiple genes sequentially at predefined genomic sites. The current climate change scenario highlights the use of such technologies so that gigantic environmental issues can be tackled by several traits in a single step. After evaluating virus resistance in the resulting plants, the lines can be a primer to initiate stacking of further genes in Cotton for other traits as well as molecular breeding with elite cotton lines.

Keywords : cotton, CRISPR/Cas9, gene stacking, genome editing, recombinases
Conference Title : ICGBGE 2022 : International Conference on Green Biotechnology and Genetic Engineering
Conference Location : Dubai, United Arab Emirates
Conference Dates : January 28-29, 2022