Molecular analysis of the stay-green mutant *dye1* induced by carbon ion beams in rice†

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Chlorophyll synthesis and degradation are carefully regulated in plants not only because chlorophyll is an essential photosynthetic molecule, but also because in its free form it can damage cells photo-oxidatively.[1)] Mutants that retain the green of leaves under senescence-inducing conditions are known as stay-green mutants. We isolated a stay-green mutant from a rice M_2 population (Oryza sativa L.) irradiated with carbon ion beams (1.6 GeV, 23 keV/μm, 20 Gy). It exhibited delayed yellowing during natural senescence in the paddy field. This recessive mutant, named *delayed yellowing1-1* (*dye1-1*), was greener than the wild-type cultivar, Nipponbare, five weeks after heading, when most leaves are senescent (Fig. 1).

We performed map-based cloning of *DYE1*. An analysis of the F_2 population and its progeny revealed that *DYE1* is located on chromosome 8. Fine mapping of *DYE1* delimited the candidate region within 43.1 kb, which contained seven predicted genes (Fig. 2). A next-generation sequence analysis revealed that *dye1-1* has a G-to-A substitution at the second exon of Lhca4, causing amino acid substitution from glutamic acid to lysine (E146K). Lhca4 is a subunit of the light-harvesting complex for photosystem I (PSI). This residue corresponds to E154 in Arabidopsis Lhca4, which is a pigment-binding site conserved not only among Lhca subunits but also among Lhcb subunits (light-harvesting complex for photosystem II) of different species.[2)]

A blue native-PAGE analysis revealed a significant change in the conformation of PSI-LHCI supercomplex in *dye1-1* (Fig. 3). Nevertheless, the biomass of *dye1-1* was comparable to that of the wild-type. Interestingly,

Fig. 1. *dye*1 exhibits a stay-green phenotype during natural senescence (five weeks after heading).

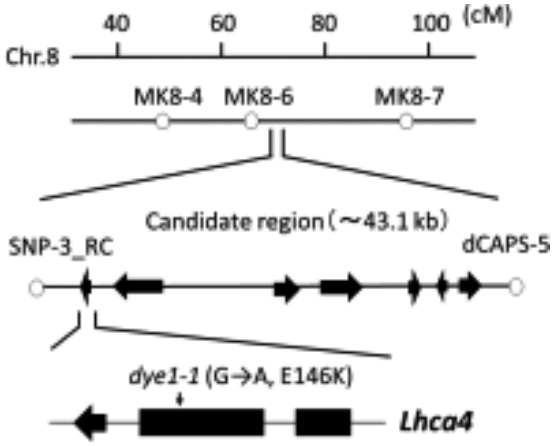

Fig. 2. *DYE1* encodes Lhca4, a subunit of the PSI antenna complex LHCI.

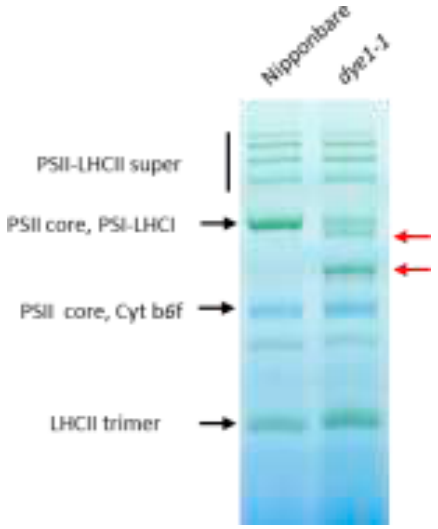

Fig. 3. Blue native-PAGE analysis of photosystems. Arrows indicate newly merged bands in *dye1-1*.

Lhcb1, a subunit of the trimeric LHCII, was highly accumulated in *dye1-1*. The high accumulation of LHCII in the LHCI mutant *dye1-1* may compensate the reduced PSI activity caused by the impairment of LHCI antenna (Lhca4), suggesting a novel functional interaction between LHCI and LHCII.

Higher chlorophyll content in leaves is observed before and during senescence in *dye1-1*, indicating that the impairment of Lhca4 influences chlorophyll synthesis and/or degradation during the development and senescence of leaves in rice. It will be of great interest to examine whether mutants of other LHCI subunits show a high chlorophyll content/stay-green phenotype.

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