Available online at www.sciencedirect.com

ScienceDirect

Journal of Genetics and Genomics 41 (2014) 353−356

JGG

LETTER TO THE EDITOR

Characterization and Molecular Mapping of a New Virescent Mutant in Rice

Plant leaves play a significant role in photosynthesis. Normal chloroplast development is critical for plant growth and yield performance. Defect of the chlorophyll in chloroplasts may cause abnormal leaf colors, such as yellow, white, or stripe. Chloroplasts have their own genomes encoding for about 100 genes that are essential for plastid protein synthesis and photosynthesis (Kanno and Hirai, 1993; Sato et al., 1999). Moreover, over 3000 proteins encoded by plant nuclear genomes target to the chloroplasts and participate in the chloroplast development and/or photosynthesis. Hitherto, a number of plant genes, which encode for enzymes involved in chlorophyll biosynthetic pathways, have been identified (Beale, 2005). Chloroplast biogenesis and development are highly based on the coordination of activities between the nuclear and chloroplast genomes, including gene transcription and RNA processing, translation, protein folding and location, and metabolite flux (Dyall et al., 2004; Woodson and Chory, 2008). Due to the complex interaction between nuclear and chloroplast, mutations of some critical genes within the network will influence the chloroplast development, and ultimately result in the leaf color mutant phenotypes or even lethal. For examples, the chloroplast ribosomal protein L21 of *Arabidopsis thaliana* is essential for plastid development and embryogenesis, and the mutation of this gene displays a lethal phenotype (Yin et al., 2012). The pentatricopeptide repeat (PPR) proteins, such as YS1 (*Yellow Seedlings 1*) of *Arabidopsis* and YSA (*Young Seedling Albino*) of rice (*Oryza sativa* L.), are involved in chloroplast RNA editing. The mutations of these genes lead to abnormal seedling color (Zhou et al., 2009; Su et al., 2012). The mutation of *Belaya smert* (*Bsm*) in *Arabidopsis*, encoding for a member of the mitochondrial transcription termination factor (mTERF) family, can cause albino and suffers defects in global plastidic gene expression (Babiychuk et al., 2011). The *Arabidopsis VAR2* gene, encoding for Ftsh2 protein, is involved in photosystem II (PSII) repair and degradation of unassembled Fe−S proteins of the cytochrome *b6f* complex, and the *var2* mutant shows green and white sectors in leaves (Chen et al., 1999, 2000; Takechi et al., 2000).

Up to date, four rice virescent mutant genes have been cloned. *Virescent 1* (*V1*) encodes for a chloroplast-localized protein NUS1, and its mutant *v1* is temperature-sensitive and develops chlorotic leaves under low temperature condition (Kusumi et al., 2011). *Virescent 2* (*V2*) encodes for a guanylate kinase and is essential for chloroplast differentiation, and its mutant is temperature-sensitive and develops chlorotic leaves at restrictive temperatures (Sugimoto et al. 2004, 2007). *Virescent 3* (*V3*) encodes for the large subunit of ribonucleotide reductase RNRL1, and its mutant is also temperature-conditional, which produces bleached leaves at a constant 20°C or 30°C but almost green leaves under diurnal 30°C/20°C conditions (Yoo et al., 2009). *Young seedling albino* (*YSA*) encodes for a PPR protein and is involved in RNA editing; the *ysa* mutant develops albino leaves before the three-leaf stage, but the mutant gradually turns green and is recovered to normal green at the six-leaf stage (Su et al., 2012). Here we report a new *virescent* mutant in rice, *virescent 14* (*v14*), of which the second and third leaves displayed albino phenotype, but from the fourth leaf stage, all leaves including the albino ones were recovered to normal green. We mapped this mutant locus in a region on chromosome 7, and identified a candidate gene for *v14*, which was caused by a sequence deletion in a putative *mTERF* gene.

In normal growth conditions (ca. 25°C), the first leaf of *v14* was normal green, while the second and third leaves of *v14* were albino, and these leaves were rescued to green after the forth leaf stage (Fig. 1A). After the de-albino stage, the growth of *v14* was not obviously different from the wild-type plant T65. To investigate whether the *v14* mutation affects chloroplast development, we compared the ultrastructures of plastids at the second and fourth leaves in *v14* and T65 using transmission electron microscope (Fig. 1B). The second leaf of *v14* had proplastids that contained vesicular structure; however, no mature thylakoids membrane system and starch grains have been detected. Interestingly, the fourth leaf of *v14* had nearly normal chloroplast structure. These results coincide with the green-reversible albino mutant phenotype. The contents of chlorophyll *a*, chlorophyll *b*, and carotenoid in the second

http://dx.doi.org/10.1016/j.jgg.2014.01.010

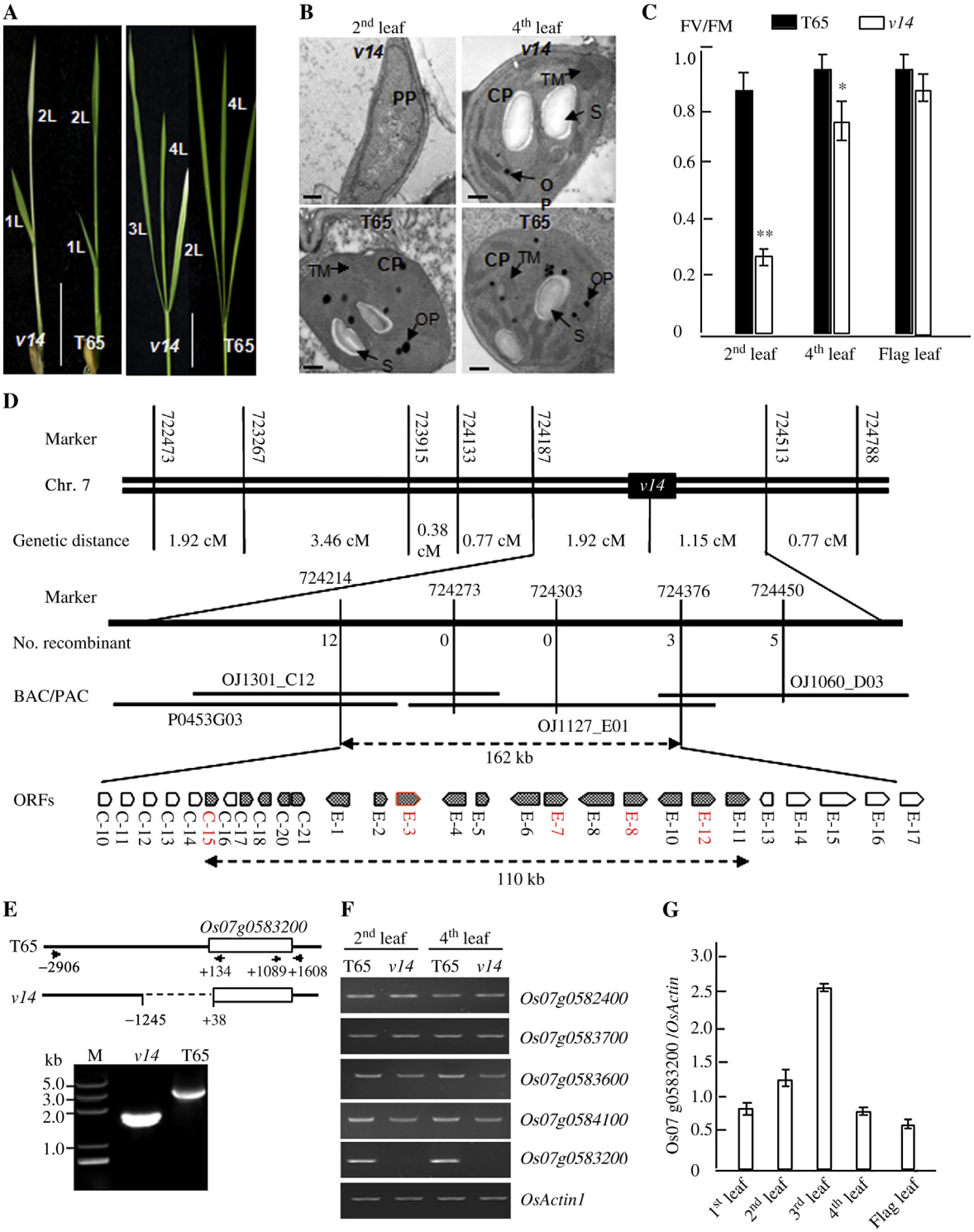

Fig. 1. Characterization and molecular mapping of a new virescent mutant, *virescent 14* (*v14*) in rice.

A: Phenotypic characterization of *v14* mutant. *v14* and wild-type (T65) plants grew at 25°C. Bars = 2 cm. **B:** Ultrastructures of chloroplasts in the second and the fourth leaf mesophyll cells of *v14* and T65 plants ($n = 10$). PP, proplastid; CP, chloroplast; OP, eosinophilic granule; TM, thylakoid membrane; S, starch grain. Bars = 0.5 μm. **C:** The activities of photosystem II in different leaves of *v14* and T65. The Fv/Fm value (ratio of variable to maximum fluorescence) reflects the PSII activity. **D:** Fine mapping of *v14* using a total of 3427 albino F_2 plants from both of the crosses (*v14* × PA64S and *v14* × Dular). *v14* locus was located between markers 724214 and 724376 in a physical distance of 162 kb which contains 28 predicted genes. Eighteen of them were sequenced (black box) which include five chloroplast-target protein genes (red font: C-15: *Os07g0582400*; E-3: *Os07g0583200*; E-7: *Os07g0583600*; E-8: *Os07g0583700*; E-12: *Os07g0584100*). **E:** Screening of candidate gene for *v14*. Schematic map showing the candidate gene, *Os07g0583200*, of which in *v14* had a sequence deletion from − 1245 bp to +38 bp. **F:** RT-PCR of five chloroplast-targeting protein genes in the mapped region, only *Os07g0583200* in *v14* was silenced. **G:** The expression pattern *of Os07g0583200* was examined at different leave developmental stages.