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PHOTOSYNTHETIC CARBON METABOLISM AND PROLINE BIOSYNTHESIS, LOOKING FOR A CONNECTION

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Among the wide range of abiotic stresses, drought has one of the greatest impacts on plants in a world perspective, affecting both productivity and plants survival. The beneficial effects of compatible osmolytes (e.g. proline, glycine betaine, and sugar alcohols) accumulating under drought is a well documented phenomenon. On the contrary less is known about the origin and the pathways connecting primary carbon metabolism with osmolytes production.

β-amylase 1 (BAM1) catalyses the hydrolysis of leaf starch in response to osmotic stress. Recently it has been demonstrated that in response to drought, Arabidopsis mutants lacking *BAM1* fail to accumulate Pro at the same level as wild-type plants, suggesting a connection between transitory starch degradation and Pro biosynthesis. With the aim of studying this possible connection an *in silico* analysis was performed to identify candidate genes that either respond to osmotic stress and belong to the putative connecting pathway. Ten homozygous T-DNA lines corresponding to the candidate genes were tested by quantifying the oxidative stress (TBA assay) induced by osmotic treatment (150 mM mannitol). Seven lines behaved like the wild type, on the remaining three, namely glucan, water dikinase 2 (*gwd2*), sucrose synthase 1 (*sus1*) and Δ^1-pyrroline-5-carboxylate synthetase 1 (*p5cs1*), further analyses were conducted.

The exposure to osmotic stress increased Pro concentration in all genotypes but *pc5s1*. However, in *gwd2* Pro increased less than in wild type plants between 4.5 and 6.5 days after treatment (DAT), and in *sus1* Pro increased 1.7-fold less than in wild type plants at 4.5 DAT but not at 6.5 DAT. These results underpin a positive correlation between GWD2, SUS1 and Pro biosynthesis in osmotic stress, with SUS1 being transiently involved in early metabolic responses. Oxidative damage (TBA assay) was 2-fold higher in *gwd2* at both 4.5 and 6.5 DAT but only at 4.5 DAT in *sus1*, suggesting a negative correlation between Pro accumulation and oxidative damage under osmotic stress. However, no similar correlation under osmotic stress was observed in *pc5s1* mutants in which the level of oxidative damage was similar to wild type plants in spite of the strong decrease in Pro concentration.

Under the same stress conditions, the total amount of soluble sugars (anthrone assay) and the fraction of reducing soluble sugars (dinitrosalicylic acid assay) were measured. Total soluble sugars were lower in all mutants, including *p5cs1*, in respect to wild type. Reducing soluble sugars were lower in *gwd2* and *sus1* but not in *p5cs1*. None of these mutants were impaired in starch accumulation.

Altogether, our work suggests that GWD2 and SUS1 could be involved in drought-induced Pro biosynthesis providing the carbon skeletons required for this metabolism. Defining a more precise role of these enzymes in the metabolic response of Arabidopsis plants to drought stress will be the subject of further studies.