Genetic Analysis of Green Plant Regeneration in Anther Culture of an F1 Hybrid between Japonica and Indica Rice (*Oryza sativa* L.)

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Introduction: Because anther culture can make doubled haploid for a short period, anther culture is used for breeding and making the plant materials for study. In particular, gene fixation of hybrid plants by anther culture can spread a possibility of breeding. But green plant regeneration rate is low and the reason has been unclear. In this study, we cultivated rice anthers of the F1 progeny between Nipponbare (*Oryza sativa* L ssp. *japonica*) and Kasalath (*Oryza sativa* L ssp. *indica*), obtained a lot of regenerated plants, and investigated the segregate distortions.

Aim: We identify genetic loci associated with green plant regeneration.

Methods: The reciprocal F1 hybrids between the rice japonica cultivar 'Nipponbare' and the indica cultivar 'Kasalath' were planted in the greenhouse in Hokkaido University. Spikes were sampled at the booting stage, and were conducted low temperature pre-treatment at 10°C for 10 days. After pre-treatment, the spikes were surface sterilized in 70% ethanol. About 40-70 anthers were placed randomly in a petri dish containing about 20 ml anther callus induction medium. (SK-1 solid medium) The anthers were incubated in the dark for 35-50 days at 25°C. We placed 4000-5000 anthers for each line. About 1000 produced calli were transferred to test tubes containing about 8 ml regeneration medium. (N6 solid medium) The calli were incubated in the light for 60 days at 25°C. We detected genotypes of the green and albino plants derived from each F1 hybrids, by using 42 SSR markers and 41 STS markers, which covered whole genome. We calculated significant difference by χ^2 test to investigate whether the segregation ratio of each marker showed the distortion from 1:1 or not.

Results and Discussion: In all chromosomes except for the chromosome 2, 5, and 7, at least 1 marker showed the segregate distortion by the 1% significant difference. In regenerated plants derived from Nipponbare x Kasalath (in the following, K/N) and Kasalath x Nipponbare (in the following, K/N) populations, common segregate distortions by the 1% significant difference in green regenerated plants were detected at 6 genetic loci, and common in albino regenerated plants were detected at 4 genetic loci. Segregate distortions common to all regenerated plants were detected in chromosome 1 and 6. Segregate distortions of 4 genetic loci in K/N plants were detected common to green and albino regenerated plants. We've investigated whether these distortions relates to the reproduction barrier system occurred by crossing. It is necessary to narrow distorted regions by more in-depth genetic mapping, and to consider plasma type and a relation among genetic regions.

Key Words: anther culture, plant regeneration, segregate distortion

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