Targeted manipulation of *SPL14/IPA1* gene for improving yield parameters in rice *via* CRISPR/Cas9 system

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Abstract

To meet the ever increasing demand for rice, it is necessary to breed elite rice varieties with improved agronomic parameters such as ideal plant architecture and higher yield. A master gene controlling ideal plant architecture trait in rice is *SPL14* (*SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE 14)* gene or *IPA1* (*IDEAL PLANT ARCHITECTURE 1*), which is a pleiotropic gene controlling various yield parameters in rice. In addition, *SPL14* transcripts are cleaved by the highly conserved *miR156* leading to reduced expression of *SPL14* gene. Hence the mutations in the miR156 binding site will lead to an enhanced expression of *SPL14* gene, thereby contributing to higher grain yield in rice. CRISPR/*Cas9* offers us a powerful means of creating novel alleles of any candidate gene of interest. Our present study was carried out with an aim of improving yield parameters in two adapted rice varieties, ASD16 and CO51 through precise editing of *miR156* binding site located in the third exon of *SPL14/IPA1* gene using CRISPR/Cas9 technology. *Agrobacterium*-mediated genetic transformation of immature embryos of rice varieties, ASD16 and CO51 using pRGEB32 vector harboring *SPL14*-sgRNA resulted in the generation of 97 putative independent events. PCR analysis and Sanger sequencing reveals the presence of deletions ranging from 1 bp to 11 bp in the miRNA binding site in the *SPL14* gene. Molecular and agronomic characterization of *ipa1* mutants in T_1 generation are underway. Development of the genome edited rice lines possessing the miR156-resistance allele of *IPA1* locus has the potential for higher yield.

Key words: SPL14/ IPA1 gene, miR156 binding site, CRISPR/Cas9.