

Changes in GABA shunt metabolites in calmodulin mutants of *Arabidopsis thaliana* under abiotic stresses

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Calmodulin (CaM) binds to glutamate decarboxylase (GAD) resulting in its activation and the synthesis of GABA in plants during episodes of environmental stress. Glutamate and alanine, other amino acids related to the GABA shunt pathway, also accumulate in plants given environmental stresses. Changes in the steady-state levels of these 3 amino acids related to GABA shunt metabolism were investigated in seven *cam* mutants and in wild-type *Arabidopsis thaliana* (Col-0). The levels of these metabolites were investigated in root and shoot tissues of plants administered cold, salt, osmotic, UV-A, UV-B, and oxidative stresses resulting from either Paraquat or H₂O₂. In root tissues of non-stressed controls, glutamate, GABA, and alanine levels are relatively unchanged in all of the *cam* mutants compared to wild type. However, in shoot tissues of unstressed plants, glutamate and alanine levels were 1.5 to 3-fold higher in plants bearing mutations of *CAM1*, *CAM4*, *CAM5*, *CAM6*, and *CAM7*, while *cam2-3* and *cam3-2* showed nearly the same levels of glutamate or alanine as controls. GABA levels in shoots of unstressed plants were 1.5 to 2-fold higher in plants bearing *CAM2*, *CAM3*, *CAM6*, and *CAM7* mutations, while GABA levels were lower than wild type levels in plants bearing mutations in *CAM1*, *CAM4*, and *CAM5*. For all of the stresses, glutamate levels increased with time or intensity of the stress typically rising by approximately 5 to 10-fold compared to unstressed plants (depending on the *cam* mutant) in root or shoot tissues regardless of the specific stress. The tissue concentrations of glutamate accumulated in roots were higher than that in shoots for nearly all stress and mutant combinations. As expected, some *cam* mutations caused glutamate to rise to levels substantially above control levels in some stress combinations, but there was no consistent *cam* mutant associated with this observation in all stresses. GABA levels also changed in response to various stress treatments. In general, GABA levels increased by approximately the same fold as glutamate levels during stress, but the tissue concentrations of GABA achieved in maximal treatments were typically 200 to 500-fold lower than for glutamate. In some *cam* mutant/stress combinations GABA levels fell as hypothesized, but surprisingly in other treatment combinations GABA levels increased in some of the *cam* mutants. Alanine levels increased by 1.1 to 5-fold in both roots and shoots depending on tissue and stress combination. However, the tissue levels of alanine accumulated were in general 1/5 the concentrations of glutamate and 10 to 20 times the levels of GABA accumulated. Taken together the above data suggest that no single *cam* mutant is specifically associated with GAD in either root or shoot tissue, and that the effects of *CAM* paralogs on GABA shunt metabolism are more complex than simply the interaction with GAD likely involving effects on glutamate accumulation and the conversion of glutamate to alanine independently of GAD and the GABA shunt. . These preliminary mutant characterizations await complementation experiments to confirm causality between phenotype and gene function.