주제-05

Improvement of Grain Yield by Editing Gene Related to Amino Acid Transporter Using CRISPR / cas9 System in Rice

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Amino acid transporters are the major mediators of N distribution and are important regulators of resource allocation in plants. Inorganic nitrogen (N) is mainly absorbed by plants in the form of nitrate and ammonium and then converted directly into amino acids at the roots or after conversion to leaves. Amino acids are then transported to roots, leaves, flowers, pollen and embryos (Fischer et al., 1998). Amino acids require transporter proteins to transfer them from source to sink organs (Coruzzi and Bush, 2001; Tegeder, 2012). These amino acid transporters (AATs) are cellular membrane proteins that transport specific amino acids. Transporters play an important role in the development of seeds and in various processes in plants such as abiotic and pathogen stresses. Although the functions of AtAATs have been extensively studied in Arabidopsis, the roles of OsAATs in rice are much less well understood (Zhao et al., 2012). Whole genome analyses have suggested the presence of 79–85 AAT homologous genes in rice (Lu et al., 2012; Zhao et al., 2012). It has been shown that biomass and yield of rice are altered signifi cantly when OsAAT genes are knocked out (Lu et al., 2012; Peng et al., 2014). In these studies, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated (Cas) systems have been successfully used as efficient tools for genome editing in a variety of species. We used the CRISPR/Cas9 system to mutate the AAT5 (Os08g0509600), AAT7 (Os08g0509600), AAT24 (Os08g0509600), AAT 49 (Os08g0509600), AAT60 (Os08g0509600) of Donggin cultivar, these genes which have been reported to function as regulators of the grain number, panicle architecture, grain size and plant architecture, respectively. Analysis of the phenotypes and frequencies of edited genes in the first generation of transformed plants (T0) showed that the CRISPR/Cas9 system was highly efficient in inducing targeted gene editing, with the desired genes being edited in 47.5% (AAT5), 77.5% (AAT7), 57.5% (AAT24), 53.7% (AAT49) and 57.5% (AAT60) of the transformed plants. The T1 generation of the AAT7, AAT49, and AAT60 mutants featured enhanced grain number and grain size, respectively. In addition, we found that the deletion mutants obtained by AAT gene editing rarely off-target in similar target sequences. These results proved that multiple regulators of important traits can be modified in a single cultivar by CRISPR/Cas9, and thus facilitate the dissection of complex gene regulatory networks in the same genomic background and the stacking of important traits in cultivated varieties. These results suggest that manipulation of AAT gene expression could be used to increase grain yield in rice.

Keywords: CRISPR/Cas9 system, gene editing, *Oryza sativa* L., amino acid transport, tiller, grain yield

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