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Profiling of Candidate Genes Associated with Cuticular Wax in Rice Mutant Tolerance to Drought Stress

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[Introduction]

Abiotic stresses, in particular salt and drought stresses that modifying the physiological and biochemical processes of plants lead to severely reduce the productivity of crops worldwide. The development of new varieties of rice that can adapt to changing circumstances is important to sustainable rice production for worldwide (Vinocur and Altman 2005, Zhu 2016). To screening of drought tolerant lines, M8 lines of TILLING rice mutants were developed.

[Materials and Methods]

Plant materials and growth conditions: The germinated seeds of Line 36 mutant and WT were transferred to plastic container with floating nylon mesh containing half-strength kimura B nutrient solution (pH=5.6) (Chen et al. 2006). In a growth chamber, two-week-old plants under cycle (16 h light/8 h dark) at 28/25°C were used. To drought stress treatment, plants were dehydrated for 8 h and re-watered 5 days.

Chlorophyll leaching assay: Chlorophyll leaching assay were performed for 3rd leaves of two-week-old plants of Line 36 mutant and WT. The leaves were cut into 8 cm length and measured for fresh-weight with five leaves.

Toluidine blue assay: The toluidine blue staining was performed as described method (Tanaka et al. 2004). 3rd leaves of two-week-old plants of Line 36 mutant and WT were immersed for 1, 2 h in 0.05% toluidine blue with and washed with water.

[Results and Discussion]

Measurement of insensitivity from the drought stress: Re-watered after dehydration, Line 36 mutant showed more recovered than WT. It was confirmed by the fresh weight and survival rate. And then the water loss assay was conducted. As a result, the Line 36 mutant showed lower water loss than WT.

Candidate gene analysis: Based on the Whole Genome Sequencing, we confirmed the frameshift variation between WT and Line 36 mutant. As a result, Os05g48260 that associated wax synthase, was selected as candidate which affect to the Line 36 mutant insensitivity from the drought stress.

Chlorophyll leaching and Toluidine blue assay: We performed Chlorophyll leaching assay and Toluidine blue assay, because cuticle permeability is strongly influenced by the quantity of cuticular wax present. As a results, chlorophyll was more readily extracted and stained from WT leaves than from Line 36 mutant.

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