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Metabolism of γ -aminobutyric acid during cold acclimation and freezing and its relationship to frost tolerance in barley and wheat

E. Mazzucotelli*, A. Tartari**, D. Guerra***, L. Cattivelli*** and G. Forlani**

*CRA- Centre for Cereal Research, S.S 16 Km 675, Foggia (FG) 71100, Italy

**Department of Biology, University of Ferrara, via L. Borsari 46, 44100 Ferrara (FE), Italy

***CRA- Centre for Genomic Research, Via S. Protaso 302, 29017 Fiorenzuola d'Arda (PC), Italy

SUMMARY – We studied the possible involvement of the GABA shunt in the plant response to low temperatures by monitoring GABA levels in barley and wheat seedlings during cold acclimation and freezing response. In frost-resistant barley seedlings, cold acclimation caused a significant increase in amino acid pools, and induced the expression of the GABA shunt genes. As a consequence, GABA was found accumulated to a higher extent during the subsequent exposure to freezing temperatures. A different picture was obtained with a frost-sensitive genotype, in which GABA accumulation occurred during the stress as well, but the activation of the GABA shunt seemed not to take place, and the substrate glutamate was almost depleted. Analogous results were found in frost-resistant and frost-sensitive wheat cultivars. Feeding non-hardened plants with exogenous glutamate resulted in increased GABA accumulation under low temperature. The overall evidences suggest that GAD activity might contribute to frost tolerance in acclimated plants.

Introduction

The γ -aminobutyric acid (GABA) is a non-protein four-carbon amino acid, synthesized by a glutamate decarboxylase (GAD), a Ca^{++} -calmodulin dependent enzyme localized in the cytosol (Snedden *et al.*, 1997). Following irreversible glutamate decarboxylation, GABA may be further metabolized to succinic acid in the so-called GABA shunt, a short pathway bypassing two steps in the Krebs cycle. The GABA shunt (Fig. 1) takes place in the mitochondrion by means of two enzymes, a GABA transaminase (GABA-T) using either α -ketoglutarate or pyruvate as amino acceptor, and a succinic semialdehyde dehydrogenase (SSADH) (Bouchè and Fromm, 2004). Although the GABA shunt is widely distributed in most prokaryotes and eukaryotes, only in animals a major role for GABA is well established as the predominant inhibitory neuro-transmitter of the brain. In plants during the last decade some experimental evidences have been reported suggesting a possible involvement of GABA in several physiological processes, including stress responses (Bouchè and Fromm, 2004).

GABA intracellular levels are typically low, but largely and rapidly increase in response to abiotic stresses, such as hypoxia, drought, cold, heat shock and mechanical stimulation (Shelp *et al.*, 1999). The GAD response to Ca^{++} influx elicited by stress could be cause of this GABA increase. In this context, given also some peculiar properties like zwitterionic form at neutral pH and high solubility, a role of GABA as a compatible osmolyte has been hypothesized (Shelp *et al.*, 1999). The finding that proline transporters (e.g. AtProT2 and LeProT1) are able to recognize GABA as a substrate (Grallath *et al.*, 2005) strengthen its role as osmoprotectant. Notwithstanding, since GAD activity is low pH-dependent and consumes H^+ , GABA synthesis might function as a pH-stat, being activated under conditions that cause cytosolic acidification (Crawford *et al.*, 1994).

GABA might function as an endogenous signalling molecule as well, since there are indications that glutamate/GABA receptors do exist also in plants (Kang and Turano, 2003). GABA levels were shown to increase also under long term conditions that limit glutamine production, reduce protein synthesis or enhance protein degradation, thus GABA might be a temporary nitrogen storage, and play a role as a sensor of nitrogen status and C:N balance, or a long distance inter-organ signal molecule (Beuve *et al.*, 2004).

The role of GABA metabolism in plant cell life has been elucidated also by studies aimed to characterise the two other GABA shunt enzymes. *Arabidopsis pollen–pistil interaction2* mutant, which

lacks a functional *GABA-T*, showed growth inhibition and misguidance of pollen tubes in pistils, suggesting that a gradient of GABA concentration is essential for the growth and guidance of pollen tubes (Palanivelu *et al.*, 2003). The disruption of the unique *SSADH* gene in *Arabidopsis* resulted in plants undergoing necrotic cell death caused by an abnormal accumulation of reactive oxygen species (ROS). Such a behaviour might rely upon the ability of the GABA shunt to supply NADH and/or succinate to mitochondrial metabolism under conditions that inhibit the TCA cycle, impair respiration and enhance the production of ROS (Bouché *et al.*, 2003). A novel evidence for an intriguing link between the GABA shunt, its byproduct γ -hydroxybutyric acid (raising from the activity of a succinic semialdehyde reductase) and ROS was also provided recently (Fait *et al.*, 2005). However, in most cases GABA accumulation can be simply associated with the cited physiological processes, and the speculated links are far to be proved definitely. Overall, the exact role of the GABA shunt in plants still awaits elucidation.

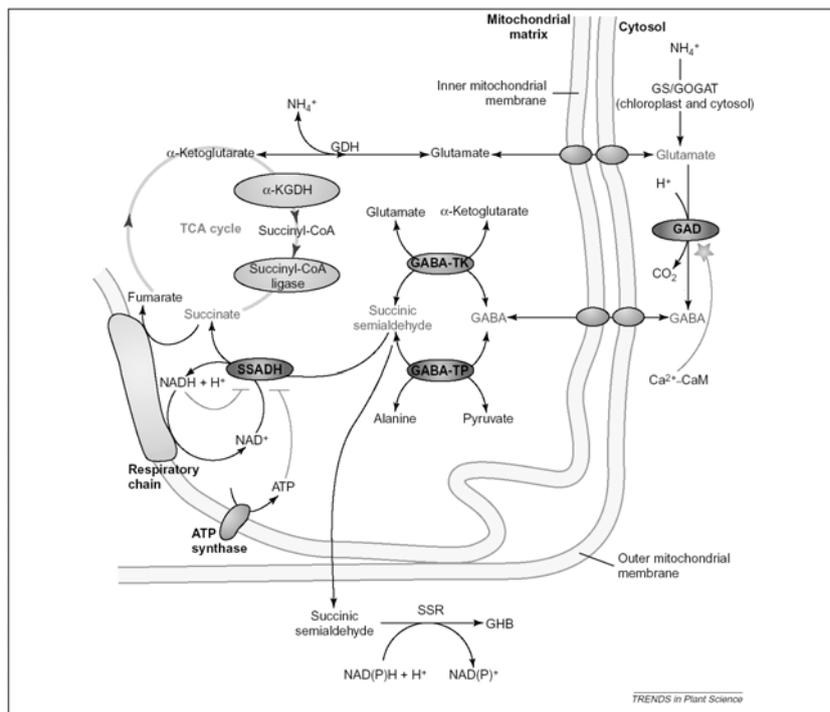


Fig. 1. The GABA shunt metabolic pathway and its regulation in plants (from Bouché and Fromm, 2004).

Summary of the work

We studied the possible involvement of the GABA shunt in the plant response to low temperatures by monitoring GABA levels in barley and wheat seedlings during cold acclimation and freezing response. In frost-resistant barley seedlings, cold acclimation caused a significant increase in amino acid pools, and induced the expression of the GABA shunt genes. As a consequence, GABA was found accumulated to a higher extent during the subsequent exposure to freezing temperatures. A different picture was obtained with a frost-sensitive genotype, in which GABA accumulation occurred during the stress as well, but the activation of the GABA shunt seemed not to take place, and the substrate glutamate was almost depleted. Analogous results were found in frost-resistant and frost-sensitive wheat cultivars. Feeding non-hardened plants with exogenous glutamate resulted in increased GABA accumulation under low temperature. The overall evidences suggest that GAD activity might contribute to frost tolerance in acclimated plants.

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