



DNA methylation involved in proline accumulation in response to osmotic stress in rice (*Oryza sativa*)

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ABSTRACT. Proline accumulation is involved in plant osmotic stress tolerance. Given that DNA methylation is related to almost all metabolic processes through regulation of gene expression, we suspected that this epigenetic modification and proline biosynthesis are probably related. To test this, we investigated whether osmotic stress-induced proline accumulation is associated with DNA methylation modifications in rice. We assessed DNA methylation and expression of 3 key genes (*P5CR*, *P5CS*, and δ -*OAT*) involved in proline biosynthesis, and measured proline content under both osmotic stress (15% polyethylene glycol) and control conditions. After osmotic stress, selfed progenies of osmotic-stressed plants accumulated higher concentrations of proline in leaves under both normal conditions and under osmotic stress than the unstressed control plants. Concomitantly, under osmotic stress, the selfed progeny plants showed higher expression levels of *P5CS* and δ -*OAT* than the control. This up-regulated expression was stably inherited by the subsequent generation. Methylation-sensitive

Southern blotting indicated that 2 of the 3 genes, *P5CS* and δ -*OAT*, had greater DNA demethylation in the selfed progenies than in the control. Apparently DNA demethylation facilitated proline accumulation by up-regulating expression of the *P5CS* and δ -*OAT* genes in response to osmotic stress.

Key words: Rice; Proline; Osmotic stress; DNA methylation