Entry No. 8RGS-0017

Category	: 8th Rice Genetics Symposium
Select Theme	: Genome and Gene editing: Novel tools and technologies
Endorsement email	:
Genome and Gene editing Novel tools and technologies Keyword 1	: CRISPR
Genome and Gene editing Novel tools and technologies Keyword 2	: Plant transformation
Genome and Gene editing Novel tools and technologies Keyword 3	: site-directed mutagenesis
Title of Entry	: Genome editing of a novel gene of Oryza sativa cv. Nagina 22 using CRISPR/Cas approach
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Affiliation 1	: ICAR-Indian Institute of Rice Research, Hyderabad
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Select only one type of presentation	: 15 minute oral presentation
Abstract	: Genome editing of a novel gene of Oryza sativa cv. Nagina 22 using CRISPR/Cas approach Supriya B. Aglawe1¥, Shaik Abdul Muzammil1, V. Venkatesh1, S.M. Balachandran1, N. Sarla1, Shelly Praveen2, and Satendra Kumar Mangrauthia1* 1ICAR-Indian Institute of Rice Research, Hyderabad 2ICAR-Indian Agricultural Research Institute, New Delhi *Corresponding author: skmdrr@gmail.com Phone: +91-40-24591342 ¥ Presenting author: sumukti003@gmail.com Mobile: +91-9030744353 The genome editing for making desired changes in the genome has been widely adopted after the emergence of the clustered, regularly interspaced, short palindromic repeat (CRISPR) technology. CRISPR and CRISPR-associated (Cas) is the latest tool of genome editing which became popular due to its ease, flexibility, and versatility. Optimization of bench level cloning protocol for CRISPR/Cas and regeneration protocol for genotype under study is a prerequisite to exploit advantages of CRISPR/Cas tool. So far, all studies on genome editing of rice genes deals with japonica and indica cultivars. The aus subgroup of O. sativa consists of elite genotypes which possess extremely useful genes for abiotic stress tolerance. In this study, we optimized CRISPR/Cas bench level protocol for editing of a novel gene of Nagina 22, an aus genotype of rice tolerant to drought and heat. A candidate gene annotated as cell wall integrity protein (CWIP) was selected for knock-out using CRISPR/Cas9. In our previous study, this gene

showed significant association with tolerance to high temperature. Two gRNAs were designed using CRISPR-P and CRISPR PLANT online bioinformatics tools to target the first exon of gene for complete loss of its function. The designed gRNAs having no off targets were synthesized and cloned in psgR-Cas9-Os vector which contains Cas9 gene from Streptococcus pyogenes. The entire expression cassette including gRNA and Cas9 gene along with their respective promoters was sub-cloned into pCAMBIA1300 binary vector. pCAMBIA1300 containing Cas9 and gRNAs was mobilized into Agrobacterium tumefaciens strain EHA105 which was used for genetic transformation of Nagina 22 using embryogenic calli. Transformation and regeneration protocol was optimized for Nagina 22. Primary transformed lines have been obtained which are being evaluated for targeted editing of the novel gene.

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