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Altered Anthranilate Synthase in 5-Methyltryptophan Resistant Rice Mutants

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Objectives

Rice M_4 mutant lines resistant to growth inhibition by 5-methyltryptophan (5MT) were selected from the callus irradiated with a gamma ray of 50 Gy through embryo culture. We investigated alteration of the key feedback control enzyme, anthranilate synthase, in tryptophan biosynthesis

Results and Discussion

For increasing the contents of specific free amino acids in rice (Oryza sativa L.) cultivar Donganbyeo, the mutant cell lines resistant to growth inhibition by 5MT were selected from the callus irradiated with a gamma ray of 50 Gy through embryo culture. Four homozygous M4 lines, MR I-40, MR I-110, MR II-8, and MR II-12, were obtained through consecutive selection of 5MT resistant cell lines, regeneration, and successive selfing and segregation tests. Anthranilate synthase activity was measured by direct fluorometric detection of the formed anthranilate in the control plants and mutant lines grown on 500 μM 5MT. Anthranilate synthase activity of mutant plants showed 2.2 ~ 3 times higher than that of the control. In kinetic analysis with tryptophan, anthranilate synthase of mutant lines showed insensitive to feedback inhibition.

The transcript levels of *OsASA* in the control was restricted by 5MT treatment. However, 5MT caused a high accumulation of the *OsASA* transcripts in the mutant lines. Northern blot analyses were also conducted with the time periods of 3h, 6h, 12h, 24h, and 48h under the stresses of two tryptophan analogs (500 μM 5MT and 200 μM aMT) and abiotic stresses (10 μM ABA, 200mM NaCl, 4°C cold). In the tryptophan analog treatments, there was a significant difference between the control and the mutants. The transcript levels of the control were continuously decreased according to the time passage, whereas the mutant plants revealed steady states of expression. These results show that *OsASA* of the mutant plants was insensitive to 5MT and aMT, therefore, the tryptophan biosynthesis was hold on during the time periods examined. In abiotic stress treatments, the transcript levels of *OsASA* in the mutant plants were gradually decreased during the time passage. However, the minor increases of *OsASA* mRNA were observed in the control plants.

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