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Os4bglu18, a Monolignol β-Glucosidase, Positively Affect Salinity Stress in a *sitl2* Mutant Rice

Su Hong Oh[1], Cheol Seong Jang[1]*

[1]Plant Genomics Laboratory, Department of Applied Plant Sciences, Kangwon National University, Chuncheon 200-713, Republic of Korea

[Introduction]

Salinity stress is one of the major abiotic stress factors that affect global rice (*Oryza sativa L.*) production worldwide. Using a forward genetics way, a salt insensitive TILLING line 2 (sitl2) rice mutant was isolated from a gamma-ray irradiated TILLING population.

[Materials and Methods]

Plant growth condition: To screen for salinity-tolerant mutant lines, seeds of the M10 core collection germplasm were germinated at 28 °C for 2 d. The germinated seeds were transferred to which contained half-strength Kimura B solution (pH 5.6; Ma et al., 2001)

Measurement of insensitivity from the salinity stress: The 7 day-olds seedlings WT and instl2 mutant were treated with 100mM NaCl for 7 days and then measured the plant length, weight, chlorophyll, hydrogen peroxide (H_2O_2) and ionomic analysis.

Candidate gene analysis: the Illumina TruSeq Nano DNA Library Kit (Illumina, USA) was used to construct DNA libraries. Whole genome sequencing was then performed using the Illumina HiSeq4000 platform (Macrogen Inc, Seoul, Korea) and a paired-end sequencing method.

[Results and Discussion]

Plant growth condition :The *sitl2* mutant showed reduced root and leaf growth in the early stage but after 3 weeks after germination, the *sitl2* mutant showed enhanced leaf growth and chlorophyll content when grown under normal conditions was not differentiated between WT and *sitl2.*

Measurement of insensitivity from the salinity stress: first in the soil condition, sitl2 mutant showed reduced leaf curling lower than WT. It was confirmed by the survival rate and fresh weight. In the germination stage, sitl2 mutant showed higher percentage of germination compared with WT in the 100mM NaCl 1/2 MS media. And then the ionomic analysis using ICP-OES was conducted. As a result, the sitl2 mutant absorbed the Na^+ ion lower than WT but absorbed the K^+ ion more than WT.

Candidate gene analysis: Based on the WGS we confirmed the frameshift variation between WT and *sitl2, and* selected candidate gene which affect to the *sitl2*' insensitivity from the salinity stress. As a result, Os4bglu18, a monolignol β -Glucosidase, was selected as candidate. Os4bglu18 has been found in previous studies that Os4BGlu14, Os4BGlu16, and Os4BGlu18 are supposed as rice monolignol β-glucosidases and can hydrolyze the monolignol glucosides coniferin and syringin. In that reasons, we predicted the sitl2 mutant have more lignin content than WT which affect to the *sitl2*' insensitivity from the salinity stress.

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*Corresponding author: Tel. 070-7135-9637, E-mail. csjang@kangwon.ac.kr