Involvement of Calmodulin in GABA Shunt Regulation and Thermotolerance in *Arabidopsis thaliana* under Heat Stress

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Plant cells accumulate glutamate, GABA, and alanine in response to stressful high temperatures. GABA is synthesized from glutamate in a decarboxylation reaction catalyzed by glutamate decarboxylase (GAD). GAD activity *in situ* is thought to be modulated by the binding of calcium-activated calmodulin (CAM) to GAD. GABA aminotransferase, the second enzyme of the GABA shunt, leads to the conversion of GABA and pyruvate into succinate semialdehyde and alanine in plants. Thus, glutamate, GABA, and alanine are all GABA shunt-related amino acid metabolites, and the levels of these amino acids may be regulated by calcium and CaM-dependent activation of GAD. We have investigated this hypothesis by examining the heat-stress-tolerance/sensitivity phenotype and GABA shunt metabolite levels in plants of seven *CAM* mutants compared to wild type *Arabidopsis thaliana* (Col-0). Seed germination, seedling survival, and production of reactive oxygen species under heat stress at 42^{o}C were examined. Mutations in *CAM5* and *CAM6* resulted in significant defects in seed germination and seedling survival, and increased levels of malonaldehyde accumulation, an indicator of reactive oxygen species (ROS) production, during heat stress. After 2 hr exposure at 42^{o}C, *cam2-3* and *cam5-4* showed reduced levels of GABA in roots. Lower levels of shoot GABA were observed in all the *CAM* mutants examined relative to wild type. *cam5-4* seedlings showed increased glutamate and alanine in roots and increased glutamate in shoots. In *cam2-3*, levels of glutamate and alanine in roots were reduced. After 3 hr at 42^{o}C the level of GABA increased substantially in both roots and shoots of *cam5-4*, and only in shoot of *cam2-3*. GABA levels were lowest in shoots bearing *CAM1* mutations. After 3 hr at 42^{o}C, *cam2-3* and *cam5-4* seedlings had high levels of alanine and glutamate in both roots and shoots. Our results suggest an important role for *CAM5* in thermotolerance of seedlings and the GABA shunt pathway. Although *cam6-1* had significant phenotypic defects in survival, germination, and accumulation of reactive oxygen species, no significant metabolite level changes were observed. This result suggests that phenotypic defects of *cam6-1* may be mediated through mechanisms other than the GABA shunt. Lack of phenotypic differences in *cam2-3* despite the apparent alteration of the GABA shunt regulation suggests that there is not a causal relationship between GABA shunt metabolism and the observed phenotypic differences in heat-stress tolerance/sensitivity. These preliminary mutant characterizations await complementation experiments to confirm causality between phenotype and gene function.