The varied functions of aluminium-activated malate transporters—much more than aluminium resistance

Antony J. Palmer*,†‡, Alison Baker†‡ and Stephen P. Muench*,†§

*School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, LS2 9JT, U.K.
†Astbury Centre for Structural Molecular Biology, University of Leeds, LS2 9JT, U.K.
‡Centre for Plant Science, University of Leeds, LS2 9JT, U.K.
§School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, LS2 9JT, U.K.

Abstract

The ALMT (aluminium-activated malate transporter) family comprises a functionally diverse but structurally similar group of ion channels. They are found ubiquitously in plant species, expressed throughout different tissues, and located in either the plasma membrane or tonoplast. The first family member identified was TaALMT1, discovered in wheat root tips, which was found to be involved in aluminium resistance by means of malate exudation into the soil. However, since this discovery other family members have been shown to have many other functions such as roles in stomatal opening, general anionic homoeostasis, and in economically valuable traits such as fruit flavour. Recent evidence has also shown that ALMT proteins can act as key molecular actors in GABA (γ-aminobutyric acid) signalling, the first evidence that GABA can act as a signal transducer in plants.

Introduction

The aluminium-activated malate transporter (ALMT) family is found ubiquitously in sequenced genomes throughout the plant kingdom [1] and was named when the first member of the family to be discovered was found to be involved in aluminium resistance in wheat [2]—although proteins of this family are channels, rather than transporters as their name suggests. The first characterized homologue in Arabidopsis thaliana, AtALMT1 [3], was similarly found to be involved in Al-resistance. However, in addition to ALMT1, the gene family in this species contains 13 other members suggesting that they are involved in more than just Al-resistance, and some of these have already shown to have a wide range of other roles [4]. To date, the ALMT family has been shown to be central to physiological processes such as control of stomatal aperture [5,6] and anion homoeostasis [7]. Furthermore, increasing attention is being paid due to their potential role in economically valuable traits such as fruit flavour [8] and grain filling [9], and more recently they have been shown to have many other functions such as roles in stomatal opening, general anionic homoeostasis, and in economically valuable traits such as fruit flavour. Recent evidence has also shown that ALMT proteins can act as key molecular actors in GABA (γ-aminobutyric acid) signalling, the first evidence that GABA can act and thus, a comprehensive review of all currently known functions is a timely addition to the literature.

Aluminium resistance

Overcoming aluminium toxicity on acid soils

Acid soils are prevalent worldwide, comprising around half of all potentially arable land [12]. In these soils, aluminium ions become solubilized and damage crops via root growth inhibition [13] and, to compound the problem, nutrients such as phosphate become less available [14]. Several plant species have been identified as aluminium-resistant and they use a variety of mechanisms including thickening of cell walls [15], active transport of aluminium away from sensitive organs [16], or, prominently, organic acid exudation, chiefly either by release of malate or citrate [17,18]. Studies on crosses of near isogenic Al-resistant and Al-sensitive wheat (Triticum aestivum) cultivars alongside electrophysiological studies using Xenopus oocytes [19,20] first identified TaALMT1 as the channel responsible for malate exudation from root tips, providing the primary mechanism of aluminium resistance. TaALMT1 is constitutively expressed in the root apices of Al-resistant wheat and malate chelates Al³⁺ forming a 2:2 complex with the trivalent aluminium ions, thus encasing the ions and rendering them non-toxic [21]. This allows longer root growth and greater yields compared with a sensitive cultivar grown on acid soils. In addition to protection from Al³⁺ toxicity, malate extrusion has the benefit of increasing phosphate availability in the soil—since Al³⁺ binds and complexes phosphate [22]. This is
part of a host of processes activated in plants for improved phosphorous usage [14,23]. Heterologous expression of TaALMT1 in cultured tobacco cells, Xenopus oocytes, and transgenic rice plants has shown efflux of malate activated by the presence of Al$^{3+}$ and expression confers Al-resistance to tobacco cells [2]. Importantly, transgenic expression of TaALMT1 in Al-sensitive barley plants rendered them resistant to aluminium toxicity [24]. In one study, transgenic plants grown on acid soils displayed root growth similar to that seen in neutral soils and a doubling in yield when expressing just the single gene [23], making it a powerful tool for transgenic crop development. A thorough review of transgenic approaches using ALMT1 and other genes can be found in Ryan et al. [25].

Other ALMTs involved in aluminium resistance

Since the characterization of TaALMT1, several ALMTs from other species including oilseed rape, Arabidopsis, rye, soybean and Yorkshire fog have been shown to be vital for Al-resistance and characterized (see Figure 1 and Table 1). These channels are activated by Al$^{3+}$ and in some cases their expression is also up-regulated upon sensing Al$^{3+}$ [26].

AtALMT1 is a malate channel critical for aluminium resistance in Arabidopsis, expressed at the plasma membrane of the epidermal cells of the root tip [3]. This protein shows 41% sequence identity and 63% similarity to TaALMT1. However, in contrast, AtALMT1 is not constitutively expressed; instead, expression is up-regulated by aluminium [26,27], mediated by the transcription factors STOP1, STOP2 and WRKY, which also regulate other genes critical for tolerance of acid soils [28–30]. In addition, two genes with 95% sequence identity with one another were identified in oilseed rape (Brassica napus) [31,32], as well as GmALMT1 in soybean (Glycine max), and ScALMT1 in rye (Secale cereale) [33,34]. These genes are expressed at root tip plasma membranes, and the corresponding proteins are permeable to malate and are activated by aluminium, showing this mechanism for Al-resistance is widespread among

---

**Figure 1 | Phylogenetic tree of ALMTs with known functions plus family members from Arabidopsis with unknown function**

Numbers on nodes denote likelihood of correctness. Tree made using MEGA6 software [51], sequences were aligned by the inbuilt MUSCLE functionality, and constructed with the maximum likelihood method and 500 iterations of bootstrapping.

**Key**

+ PM-localised but not Al-activated
  ▲ Al-activated, root PM-localised.
  ▼ Involved in guard cell movements
  ◆ Tonoplast localised
  ? So far uncharacterised
  X Expressed at root PM but not Al-activated
  *Multiple known roles, PM-localised
Summary of known functions and localization of ALMTs from several species

| Gene       | Organism               | Localization                        | Al-activated? | Function                          |
|------------|------------------------|-------------------------------------|---------------|-----------------------------------|
| AtALMT1    | Arabidopsis thaliana   | Root cell plasma membranes           | Y             | Aluminium resistance              |
| AtALMT6    | Arabidopsis thaliana   | Tonoplast                           | N             | Guard cell malate currents        |
| AtALMT9    | Arabidopsis thaliana   | Tonoplast                           | N             | Chloride currents in guard cells  |
| AtALMT12   | Arabidopsis thaliana   | Plasma membrane of guard cells      | N             | Stomatal closing                  |
| TaALMT1    | Wheat, Triticum aestivum | Root cell plasma membranes           | Y             | Aluminium resistance              |
| HIALMT1    | Holcus lanatus         | Root plasma membrane                 | Y             | Aluminium resistance              |
| VvALMT9    | Grape, Vitis vinifera  | Tonoplast of berry mesocarp          | N             | Fruit flavour/vacuolar malate     |
| MdALMT1    | Apple, Malus domestica | Tonoplast                           | N             | Fruit flavour/vacuolar malate     |
| BrnALMT1   | Oilseed rape, Brassica napus | Root cell plasma membranes           | Y             | Aluminium resistance              |
| BrnALMT2   | Oilseed rape, Brassica napus | Root cell plasma membranes           | Y             | Aluminium resistance              |
| ScALMT1    | Rye, Secale cereale    | Root cell plasma membranes           | Y             | Aluminium resistance              |
| GmAALMT1   | Soybean, Glycine max   | Root cell plasma membranes           | Y             | Aluminium resistance              |
| HvALMT1    | Barley, Hordeum vulgare| Root cell plasma membranes and guard cells | N             | Maintaining turgor in growing cells, guard cell movements |
| ZmAALMT1   | Maize, Zea mays        | Plasma membranes throughout plant    | N             | Inorganic anion homeostasis       |
| ZmAALMT2   | Maize, Zea mays        | Root plasma membrane                 | N             | Constitutive malate efflux, not Al related |

plant species. In addition, GmAALMT1 channel activity has also been shown to be regulated by pH changes and phosphorous concentration [35]. Yorkshire fog (Holcus lanatus) HIALMT1 has also been identified as an important resistance gene in the model grass species, and has Al-activated expression similar to AtALMT1 controlled by a Al-responsive transcription factor ART1, with expression levels in different accessions controlled by the number of binding regions for ART1 in the promoter region [36].

Other root-related functions
ZmAALMT2 from maize (Zea mays) has been shown to be root localized and to release malate into the soil. Unlike AtALMT1 and TaALMT1, however, this is not correlated with Al-resistance, but instead is likely to provide solubilization for soil nutrients, such as phosphate, as discussed above for TaALMT1. As it is also found in vascular tissue, ZmAALMT2 could also play a role in the transport of organic acids or mineral anions in the xylem [37].

Guard cell movements
Another vital role played by some ALMT family members is as molecular components of the guard cell movements that regulate gas exchange across leaf surfaces. Plants control CO₂ uptake and water loss by regulating the aperture of the stomatal pores. Three family members from Arabidopsis, AtALMT6, AtALMT9 and AtALMT12, are involved in opening and closing of stomata, with movement driven by osmotically active inorganic and organic ions [38]. Upon stomatal opening, K⁺ enters the guard cell via voltage-gated inward rectifying potassium channels, driven by the electrochemical gradient maintained by ATP-driven proton pumping. Malate synthesis from stored starch provides a charge-balancing ion, and is taken up into the vacuole via AtALMT6, and similarly AtALMT9 acts to permit entry of chloride counterions into the vacuole. The increase in solutes draws water into the cell down the water potential gradient, swelling the cell. The process is inverted during stomatal closing. The membrane is depolarized, prompted by the action of AtALMT12/QUAC1 (quick anion channel 1) releasing malate rapidly, this allows K⁺ to flow out of the cell, accompanied by Cl⁻ and NO₃⁻ anions via SLAC1 (slow anion channel 1). In addition, AtALMT6 activity is regulated in part by cytosolic malate concentration, and so malate can flow out of the vacuole to either be lost via AtALMT12/QUAC1 or used in metabolism. This loss of osmotica drives a loss of water and closing of the stomata.
AtALMT6 is expressed in guard cell vacuoles and is a malate channel, specific for divalent malate involved in stomatal movements. It is not aluminium activated but instead is controlled by light, ABA [39], pH and cytosolic malate concentration [4]. As transport is dependent on the concentration of malate in the cytosol and the tonoplast membrane potential, AtALMT6 can mediate both malate uptake into and release from the vacuole in guard cells, with uptake during stomatal opening, and release during stomatal closing as shown in Figure 2. Interestingly, in A. thaliana expression is also seen in floral organs, suggesting another role yet to be elucidated. Knockout plants did not show phenotypic differences, indicating functional redundancy in vacuolar malate channels, perhaps from the action of...
Schematic of ion currents in stomatal opening and closing

K⁺ ions enter the cell during opening, and the charge is balanced by malate synthesis from starch, which is then taken up into the vacuole via ALMT6 and activates ALMT9 to permit Cl⁻ uptake into the vacuole. This increase in osmotic potential draws water into the cell, leading to swelling and stomatal opening. Stomatal closing, conversely, is driven by release of K⁺ ions, which is permitted during membrane depolarization and driven by ALMT12. Additionally, anions are released by SLAC1 over a longer period of time. Loss of osmolytes leads to concomitant loss of water and thus stomatal closing.

AtALMT9 and perhaps also AtALMT5, which has been shown to be expressed in guard cells, and to be closely related to AtALMT6 (see Figure 1).

AtALMT9 is a vacuolar chloride channel in guard cells and AtALMT9 knockouts show impaired stomatal opening [6]. It permits chloride to enter the vacuole, providing a charge-balance for K⁺ in the same way as malate. Malate in the cytosol (synthesized from starch) activates the channel, meaning malate can act both as an osmolyte and as a signalling molecule in guard cells. Interestingly, kinetic data suggest AtALMT9 is multimeric, with a number of subunits >2.5 with more recent work suggesting that it forms a tetramer [40]. Furthermore, cytosolic nucleotides such as ATP block the activity of AtALMT9, competing with malate for a binding site [41]. The H⁺-V-ATPases that maintain a hyperpolarized tonoplast consume ATP, provoking lowered cytosolic ATP concentrations, removing this block and thus permitting currents via AtALMT9 and facilitating anion uptake into the vacuole.

AtALMT12 (also known as QUAC1) is expressed in guard cell plasma membranes and operates as an R-type (rapid-type) channel crucial for stomatal closing [5], allowing rapid malate release. One study also found localization to endomembranes, although this is possibly an artefact of overexpression [42]. The channel opens with fast kinetics upon membrane depolarization, releasing malate into the apoplasm in parallel with K⁺ release through potassium channels to maintain the depolarization. Moreover, external malate causes increased activation of the channel and thus may represent a positive-feedback loop. Loss-of-function mutants confer a wilty phenotype due to their impaired stomatal closing. Rather than being ligand gated, as seen in ALMT1, ALMT12 activity is voltage gated; however, the voltage sensor is yet to be identified, although it is likely to be in the CTD (C-terminal domain), which has been shown to be vital for regulation [43].

Other roles

Malate storage and homoeostasis

AtALMT9, in addition to its role in guard cells (discussed above and summarized in Table 1), has been shown to be permeable to both chloride and malate and expressed strongly throughout leaf mesophyll tissue. It is likely to have a role in homoeostasis: ensuring that the concentration of malate – which plays an essential role in metabolism as part of the citric acid cycle – remains stable within the cytoplasm. Similar to AtALMT6, the channel is likely to work in both directions, by storing excess in the vacuole and releasing it when required to regulate osmotic potential and C-metabolism [44].

Cell elongation and nutrient storage

Barley (Hordeum vulgare) HvALMT1 is a malate channel expressed in guard cells and the root elongation zone, as well as floral tissues and seeds [45]. Although it has the greatest sequence similarity to TaALMT1 of any barley gene and localizes to the plasma membrane, it is not involved in Al-resistance but seems to have several distinct roles within the plant. HvALMT1 over-expressing lines take longer to close their stomata [46] and RNAi knockouts show a similar phenotype to Atamlt12 knockouts [9], so HvALMT1 is likely to be a functional homologue of AtALMT12. In expanding cells, HvALMT1 may help provide an osmotic balance and regulate turgor. Additionally, later studies have shown that this channel plays a role in seed development during acidification of the starchy endosperm, which is required for enzyme activity [9]. Rather than directly causing acidification itself (this is probably caused by a H⁺-ATPase pumping protons from the aleurone) release of malate is suggested to act as a counterion for H⁺ and other positively charged nutrients such as K⁺, helping to maintain electroneutrality and osmotic balance in a similar manner to the role of malate in guard cell movements. The significant difference in function between HvALMT1 and TaALMT1 despite strong sequence similarity again highlights that small differences in sequence can underlie large changes in function.
Fruit flavour
Malic acid is an important component of apple taste, grape quality and wine production: an economically significant set of traits. A malic acid channel with high homology to AtALMT genes has been shown to be responsible for the acidity of apples, and functions to accumulate malic acid in the vacuole [8,47]. Similarly, grape berries contain an ALMT family member expressed in the tonoplast of berry mesocarp tissue responsible for malate and tartrate accumulation [48]. Understanding of the action of these genes could be valuable for fruit and wine development [47].

Inorganic anion homoeostasis
ZmALMT1 from maize was one of the first family members to be described that did not have a role in aluminium resistance. It localizes to plasma membranes throughout the plant, but is less permeable to organic anions and instead probably involved in inorganic anion homoeostasis and mineral nutrition [7].

GABA signalling
A recently discovered role of ALMTs is in mediating GABA signalling in plants. This is the first evidence for GABA signalling in plants in addition to its established role as a metabolite [10]. GABA – a non-protein amino acid – accumulates in plant tissues in response to biotic and abiotic stresses, and has a central role in pollen tube growth and regulation of root growth [49]. Recently, it has been shown that GABA’s influence is exerted by interaction with ALMT proteins, and a putative GABA-binding motif has been identified in the CTD. GABA binding negatively regulates ion flux through the channel, i.e. decreasing carbon flux from roots in the case of TaALMT1. Additionally, as plant anion equilibrium potentials are strongly positive and plant action potentials are generated by voltage-gated ion channels, GABA inhibition of ALMTs will hyperpolarize membranes and decrease excitability.

Moreover, to advance studies of ALMTs, perhaps parallels can be drawn with the more well-studied GABA(A) receptors from mammals, which have a greater level of structural detail elucidated already. Although they are different gene families, it may be possible to apply insights from mammalian proteins to design experiments to study ALMTs as GABA(A) receptors are predicted to have a similar overall architecture, being ∼450AA long and divided into a membrane-embedded half and a soluble half [50]. In the mammalian system the channel is formed by a central pore between five monomers, and the GABA-binding site is found at the interface of monomer subunits; perhaps a similar multimeric structure will be found for ALMTs as suggested by recent results from AtALMT9 [40].

Conclusions
ALMTs have been shown to be involved in many vital roles in plants (summarized in Table 1), and there are many more likely to be found. For example, ten genes in Arabidopsis still have unassigned functions as seen in Figure 1. Some of these may have similar or redundant roles, for example AtALMT4 and AtALMT5 are closely related to AtALMT6 and so may also have a role in the guard cell vacuolar membrane. Indeed, some of these channels may account for residual activity seen in knockout mutants that still retain some function. Alternatively, ALMTs may form hetero-multimers to provide diversified functions, as seen in GABA(A) receptors in mammals. In addition, several members have expression patterns that show they must have a wider range of roles than is currently known – for example, AtALMT6 has a role in guard cells, but is also expressed in floral tissues with an as-yet undefined function. It is possible that ALMTs are involved in shuttling malate in C4 and CAM metabolism as the channels involved have not yet been identified. However, as Arabidopsis is a C3 plant these experiments will have to be done in another species.

Although there is a growing body of knowledge about the physiological functions of ALMTs, much less is known about their structure and mechanism. Relatively small differences in sequence can lead to large changes in localization, substrate specificity, gating and physiological function. In many cases, evidence is either scant or directly contradictory. No detailed 3D structure is available of any family member, but would help guide biochemical and functional studies and elucidate further details of mechanism and regulation, and thus detailed, high quality structural studies are vital for a full understanding of ALMTs.

Finally, the ALMT family has been shown to have many members that are not aluminium activated, to have members permeable to anions other than malate, and to be channels rather than active transporters. Thus, the name aluminium-activated malate transporters does not fully reflect this family of proteins and is potentially confusing. It is perhaps advisable to take up the previously suggested QUAC nomenclature, which better reflects the characteristics of the family members.

Acknowledgements
Antony Palmer acknowledges the mentorship and support of Steve Baldwin, without whom this work would not have been possible.

Funding
This work was supported by the Biotechnology and Biological Sciences Research Council White Rose DTP [grant number BB/J014443/1 (T. Palmer)].

References
1 Dreyer, I., Gomez-Porras, J.L., Riano-Pachon, D.M., Hedrich, R. and Geiger, D. (2012) Molecular evolution of slow and quick anion channels (SLACs and QUACs/ALMTs). Front. Plant Sci. 3, 263 ( CrossRef PubMed)
2 Sasaki, T., Yamamoto, Y., Ezaki, B., Katsuhara, M., Ahn, S.J., Ryan, P.R., Delhaize, E. and Matsumoto, H. (2004) A wheat gene encoding an aluminum-activated malate transporter. Plant J. 37, 645-653 CrossRef PubMed

3 Hoekenga, O.A., Maron, L.G., Piñeros, M.A., Cançado, G.M.A., Shaff, J., Kobayashi, Y., Ryan, P.R., Dong, B., Delhaize, E., Sasaki, T. et al. (2006) TaALMT1, which encodes a malate transporter, is identified as one of several genes critical for aluminum tolerance in Arabidopsis. Proc. Natl. Acad. Sci. U.S.A. 103, 9738-9743 CrossRef PubMed

4 Meyer, S., Scholz-Starke, J., De Angelis, A., Koversmann, P., Burlo, B., Gambino, P., Uahir, S. and Martinou, E. (2011) Malate transport by the vacuolar ALMT6 channel in guard cells is subject to multiple regulation. Plant J. Cell Mol. Biol. 67, 247-257 CrossRef

5 Meyer, S., Mumma, P., Imes, D., Endler, A., Weder, B., Al-Rashed, K.A.S., Geiger, D., Marten, I., Martinou, E. and Hedrich, R. (2010) ALMT12 represents an R-type anion channel required for stomatal movement in Arabidopsis guard cells. Plant J. 63, 1058-1062 CrossRef PubMed

6 De Angelis, A., Zhang, J., Meyer, S. and Martinou, E. (2015) TaALMT9 is a malate-activated vacuolar chloride channel required for stomatal opening in Arabidopsis. Nat. Commun. 4, 1804 CrossRef PubMed

7 Piñeros, M.A., Cançado, G.M.A., Maron, L.G., Lyi, S.M., Memossi, M. and Kochian, L.V. (2008) Not all ALMT-type transporters mediate aluminum-activated organic acid responses: the case of ZmALMT1 – an anion-selective transporter. Plant 53, 352-367 CrossRef PubMed

8 Khan, S.A., Beekwilder, J., Schacht, J.G., Mumm, R., Soria, J.M., Jacobson, E. and Schouten, H.J. (2013) Differences in acidity of apples are probably mainly caused by a malic acid transporter gene on G16. Tree Genet. Genomes 9, 475-487 CrossRef

9 Xu, M., Gruber, B.D., Delhaize, E., White, R.G., James, R.A., You, J., Yang, Z. and Ryan, P.R. (2015) The barley anion channel, HvALMT1, has multiple roles in guard cell physiology and grain metabolism. Physiol. Plant. 153, 183-193 CrossRef PubMed

10 Ramesh, S.A., Tyerman, S.D., Xu, B., Böse, J., Kaur, S., Conn, V., Domingos, P., Uahir, S., Wege, S., Shabala, S. et al. (2015) GABA-sigalling modulates plant growth by directly regulating the activity of plant-specific anion transporters. Nat. Commun. 6, 7879 CrossRef PubMed

11 Soto-Cerrada, B.J., Histothe-Zanclezhue, C., Mathias, M., Penaloza, E., Zumpa, J., Taboada, G.G. and Tamariz, A. (2015) Maize root hair cell breeding for TaALMT1, a major gene conferring aluminum tolerance to wheat. Biol. Plant. 59, 83-91 CrossRef

12 Kochan, L.V., Piñeros, M.A., Liu, J. and Magalhães, J.V. (2015) Plant adaptation to acid soils: the molecular basis for crop aluminum resistance. Annu. Rev. Plant Biol. 66, 571-598 CrossRef PubMed

13 Kochan, L.V. (1995) Cellular mechanisms of aluminium toxicity and resistance in plants. Ann. Rev. Plant Physiol. Plant Molec. Biol. 46, 237-260 CrossRef

14 Baker, A., Cesari, S.A., Palmer, A.J., Paterson, J.B., Qian, W., Muñiz, S.P. et al. (2015) Replace, reuse, recycle: improving the sustainable use of phosphorus by plants. J. Exp. Bot. 66, 3523-3540 CrossRef PubMed

15 Wang, C., Zhao, X.-Q., Aizawa, T., Sunairi, M. and Shen, R.-F. (2013) High 

16 Zhang, J., Martinoia, E. and De Angeli, A. (2014) Cytosolic nucleotides provide opportunities for enhancing crop production on acid soils. J. Exp. Bot. 65, 29-42 CrossRef PubMed

17 Delhaize, E., Mu, J.F. and Ryan, P.R. (2012) Transcriptional regulation of aluminum tolerance genes. Trends Plant Sci. 17, 341-348 CrossRef PubMed

18 Kobayashi, Y., Hoekenga, O.A., Itoh, H., Nakashima, M., Saito, S., Shaff, J.E. et al. (2007) Characterization of AtALMT1 expression in aluminum-inducible malate release and its role for rhizotoxic stress tolerance in Arabidopsis. Plant Physiol. 145, 843-852 CrossRef PubMed

19 Sasaki, Y., Iuchi, S., Kobayashi, Y., Kobayashi, Y., Ikka, T., Sakurai, N. et al. (2009) STOP1 regulates multiple genes that protect arabidopsis from proton and aluminum toxicities. Plant Physiol. 150, 261-294 CrossRef PubMed

20 Kobayashi, Y., Ohyama, Y., Kobayashi, Y., Ito, H., Iuchi, S., Fujita, M. et al. (2014) STOP2 activates transcription of several genes for Al- and Low pH-tolerance that are regulated by STOP1 in Arabidopsis. Mol. Plant. 7, 311-322 CrossRef PubMed

21 Dinz, J.Z., Yan, J.Y., Xu, X.Y., Li, G.X. and Zheng, S.J. (2013) WRKY46 functions as a transcriptional repressor of ALMT1, regulating aluminum-induced malate secretion in Arabidopsis. Plant J. 76, 825-835 CrossRef PubMed

22 Ligaba, A., Katsuhara, M., Ryan, P.R., Shibasaki, M. and Matsumoto, H. (2004) The BnALMT1 and BnALMT2 genes from rape encode aluminum-activated malate transporters that enhance the aluminum resistance of plant cells. Plant Physiol. 142, 1294-1303 CrossRef PubMed

23 Hoffland, E., Findenegg, G.R. and Neflemens, J.A. (1989) Solubilization of rock phosphate by rape. Plant Soil 113, 155-160 CrossRef

24 Fontecha, G., Silva-Nava, J., Benito, C., Nesvest, M.A., Espino, F.J., Hernández-Riquer, M.V. et al. (2007) Candidate gene identification of an aluminum-activated organic acid transporter gene at the Alt4 locus for aluminum tolerance in rye (Secale cereale L. Tag Theor. Appl. Genet. Theor. Angew. Genet. 114, 249-260 CrossRef

25 Collins, N.C., Shirley, N.J., Saeed, M., Pallotta, M. and Gustafson, J.P. (2008) An ALMT gene cluster controlling aluminum tolerance in barley at the Alt4 locus of rye (Secale cereale L.). Genetics 179, 669-682 CrossRef PubMed

26 Liang, C., Piñeros, M.A., Tian, J., Yao, Z., Sun, L., Liu, J. et al. (2013) Low pH, aluminum, and phosphorus coordinately regulate malate exudation through GmALMT1 to improve soybean adaptation to acid soils. Plant Physiol. 161, 1347-1361 CrossRef PubMed

27 Chen, Z.C., Yokosh, K., Kashino, M., Zhao, F.-J., Yamaji, N. and Ma, J.F. (2013) Adaptation to acid soil is achieved by increased numbers of cis-acting elements regulating ALMT1 expression in Holcus lanatus. Plant J. 76, 10-23 PubMed

28 Li, J., Shafer, M., Delhaize, E. and Tyerman, S.D. (2012) Maize ZmAlmt2 is a root anion transporter that mediates constitutive root malate efflux. Plant Cell Environ. 35, 1185-1200 CrossRef PubMed

29 Kollist, H., Nuhakt, M. and Roelfsema, M.R.G. (2014) Closing gaps: linking elements that control stomatal movement. New Phytol. 203, 44-62 CrossRef PubMed

30 Roelfsema, M.R.G., Levenkov, V. and Hedrich, R. (2004) ABA depolarizes guard cells in intact plants, through a transient activation of R- and S-type anion channels. Plant J. Cell Mol. Biol. 37, 578-588 CrossRef PubMed

31 Zhang, J., Baeza, U., Krugel, U., Martinoia, E. and Angeli, A.D. (2013) Identification of a probable pore-forming domain in the multivacuolar anion channel AALMT9. Plant Physiol. 163, 830-843 CrossRef PubMed

32 Zhang, J., Martinoia, E. and De Angelis, A. (2014) Cytosolic nucleotides block and regulate the arabidopsis vacuolar anion channel AA1MT9. J. Biol. Chem. 289, 25581-25589 CrossRef PubMed

33 Sasaki, T., Morii, I.C., Furuchi, T., Maenensis, S., Toyooka, K., Matsuka, K. et al. (2010) Closing plant stomata requires a homolog of an aluminum-activated malate transporter. Plant Cell Physiol. 51, 354-365 CrossRef PubMed

© 2016 The Author(s) This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and distributed under the Creative Commons Attribution Licence 4.0 (CC BY).
43 Mumm, P., Imes, D., Martinoia, E., Al-Rasheid, KAS, Geiger, D., Marten, I. et al. (2013) C-terminus mediated voltage gating of Arabidopsis guard cell anion channel QUAC1. Mol. Plant. 6, 1550–1563 CrossRef PubMed

44 Kovermann, P., Meyer, S., Hörtensteiner, S., Picco, C., Scholz-Starke, J., Rivera, S. et al. (2007) The Arabidopsis vacuolar malate channel is a member of the ALMT family. Plant J. 52, 1169–1180 CrossRef PubMed

45 Gruber, B.D., Ryan, P.R., Richardson, A.E., Tyerman, S.D., Ramesh, S., Hebb, D.M. et al. (2010) HvALMT1 from barley is involved in the transport of organic anions. J. Exp. Bot. 61, 1455–1467 CrossRef PubMed

46 Gruber, B.D., Delhaize, E., Richardson, A.E., Roessner, U., James, R.A., Howitt, S.M. et al. (2011) Characterisation of HvALMT1 function in transgenic barley plants. Funct. Plant Biol. 38, 163–175 CrossRef PubMed

47 Bai, Y., Dougherty, L., Li, M., Fazio, G., Cheng, L. and Xu, K. (2012) A natural mutation-led truncation in one of the two aluminum-activated malate transporter-like genes at the Ma locus is associated with low fruit acidity in apple. Mol. Genet. Genomics 287, 663–678 CrossRef PubMed

48 Angeli, A.D., Baetz, U., Francisco, R., Zhang, J., Chaves, M.M. and Regalado, A. (2013) The vacuolar channel VvALMT9 mediates malate and tartrate accumulation in berries of Vitis vinifera. Planta 238, 283–291 CrossRef PubMed

49 Palanivelu, R., Brass, L., Edlund, A.F. and Preuss, D. (2003) Pollen tube growth and guidance is regulated by POP2, an Arabidopsis gene that controls GABA levels. Cell 114, 47–59 CrossRef PubMed

50 Ernst, M., Brauchart, D., Boresch, S. and Sieghart, W. (2003) Comparative modeling of GABAA receptors: limits, insights, future developments. Neuroscience 119, 933–943 CrossRef PubMed

51 Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729 CrossRef PubMed

Received 15 January 2016
doi:10.1042/BST20160027