

## Functional analysis of a new *virescent* mutant in rice induced by heavy-ion beam irradiation

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It is evident that heavy-ion beam irradiation effectively produced many phenotypic mutants in rice (*Oryza sativa* L. cv. Nipponbare)<sup>1,2</sup>; thus, the method has been applied to rice breeding as a new technology. Although many rice mutants have been isolated to date, the functions of a number of their causative genes have not been completely elucidated. One such mutant, 22-4Y in rice, has been obtained as a *virescent* mutant by irradiating seeds with a carbon-ion beam (20 Gy, LET: 22.5 keV/ $\mu$ m)<sup>3</sup>. *virescent* is a chlorotic mutant of higher plants that reduces the chlorophyll content in young leaves, but the chlorophyll content of the leaves recover as the plants grow<sup>4</sup>. Certain classes of the *virescent* mutants are low-temperature conditional<sup>4</sup>; the 22-4Y mutant also shows chlorotic leaves phenotype when grown at 20 °C but not at 30 °C during the early growth stages<sup>3</sup>. As the 22-4Y functions are related with chlorophyll synthesis and/or chloroplast development, both of which are important for crop production, we decided to investigate the 22-4Y gene and to analyze its functions.

Genetic analysis of the 22-4Y mutant revealed that gene LOC\_Os05g34040 on the long arm of chromosome 5 showed a deletion of 13095 bp<sup>3</sup>. Morita et al.<sup>3</sup> confirmed by using a genetic complementation test that the causative gene of 22-4Y is LOC\_05g34040.

In order to know the gene functions, in many cases, it is most important and necessary to reveal the functions of the protein encoding to the corresponding gene. BLAST searches of the complete *Oryza sativa* sequence revealed that only one copy of the 22-4Y gene is present in the nuclear genome, which encodes a putative polypeptide of 720 amino acids with a calculated molecular mass of 78.2 kDa (Fig.1, the upper line). Pfam search predicted that the 22-4Y protein had a putative conserved FAD/NAD(P)-binding motif (GXGXXG) (Fig.1, red bars) and a homology to pyridine nucleotide-disulfide oxidoreductase family enzymes (Fig1, green region). This means that 22-4Y is a new class of VIRECENT protein among so far identified<sup>4</sup> (V1, chloroplast-localized RNA-binding protein; V2, guanylate kinase; V3, large subunit of ribonucleotide reductase). TargetP program predicted that the N-terminal 57 amino acids sequence of the 22-4Y protein was a chloroplast transit peptide. Actually, when the N-terminal 76 amino acids sequence of the 22-4Y protein was fused to cyan fluorescent protein (CFP) and transiently expressed in an onion epidermal cell, the fusion protein was localized to chloroplast. Thus, the N-terminal sequence of the 22-4Y protein was functional as a chloroplast targeting peptide. Electron microscopic observation revealed that chloroplast development was arrested when 22-4Y mutant was grown at 20 °C but not at 30 °C during the early growth stages. These results suggest that the 22-4Y protein was functional in chloroplast and had some important regulatory functions in chloroplast development.

To further reveal and analyze the protein function of 22-4Y, we decided to purify some recombinant proteins of 22-4Y by using an *E. coli* expression system (Fig. 1). It is important to demonstrate the enzymatic activity of the 22-4Y protein, as well as to identify the target (substrate) protein(s) to reveal the regulatory functions of the 22-4Y protein during chloroplast development.

### References

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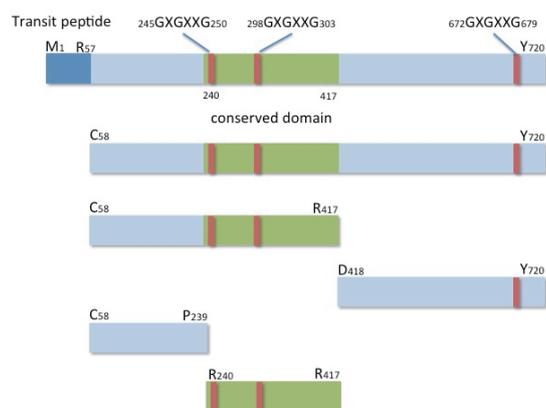


Fig. 1. Schematic representation of the 22-4Y protein and its fragments expressed in *E. coli*.

The predicted 22-4Y protein (the upper line) has 720 amino acids (M1-Y720) with a calculated molecular mass of 78.2 kDa. Each fragment of 22-4Y (C58-Y720), (C58-R417), (D418-Y720), (C58-P239) and (R240-R417) is shown. Positions of the FAD/NAD(P)-binding motif (GXGXXG) are shown in red bars. A region with homology to pyridine nucleotide-disulfide oxidoreductase family enzymes is shown in green. The predicted chloroplast transit peptide is shown in dark blue.

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