Analysis of a temperature sensitive virescent mutant of rice induced by heavy-ion beam†

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In plants, several leaf-color mutants such as albino, xantha, pale green, stripe, and virescence have been found. Among them, virescent mutants are intriguing because they exhibit white chlorotic leaves during the early growth stages, but produce normal green leaves as they grow. To date, an increasing number of genes responsible for virescent phenotypes have been identified from a number of species. For example, eight genes were recognized as causative genes for the virescent phenotype in rice. Among them, only three genes were reported until 2010, and the remaining five genes were identified recently (since 2011). However, little is known about the mechanism underlying this phenotype. To gain insight into the molecular mechanism of the virescent phenotype, we isolated and characterized a novel rice mutant named 22-4Y.

22-4Y was obtained from a mutant population generated by heavy-ion irradiation ($^{12}C^{6+}$ ions, 20 Gy, LET: 22.5 keV μm^{-1}).[1)] This mutant exhibited chlorotic leaves only during an early growth period, especially the second, third, and fourth leaves at 25°C. After the fifth-leaf stage, the mutants produced normal green leaves. In addition, the mutant shows temperature sensitivity. At 30°C, 22-4Y produced third and fourth leaves with slight chlorosis. However, at 20°C, almost all leaves of the mutant were white. Interestingly, the mutant transferred from 30°C to 20°C conditions at the fifth leaf stage produced chlorotic leaves as the sixth and subsequent leaves, indicating that this mutant responded to low temperature in the early growth and tillering stages. We performed transmission electron microscopy to compare the chloroplast ultrastructure of the mutant grown at 30°C and 20°C. At 30°C, mutant plants possessed normally developed chloroplasts. However, the chloroplasts in the mutant at 20°C displayed undeveloped membrane structures. These observations demonstrate that the chlorotic phenotype of the mutant is attributable to aberrant chloroplast development during leaf formation. Genetic analysis revealed that there are two genes altered by translocation (deletion for LOC_Os05g34040 and insertion for LOC_Os12g31810) (Fig. 1). A complementation test revealed that the causative gene for the virescent phenotype is LOC_Os05g34040.[2)] Since LOC_Os05g34040 is a newly identified causative gene for the virescent phenotype, we named this gene *Cold Sensitive Virescent1* (*CSV1*). The protein domain analysis of CSV1 showed that this protein belongs to the large family of FAD-dependent pyridine nucleotide reductases (FADPNR). Since the FADPNR family includes a broad range of oxidoreductase enzymes, it is difficult to infer the molecular function of CSV1 simply from its amino acid sequence homology. ChloroP program predicted that the N-terminal region of the CSV1 protein might function as a chloroplast transit peptide. To verify its ability, we constructed a fusion gene expressing the N-terminal region of CSV1 fused to the N-terminus of the cyan fluorescent protein (CFP), under the control of a strong promoter (named 35S::TP_{CSV1}-CFP). We co-introduced 35S::TP_{CSV1}-CFP with 35S::$TP_{AtFtsZ1}$-YFP, positive control encoding the plastid-targeted yellow fluorescent protein (YFP), into an onion epidermal cell. The fluorescent signals of CFP were clearly co-localized with those of YFP (Fig. 2). In contrast, the fluorescent signals were observed in the cytoplasm and nucleus when we introduced the plasmid DNA for a non-fused CFP or YFP as a control. These observations demonstrated that CSV1 is a plastid-localizing protein. Our findings indicate that *CSV1* is an important gene for chloroplast development under cold stress, both in the early growth stage and in the tillering stage.

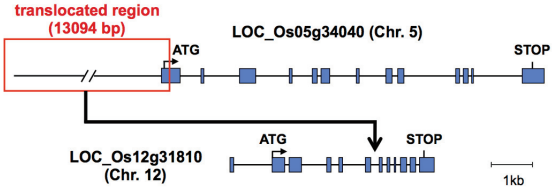

Fig. 1. Schematic representation of the translocation induced in 22-4Y. The boxes indicate exons. ATG and STOP indicate the start and stop codon, respectively.

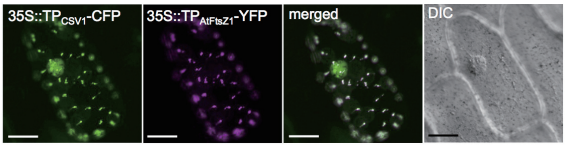

Fig. 2. Intracellular localization of CSV1. 35S::TP_{CSV1}-CFP, CFP with the putative transit peptide of CSV1 (pseudo-coloured in green); 35S::$TP_{AtFtsZ1}$-YFP, YFP with the transit peptide of plastid-targeted AtFtsZ1 (magenta); Merged, merged images of the CFP and YFP images; DIC, differential interference contrast. Scale bars = 50 μm.

References

1) M. Nakagawa et al.: RIKEN Accel. Prog. Rep. **43**, 281 (2010).
2) R. Morita et al.: RIKEN Accel. Prog. Rep. **46**, 259 (2013).

† Condensed from the article in Biosci. Biotech. Biochem. **81**, 271 (2017)

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