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# FINE MAPPING AND FUNCTIONAL CHARACTERIZATION OF THE BARLEY VIRIDIS ZB63 PHOTOSYNTHETIC MUTANT

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Barley genetic stocks offer a unique collection of chloroplast-deficient mutants that represent an important resource to explore chloroplast biogenesis and photosynthetic molecular mechanisms. Most of these mutants have been genetically and biochemically characterized in the past, among them the photosynthetic mutant viridis zb63 is a lethal mutation with pale green leaves. Biochemical studies have demonstrated that zb63 contains a functional PSII with a minimal antenna system and a depleted PSI, a condition leading to the constitutive reduction of plastoquinone even when grown at very low light intensities. No other allelic mutations for this locus have been reported so far.

zb63 was initially localized in the region between 40 and 60 Mb of chromosome 2H following a BSA carried out on a BC3F2 mapping population using an exome capture and sequencing approach. Then, about 480 BC3F2 individuals have been genotyped with KASP and CAPS markers to narrow-down the mutation to the physical interval between 43 and 45 Mb. The 22 genes annotated in this region on the reference sequence of the cultivar Morex (v2.0, 2019) were further studied in silico, and a gene coding for Pentatricopeptide Repeat (PPR) protein was identified as candidate. The gene carries a 14 bp deletion in exon 1 that creates a premature stops codon. In Arabidopsis, a knockout in the orthologous Pdm4 gene leads to albina phenotype with completely white leaves. The PPR gene was transiently silenced through Virus Induced Gene Silencing and the obtained leaves expressed the typical viridis phenotype of the zb63 mutant. Furthermore, northern and western-blot assays confirmed that silencing the PPR candidate gene reproduces a molecular phenotype identical to zb63 mutant.

The cloning of the zb63 mutant will shade light on mechanisms controlling the PSI assembly.