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Mutagenesis Reveals That the *OsPPa6* Gene Is Required for Enhancing the Alkaline Tolerance in Rice

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Alkaline stress (AS) is one of the abiotic stressful factors limiting plant's growth and development. Inorganic pyrophosphatase is usually involved in a variety of biological processes in plant in response to the abiotic stresses. Here, to clarify the responsive regulation of inorganic pyrophosphatase in rice under AS, the mutagenesis of the *OsPPa6* gene encoding an inorganic pyrophosphatase in rice cv. Kitaake (*Oryza sativa* L. ssp. japonica) was performed by the CRISPR/Cas9 system. Two homozygous independent mutants with cas9-free were obtained by continuously screening. qPCR reveals that the *OsPPa6* gene was significantly induced by AS, and the mutagenesis of the *OsPPa6* gene apparently delayed rice's growth and development, especially under AS. Measurements demonstrate that the contents of pyrophosphate in the mutants were higher than those in the wild type under AS, however, the accumulation of inorganic phosphate, ATP, chlorophyll, sucrose, and starch in the mutants were decreased significantly, and the mutagenesis of the *OsPPa6* gene remarkably lowered the net photosynthetic rate of rice mutants, thus reducing the contents of soluble sugar and proline, but remarkably increasing MDA, osmotic potentials and Na^+/K^+ ratio in the mutants under AS. Metabonomics measurement shows that the mutants obviously down-regulated the accumulation of phosphorylcholine, choline, anthranilic acid, apigenin, coniferol and dodecanoic acid, but up-regulated the accumulation of L-valine, alpha-ketoglutarate, phenylpyruvate and L-phenylalanine under AS. This study suggests that the *OsPPa6* gene is an important osmotic regulatory factor in rice, and the gene-editing of CRISPR/Cas9-guided is an effective method evaluating the responsive regulation of the stress-induced gene, and simultaneously provides a scientific support for the application of the gene encoding a soluble inorganic pyrophosphatase in molecular breeding.

Keywords: alkaline stress, CRISPR/Cas9, inorganic pyrophosphatase, metabonomics, mutagenesis, *OsPPa6* gene