**Octa Journal of Biosciences**
International peer-reviewed journal
**Octa J. Biosci. Vol. 5(2):52-55**

**ISSN 2321 – 3663**
**July-Dec 2017**


Octa Journal of Biosciences

journal homepage: www.sciencebeingjournal.com

Scientific Planet Society
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Review Article

# Aluminum-activated malate transporter protein (ALMT): "*Prime GABA receptor*" in plants under abiotic stress: A review

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ARTICLE INFO

Received 10 July 2017
Revised 12 Oct 2017
Accepted 30 Nov 2017
Available online 16 Dec 2017

**Keywords:** Gamma-aminobutyric acid (GABA), glutamate decarboxylase (GAD), aluminum-activated malate transporter (ALMT), stress

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ABSTRACT

GABA play dual role in plants. In response to stress, H+ and Ca2+ concentrations have been changed. It activates glutamate decarboxylase (GAD). GAD catalyzed the decarboxylation of glutamate to GABA or inhibition of GABA transaminase (GABA-T). In plant GADs contains calmodulin-binding domains (CaM-BDs), having 22 to 25 additional amino acids at the C-terminal end, helps in CaM binding in the presence of Ca2+. TaALMT1 gene (formerly named ALMT1) in plants regulated aluminum-activated malate transporter (ALMT) channel of GABA under stress condition (similar to mammalian anionic channels-GABAA receptors). It alters the electrical potential across membranes. ALMTs under lie a variety of processes, including metal toxicity avoidance, mineral nutrition, ion homeostasis, turgor regulation, fruit quality, and guard cell function.

## INTRODUCTION

During the last decade, our knowledge on the importance of metabolic adjustments to unfavorable growth conditions has increased considerably. Natural stress tolerance is a very complex process involving numerous metabolites and metabolic pathways. Analyses of metabolic adjustments of plants with different levels of stress tolerance and transgenic approaches provide important complementing evidence for better understanding the role of different metabolites in adjusting to harsh environments. Plants have evolved a myriad of responses to alleviate stressful conditions. In the series of protection, there are some amino acids which work as primary metabolites, and serve as osmolytes as well as precursors for synthesis of proteins and secondary metabolites for stress defense in plants. A neurotransmitter in mammals 'Gamma-aminobutyric acid (GABA)', a four carbon non-protein amino acid and a major component of the free amino acid pool, recently found in plants. It sends some signals or generates electrical signals/ potential differences across membranes via altered cycling of tricarboxylic acid (TCA) intermediates in plants under stress/threat condition similar to mammals. It induces accumulation of both carbohydrates and amino acids against stress tolerance. Out of the total 10 identified amino acid it has been now clear that application of GABA increases the level of 7 amino acids (GABA, glutamic acid, aspartic acid, threonine, serine, alanine, and valine) under plant stress conditions. GABA works as an endogenous signaling molecule. It is an important C:N metabolite, associated with carbon–nitrogen balance, regulate the cytosolic pH, protect the plant against oxidative and biotic stress developed salt tolerance, act as ROS scavenger (Bouche and Fromm, 2004; Song et al., 2010; Liu et al., 2011) and the significant source of succinate for the TCA cycle.

GABA also acts as a plant signal by way of multiple strands of evidence (Kinnersley and Lin, 2000; Michaeli and Fromm, 2015) (i) GABA concentration increases rapidly in plant tissues on any of a multitude of abiotic (anoxia, heat, cold, salt, drought, mechanical) or biotic (herbivory, pathogens, viruses) stresses (Kinnersley and Turano, 2000; Cholewa et al., 1997; Shelp et al., 2012), (ii) plants contain glutamate receptor-like proteins (GLRs) that are involved in regulation of growth, wound signaling, and plant defense signaling (Mihard et al., 2011; Mousavi et al., 2013; Forde, 2014), (iii) pollen tube growth to the ovary is directed by a GABA gradient (Palanivelu et al., 2003; Renault et al, 2011), and (iv) the presence of GABA-binding sites on plant membranes (Liang et al., 2006), (v) Instead, it was recently found that GABA regulated aluminum-activated malate transporter (ALMT) channel activity TaALMT1 gene (formerly named ALMT1), of ALMT family (Sasaki et al., 2004), which regulates plant growth under stress (Ramesh et al., 2015). In this review, we studied the plant GABA receptor in terms of their molecular identity, predicted topology, mode of action, and their roles in signaling pathway. Still role of GABA as stress signaling molecules on differential expression of genes and proteins controlling the metabolic pathways remains to be unknown for future research.

### Structure, Shunting pathway and role of GABA in Plants

Role of GABA's has been well-described as a neurotransmitter in mammals. Recently it was found that plants also send some electrical signals/ or developed potential difference across the membrane under biotic and abiotic stress condition. Under such condition TCA (tricarboxyalic acid cycle) intermediates altered their path way and regulate the plant growth under stress. GABA a four carbon non-protein amino acid is a major component of the free amino acid pool. GABA has an amino group on the γ-carbon rather on the α-carbon. It is highly soluble in water. Structurally it is a flexible molecule. Salinity stress and elevated GABA levels overlapped with the induction of glutamate decarboxylase 4 (GAD4) expression in Arabidopsis thaliana (L.) [Heynh. ecotype Col-0]. In-silico and microarray analysis revealed the over-representation of binding sites for WRKY and MYB transcription factors in the GAD4 promoter, as well as their co-expression with GAD4 resulted that GAD4 expression is associated with the inducible co-expression of WRKY28, WRKY30, WRKY40, MYB2, MYB15, MYB108, calmodulin-37 and aluminum-activated malate transporter-2, suggesting the involvement of such genes in regulation, protein activation, and anion transport in GABA accumulation. Salinity stress suggests that the GABA accumulation could involve post-translational activation of pre-existing GAD1 and GAD2 by elevated cytosolic calmodulin, as well as induction of GAD4expression. Hence under the different adverse environmental conditions, two functional calcium/calmodulin modulated isoforms of GAD are known to be existed in plants. GAD1 (expressed in root tissues only) and GAD2 (expressed in roots, leaves, inflorescence stems, and flowers). GAD expression has been shown in tissues that accumulate GABA (Fig.1). In the process of metabolisation of GABA three enzymes (cytosolic enzyme glutamate decarboxylase (GAD), mitochondrial enzymes GABA transaminase (GABA-T) and succinic-semi-aldehyde dehydrogenase (SSADH) plays a very important role. The GABA shunt has since been associated with various physiological responses, including the regulation of cytosolic pH, carbon fluxes into the TCA cycle, nitrogen metabolism, protection against oxidative stress, osmo regulation and signaling. Recent developments in arabidopsis (*Arabidopsis thaliana*) functional genomics suggest that it acts as a signal molecule and shown their role with phytohormones such as abscisic acid and ethylene (Lancien and Roberts, 2006). Further there is clear evidence that GABA accumulation plays vital role in stomatal closure (Mekonnen et al., 2016) and that ALMT membrane channels are present in guard cells (Meyer et al., 2010). During drought it caused stomatal closure via accumulation of abscisic acid (ABA). ABA stimulates Ca2+ influx and Ca2+/calmodulin activated GAD, which resulted GABA synthesis (Shelp et al., 2012) (Fig. 2).

### Plant stress perception, recognition and cytoplasmic signaling of GABA

Plant stress perception detected by the membrane-bound protein, which triggered the special kinds of protein which stimulates production of signaling molecules that can turn on/off genes that enable the plant to better cope with the deleterious effects of the stress. In response to stress H+ and Ca2+ concentrations have been changed, which activates glutamate decarboxylase (GAD). GAD catalyzed the decarboxylation of glutamate to GABA, or inhibition of GABA transaminase (GABA-T), which converts GABA to succinic semi-aldehyde. This may induce expression of specific metabolism and signaling-related genes while repressing others associated with developmental processes (Michaeli and Fromm, 2015). Acidic pH also stimulates the GAD activity. Shukuya and Schwert (1960) observed that low pH with maximal activity at GAD is activated in E. coli. Anoxia conditions (e.g. flooding) also caused cytosolic acidosis (Roberts et al., 1984; Aurisano et al., 1995A). It increases the accumulation of GABA. Plant vacuoles act as reservoirs for organic acids with a pH of 3.5 to 5.5 (Galston et al., 1980). Mechanical damage that ruptures vacuolar membranes will release organic acids into the cytosol, increasing cytosolic acidification and the likelihood of GAD activation.

## Role of specific proteins in signaling pathway

Recent studies have supported the hypothesis that GAD and GABA may be part of a signal transduction pathway occurring in plants after exposure to stress. It has been found out that plant GADs contains calmodulin-binding domains (CaM-BDs), having 22 to 25 additional amino acids at the C-terminal end, when compared with the other amino acid sequences of GADs from other kingdoms. These domains have been shown to be sufficient for the binding of CaM in the presence of Ca2+ (Arazi et al., 1995; Zik et al., 1998). There are some other groups of protein i.e. calcium dependent kinases (CDPK) that are activated only by calcium and much less well known group of calcium activated proteins 'calcineurin-B (AtCBL)' found in Arabidopsis (Kudla et al., 1999). These CDPK contains calcium binding domains and kinase domain, which could detect changes in calcium level, which regulates further protein kinase activity. Romeis et al. (Romeis et al. 2000) discovered 68-70 KD-CDPK in tomato, which was involved in defensive property of plant. Much research into role of CDPKs in CAM-BDs are still being remain and going to be investigated. Similar to CDPK AtCBL have shown strong similarity to the calcineurin-B subunit, which is similar to neuronal calcium sensory system of animals (Shi et al., 1999). There are strong possibilities that it play very important role in signal transduction in up-regulation of genes under abiotic stress condition.

## Regulation of Aluminum-activated malate transporter (ALMT) membrane channel by GABA

While examining the effect of combining stresses that can modulate plant growth individually, it were observed that acidosis, trivalent aluminium ions (Al3+) and GABA accumulation interplay an unexpected role. This finding has led us to the identification of aluminium-activated malate transporters (ALMT) as key transducers of GABA signaling in plants. GABA-mediated regulation of ALMT proteins underlies a novel signaling pathway that has the potential to translate changes in the concentration of this plant stress metabolite into physiological outputs throughout the plant. It is also interesting to note that compounds derived from plants (bicuculline) or mycorrhizal fungi (musicmol), that are known to regulate mammalian GABA receptors, can affect ion transport in plants, and as a resulted plant growth. In response to biotic and abiotic stress the non-protein amino acid, gamma-aminobutyric acid (GABA) rapidly accumulates in plant tissues, and regulates plant growth. GABA exerts its effects in plants by negatively regulating anion flux through plant aluminium-activated malate transporter (ALMT) proteins, which is activated by anions (Al+3). The aluminium activated malate transporter (ALMT) gene family is named after the first member of the family identified in wheat (Triticum aestivum L.) (Sakaki et al., 2004). The product of this gene controls resistance to aluminium (Al) toxicity. TaALMT1 was the first member of this novel family of genes encoding anion channels.

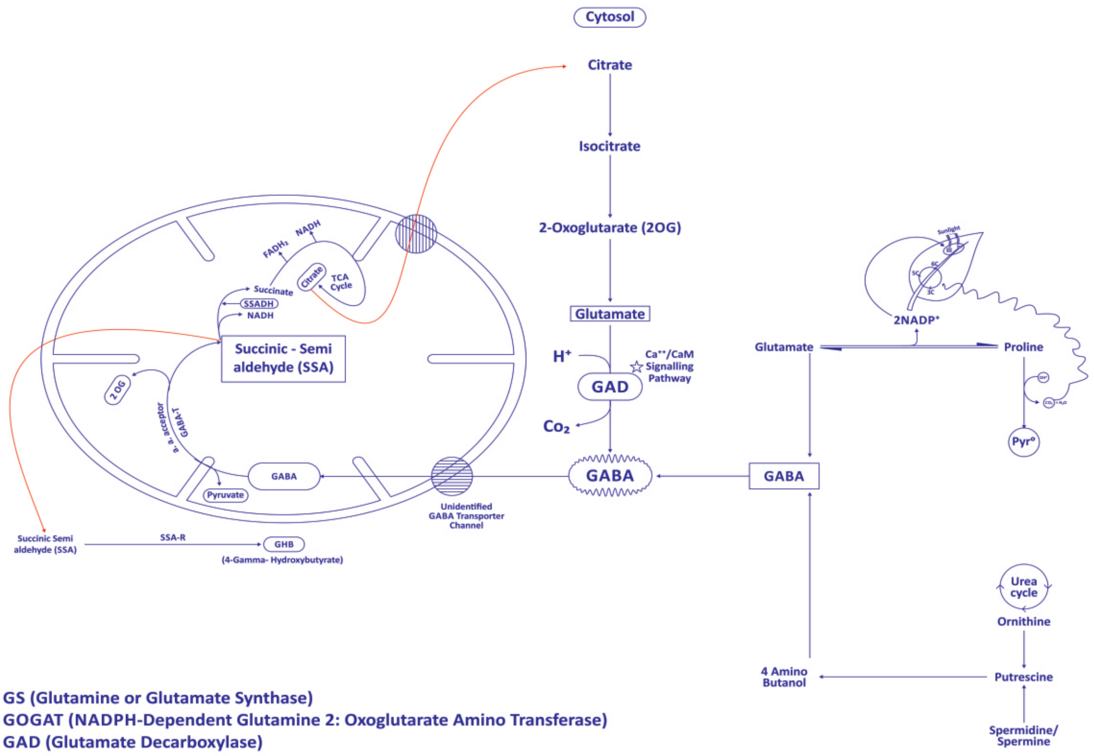


**Fig.1 Regulation and shunting pathway of $\gamma$-amino butyric acid (GABA) in Plants**

Until recently it was not known whether this occurs by regulation of carbon metabolism or via an unidentified signaling pathway. GABA modulation of ALMT activity results in altered root growth and tolerance to alkaline and acid pH and aluminium ions. Further site directed mutagenesis of residues within ALMT proteins abolishes GABA efficacy but does not alter other transport properties. Therefore GABA exerts multiple physiological effects via the ALMT proteins including regulation of root and pollen tube growth and GABA can be considered a signaling molecule in both plants similar to animal kingdoms. This implicates that ALMT protein act as prime 'plant GABA receptor', similar to mammalian anionic channels-GABAA receptors. Both of them alter the electrical potential across membranes (Table 1). ALMTs share little homology with mammalian GABAA receptors; the only region of similarity so far identified is a domain of 12 amino residues containing a phenylalanine or tyrosine required for GABA sensitivity. First of all Al tolerance-related studies led to the identification of AtALMT1 in which 14 Arabidopsis ALMT members to be functionally characterized, as well as the homologs BnALMT1 and BnALMT2 from rape, GmALMT1 in soybean, and ScALMT1 in rye. They all shared similar functional characteristics consistent with their involvement in mediating the organic acid exudation in Al-tolerance response in these plant species (Ligaba et al., 2006; Ligaba et al., 2007).

Likewise, MsALMT1 from Medicago sativa and HlALMT1 from the grass Holcus lanatus have been described as crucial genes involved in Al resistance (Table 2) (Chen et al., 2013; Chen et al., 2013a). Sub-sequent studies in relation to the role of the novel and emerging ALMT family in mediating Al-resistance responses led to the identification of the maize homolog ZmALMT1. However, although ZmALMT1 as shown to function as a plasma membrane transporter capable of mediating a selective anion efflux and influx, the gene expression data as well as bio-physical transport characteristics provided the first indication in the literature that the in planta function of some members of this ALMT family might extend beyond Al tolerance to a variety of physiological processes (Pineros et al., 2008b). Since then an increasing number of studies have clearly indicated that ALMTs under lie a variety of processes, including metal toxicity avoidance, mineral nutrition, ion homeostasis, turgor regulation, fruit quality, and guard cell function.

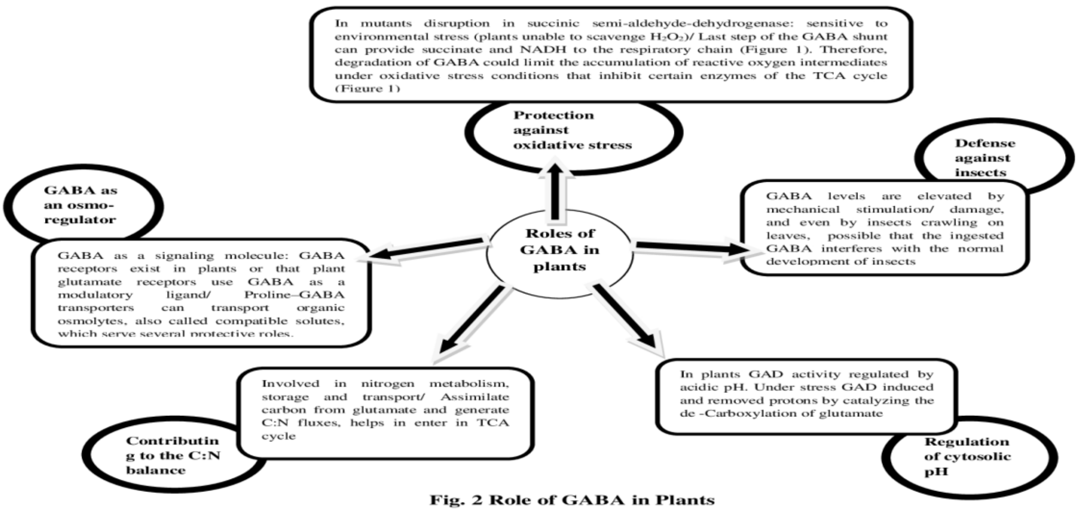


**Fig. 2 Role of GABA in Plants**

**Table 1:** Role of ALMT members in plants under stress.

| | |
|---|---|
| In plant: ALMTs are a multigenic protein family | • All are involved in multiple physiological processes. $Al^{3+}$ does not activate mo st ALMTs |
| Arabidopsis: 14 members | • ALMT members are mainly activated by anions, particularly malate. |
| Rice: 09 members | • Malate activation and GABA inhibition of ALMTs provide a mechanism for |
| Grapevine: 12 members | indicating changes in tricarboxylic acid (TCA) cycle activity via membrane signaling<br>• Activated ALMT involved i n a range of physiological processes including stomatal movement, pollen tube growth, nutrition, and grape, berry and tomato ripening etc. |

## GABA-Gated ALMT

Plants alter their internal GABA concentration in response to environmental changes within seconds. GABA follows daily rhythms or exhibit sustained increases over days (Allan and Shelp, 2006; Espinoza et al., 2010). A frequent immediate response to environmental stress is an increase in cytosolic Ca2+ concentration, which activates glutamate decarboxylase (GAD) resulting in GABA synthesis; this increase in GABA concentration is then capable of reducing ALMT activity. Candidate compounds include g-hydroxybutyrate (GHB), few amino acids and carboxylates, are also related to GABA metabolism (Ramesh et al., 2015; Shelp et al., 2012; Shelp et al., 2012). Plants could propagate long-distance electrical signals through ALMT in a GLRs (glutamate receptor-like proteins) fashion, through interaction with the signals of GABA and carboxylate (malate).

## CONCLUSION AND FUTURE RESEARCH

The characterization of the predicted GABA-binding motif in plants is still in its very early stage, and we can consider this a key research gaps. It remains to be shown: (1) whether GABA binds to the identified aromatic amino-acid residues in ALMT1; (2) what residues line the binding site and the pore; (3) the kinetics of GABA binding; (4) whether there is more than one region in the ALMT proteins involved in GABA-mediated regulation; (5) whether there are other metabolites, such as amino acids and compounds, related to GABA metabolism that are involved in regulation of ALMTs/ion channels; and (6) what the tertiary structure is of ALMTs. This review suggests that new insights into the GABA regulation of physiological, developmental, and growth processes in plants may rapidly occur in the near future.

## Disclosure statement

No potential conflict of interest was reported by the author.

**Financial and proprietary interest**: Nil

**Financial support:** Nil

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