eXtra Botany

Insight

GABA transport: beyond stress? A closer look at AtGAT2

Reuben Tayengwa*

Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742, USA

* Correspondence: reubent@umd.edu

This article comments on:

The amino acid gamma-aminobutyric acid (GABA) is a non-proteinogenic metabolite, distinguishable from the amino acids used for protein synthesis. Many living organisms contain GABA, including vertebrate animals, plants, bacteria, and insects. GABA was first discovered in potato tubers in 1949 (Steward *et al.*, 1949) and its role as a plant metabolite has been investigated ever since. A new study by Meier *et al.* (2024) sheds light on the function of the GABA transporter AtGAT2 in Arabidopsis.

GABA is a plant stress-response compound that accumulates rapidly under adverse conditions (Kinnersley and Turano, 2000; Bai *et al.*, 2019; Tarkowski *et al.*, 2020). GABA levels increase in response to insect and pathogen attacks, extreme weather, hypoxia, and wounding (Shelp *et al.*, 1999, 2021; Kinnersley and Turano, 2000; Scholz *et al.*, 2015; Sita and Kumar, 2020; Hayat *et al.*, 2023). GABA is found in virtually all plant tissues and organs, including embryos, cotyledons, leaves, shoots, roots, nodules, xylem and phloem, flowers, seeds, fruits, and various cellular compartments, namely the cytosol, mitochondria, vacuole, plastids, cell wall, and apoplast (Kinnersley and Turano, 2000; Hijaz and Killiny, 2020). The specific distribution of GABA within tissues and organelles varies depending on the plant species and developmental stage, and the environmental conditions (Ramesh *et al.*, 2017). GABA also functions as a primary metabolite in both nitrogen and carbon metabolism (Shelp *et al.*, 1999; Weigelt *et al.*, 2008; Fait *et al.*, 2011; Batushansky *et al.*, 2015). *Arabidopsis thaliana* plants can grow on media containing GABA as the sole nitrogen source, providing early evidence of the existence of GABA transporters (Breitkreuz *et al.*, 1999).

Recent studies have shown that GABA also acts as a signaling molecule in some plant development processes and appears to be transported across cellular membranes to ensure its proper and timely distribution (Kinnersley and Turano, 2000; Bouché *et al.*, 2003; Mekonnen *et al.*, 2016; Du *et al.*, 2020). The current evidence supports a common ancestry of plant and animal GABA signaling, but documentation of an analogous function in plants to its well-studied role as a mammalian anti-excitatory neurotransmitter is lacking. A gene-edited GABA-enriched tomato, now sold to consumers in Japan, contains elevated GABA levels, which are thought to promote relaxation and help lower blood pressure (Waltz, 2021). Better understanding of GABA metabolic compartmentalization and identification of GABA-regulated ion channels in plants is a current goal of GABA signaling research.

Characterizing AtGAT2: a voltage-dependent low-affinity GABA transporter

Two low-affinity GABA transporters, amino acid permease 3 (AAP3) and proline transporters 2 (ProT2), were identified in plants via heterologous complementation in yeast (Breitkreuz *et al.*, 1999). However, given that these transporters have higher affinities for other substrates, such as neutral amino acids and glutamate (AtAAP3) or glycine betaine and proline (AtProTs), it is not clear how significant their roles in GABA transport is *in planta*.

Aluminum-activated malate transporters (ALMTs), although primarily known for malate transport, are modulated by GABA and may play a role in GABA efflux (Žárský, 2015). AtGAT1, a plasma membrane-located high-affinity GABA influx transporter, is one of a two-member gene family that also includes At5g41800 (*AtGAT2*) (Meyer *et al.*, 2006). However, the transport mechanisms underlying GABA accumulation and its

broader physiological roles beyond stress remain poorly understood. As part of previously published work from the Rentsch group characterizing AtGAT1, Meyer *et al.* (2006) showed that AtGAT2 did not complement GABA transport deficiency in a GABA- and proline transport-deficient *Saccharomyces cerevisiae* mutant (strain 22574d), and the authors therefore could not proceed with functional studies.

In their latest work, the group performed two-electrode voltage clamp (TEVC) experiments (an electrophysiology technique used to study the properties of membrane transporters) using unfertilized eggs from an African clawed frog (*Xenopus laevis*) as a heterologous model system to study the transport properties of AtGAT2 (Meier *et al.*, 2024). To study how GABA moves across the cell membrane, the authors expressed AtGAT2 in oocytes and measured the electrical currents when a membrane potential between −110 mV and −30 mV was applied. The highest affinity of AtGAT2 for GABA was found at the most negative membrane potential (−110 mV, pH 5.5).

The analysis confirmed that AtGAT2 is a low-affinity GABA transporter. In addition, although GABA is its primary substrate, AtGAT2 also transports butylamine, L-alanine, glutamate, proline, and choline. Unlike AtGAT1, AtGAT2 showed strong voltage dependence, with higher affinity and maximal transport rates observed at strongly negative membrane potentials typical of plant cell membranes. The reliance on voltage suggests the crucial role of AtGAT2 in maintaining GABA homeostasis under stress conditions when membrane potentials fluctuate.

To confirm the GABA transport activity of AtGAT2 that was detected through currents in TEVC experiments, the authors measured the uptake of 50 mM [^{3}H]-GABA in oocytes expressing AtGAT2 compared with uptake into oocytes without AtGAT2 expression. After 30 min, AtGAT2-expressing oocytes contained 7.5 times more GABA than control oocytes. At all time points, AtGAT2-expressing oocytes had higher GABA concentrations. These results confirm that the currents in the TEVC experiments indeed correlate with GABA uptake.

AtGAT2 expression and localization, and the enigma of its physiological role

The expression of AtGAT2 in vascular tissues, developing pollen, and young seeds implies its involvement in

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Box 1. Comparative analysis of Arabidopsis GABA transporters AtGAT1 and AtGAT2

Summary of the key differences between the two major GABA transporters in *Arabidopsis thaliana*, AtGAT1 and AtGAT2. The characteristics compared comprise affinity for GABA, transport properties, ability to complement GABA uptake deficiency in yeast mutants, functional expression in *Xenopus* oocytes, tissue localization, subcellular localization, and proposed physiological functions. This comparison highlights the distinct roles of these transporters in GABA transport and signaling within the plant. This distinction hints at the possibility of different yet complementary roles for these transporters in managing GABA levels under varying conditions in various tissues. Despite the apparent differences between AtGAT1 and AtGAT2, surprisingly, there were no phenotypic differences in *AtGAT2* overexpression or *gat1, gat2, gat1 gat2* knockout lines compared with wild-type plants, even under varying GABA concentrations or stress conditions. This result suggests that further studies on the interaction between AtGAT1, AtGAT2, and other GABA transporters are needed.

Feature	AtGAT1[1]	AtGAT2[2]
Affinity for GABA	High ($K_{0.5}$ ~40–50 μM)	Low ($K_{0.5}$ ~8 mM at −110 mV, pH 5.5)
Transport properties	Voltage independent (in the range of −10 mV to −90 mV)	Voltage dependent, higher affinity and transport rate at more negative membrane potentials
Yeast expression	Complements GABA transport deficiency in yeast mutant (strain 22574d)	Unable to complement GABA transport deficiency in yeast mutant
Xenopus oocyte expression	Functional expression, induces inward currents upon GABA addition	Functional expression, induces inward currents upon GABA addition
Tissue localization	High expression in flowers	Expression in vascular tissues, developing pollen, young seeds, nectaries
Subcellular localization	Plasma membrane	Plasma membrane, endoplasmic reticulum
Physiological function	May play a role in GABA signaling events, possibly by removing GABA from the apoplast; GABA transport during wounding and senescence	Potential role in long-distance GABA transport, nutrient loading of sink organs, and stress adaptation

[1]Meyer *et al*. (2006).
[2]Meier *et al*. (2024).

long-distance GABA transport, nutrient loading of sink organs, and the nourishment of developing tissues, expanding our understanding of GABA transport beyond stress responses. This hypothesis aligns with the known role of GABA in plant reproduction and stress tolerance (Ramesh *et al.*, 2015; Priya *et al.*, 2019). AtGAT2 is located in the plasma membrane and internal membranes, and therefore, a function in internal membranes cannot be excluded. Meier *et al.* (2024) show that *AtGAT2* expression is up-regulated during osmotic stress and low water potential but remains unchanged under cold stress and wounding, suggesting its potential role in stress adaptation. However, the physiological significance of AtGAT2 is not clear. Surprisingly, the authors found that no significant phenotypic differences existed between *AtGAT2*-overexpressing or knockout lines and wild-type plants, even under varying GABA concentrations or stress conditions. This observation raises intriguing questions about the functional redundancy of the GABA transport system and compensatory mechanisms. Could the role of AtGAT2 be more subtle or redundant with other transporters?

The study's findings challenge us to reconsider the conventional view of GABA transporters as mere facilitators of GABA movement. The voltage dependence of AtGAT2 hints at a dynamic regulatory role, potentially fine-tuning GABA levels in response to environmental cues. In addition, the fact that AtGAT1 and AtGAT2 are, respectively, high-affinity and low-affinity GABA transporters further supports the dynamic regulatory roles of these transporters (Box 1). We can speculate that high- and low-affinity transporters allow plants to efficiently take up GABA across a wide range of concentrations. Since different plant tissues or developmental stages may contain varying GABA concentrations, high-affinity transporters may function where GABA concentrations are low. In contrast, the low-affinity transporter could function in places of GABA abundance. This discovery prompts us to delve deeper into the molecular mechanisms underlying GABA transport and to explore its broader implications in plant biology. This work provides a detailed characterization of AtGAT2 and challenges the prevailing view that GABA transporters are primarily involved in stress responses. Furthermore, understanding the tissue-specific expression of *AtGAT2* could pave the way for targeted genetic engineering strategies to enhance stress tolerance and improve crop yields in the face of climate change. This study opens exciting avenues for future research. The interaction between AtGAT2 and other GABA transporters, such as AtGAT1 and ALMTs, needs further investigation. Unraveling the regulatory mechanisms controlling the expression and activity of these transporters will deepen our understanding of the intricate GABA transport network in plants.

Conflict of interest

No conflict of interest is declared.

Data availability

No new data were generated or analyzed in support of this research.

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