Organic acid carriers in tolerance to toxic aluminum in wheat

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ABSTRACT: *Aluminum (Al) toxicity in plants is seen in about 15% of the soils worldwide, restraining yields in arable land. In Brazil, acidic soils limit production of wheat (Triticum aestivum L.) and other cereals. Al is toxic for most winter cereals when its concentration increases and soil pH is below 5. One of the main concerns with acidic soil is the increase in the mobility of Al^{3+} ions. Al binds to cell walls in roots, preventing meristematic elongation in sensitive species, causing damage to the root system and results in lower yields. Al^{3+} forms highly stable complexes with phosphorus (P), limiting its availability to plants, as well as reducing cell division and elongation. To deal with Al toxicity, plants have developed strategies such as organic acid (OA) exudation by roots; this mechanism of detoxification has been well-characterized. OAs, in turn, chelate ions Al^3, forming non-toxic compounds that do not penetrate the root system. Some genes responsible for Al tolerance in wheat have been identified, particularly TaALMT1 and TaMATE1B that transport malate and citrate OAs, respectively. In this review, we discussed the mechanisms by which Al damages roots those by which plants are protected, primarily through two genes. We also described the interaction of the ALMT1 gene with P and iron (Fe).*
Key words: *Triticum aestivum L. Al^{3+}, acid soils, ALMT1, MATE.*

Transportadores de ácidos orgânicos na tolerância ao alumínio tóxico em trigo

RESUMO: *A toxicidade do alumínio (Al) às plantas é observada em cerca de 15% dos solos no planeta, sendo um fator restritivo à produtividade em terras cultiváveis. No Brasil, os solos ácidos são limitantes à produção de trigo (Triticum aestivum L.) e outros cereais. O Al é tóxico para a maioria dos cereais de inverno, quando a sua concentração aumenta e o pH do solo atinge valores inferiores a 5. Uma das principais preocupações sobre o solo ácido é o aumento da mobilidade dos íons Al^{3+}. O Al pode se ligar as paredes celulares das raízes e, como consequência, impedir o alongamento meristemático em espécies sensíveis, provocando danos ao sistema radicular, que resulta em menor desempenho agronômico das plantas. O Al^{3+} é também capaz de formar complexos altamente estáveis com fósforo (P), limitando sua disponibilidade para as plantas, e também reduzindo a divisão e o alongamento celular. Para lidar com a toxicidade ao Al, as plantas desenvolveram algumas estratégias como a exsudação de ácido orgânicos (AOs) pelas raízes, sendo este mecanismo de destoxificação bem caracterizado. Os AOs, por sua vez, quelam íons Al^{3+} formando compostos não tóxicos que não penetram no sistema radicular. Alguns genes responsáveis pela tolerância ao Al em trigo foram identificados, com ênfase para TaALMT1 e TaMATE1B, que exsudam os AOs malato e citrato, respectivamente. Nesta revisão, discutimos os mecanismos pelos quais Al danifica raízes, bem como plantas protegem-se, através de dois genes principalmente. Também apresentamos a interação do gene ALMT1 com P e ferro (Fe).*
Palavras-chave: *Triticum aestivum L. Al^{3+}, solos ácidos, ALMT1, MATE.*

INTRODUCTION

Aluminum (Al) is the third most abundant element in the earth's crust after oxygen and silicon. Due to its complex dissociation, Al assumes various forms and solubilities according to pH. In soils with low pH, Al dissolves into ionic forms, commonly known as Al^{3+}, $Al(OH)_2^+$ and $Al(OH)_2^+$. Among these forms, Al^{3+} is the most phytotoxic for wheat rhizosphere, while $Al(OH)_2^+$ also appears to be toxic to dicotyledonous species (GARCIA-OLIVEIRA et al., 2015). The toxicity can be explained by differences in sphingolipids, especially glycosylphosphatidyl inositol, present in the plasma membranes of both types of plants. In dicotyledons, the membrane has two free hexoses in the extracellular portion, whereas the monocotyledons have three free hexoses at the same position (LENARČIČ et al., 2017). It has

therefore been suggested that these free sugars have a higher affinity for $Al(OH)^{2+}$ and Al^{3+}.

In soils where the pH is less than 5.0, the solubility of Al tends to increase, causing a limitation in the absorption of nutrients, because it inhibits the transport of potassium (K^+) and calcium (Ca^{2+}) ions, forms insoluble complexes with phosphorus (P), considerably reducing its availability to plants, as well as altering water uptake by the plant root system (KOCHIAN et al., 2015). This is mainly due to the accumulation of toxic ions in the roots, especially in the form of the trivalent Al^{3+} cation. Consequently, inhibition of root system growth and reduction of productivity occurs due to nutritional deficiency and water stress (BIAN et al., 2015).

The apex of wheat roots is considered the main target of toxic Al (RYAN et al., 1993), causing development of smaller roots with characteristic thickening. Recently, cell walls have been identified as the primary lesion sites, being loosened in the elongation zone (KOPITTKE et al., 2015). Al interacts with polysaccharides in cell walls where they are retained. Decreases in retention have been demonstrated in wheat, with increases in putrescine (polyamine) that increase the methylation degree of pectins, reducing Al's effects (YU et al., 2015). Al also causes extensive damage to other cellular processes. In sensitive cultivars, Al inhibits DNA synthesis, alters cell membrane potentials and reduces H^+ efflux in the root apex (GARCIA-OLIVEIRA et al., 2015).

Oxidative stress produced by toxic Al causes significant increases in the production of reactive oxygen species (ROS), including superoxide radicals (O^2), hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2) (MOUSTAKA et al., 2016). The ROS alter unsaturated fatty acids through membrane lipid peroxidation, in turn leading to severe cellular damage (MATSUMOTO et al., 2015). The production of nitric oxide (NO) in wheat regulates this oxidative stress (SUN et al., 2018). Programmed cell death (apoptosis) can also be induced as a result of Al accumulation, because Al^{3+} ions have high affinity for biomembranes, particularly because of the negative charge of phospholipids that irreversibly bind to Al (MATSUMOTO et al., 2015).

Aerial damage suggests that phytotoxic effects may affect leaf metabolism by reducing growth, chlorophyll contents, mineral nutrients, photosynthetic rates and transpiration (MOUSTAKA et al., 2016). Al also reduces the accumulation of divalent cations, especially Ca and Mg, causing significant losses in photosystem II, the photosynthetic component that is most sensitive to stress (MOUSTAKA et al., 2016).

In order to reduce the phytotoxicity caused by acidic soils, a common practice is the application of limestone, where the chemical species $HCO_{3\,(aq)}^-$, $CO_{3\,(aq)}^{2-}$ and $OH^-_{(aq)}$ react with $H^+_{(aq)}$ to diminish soil acidity. However, this practice is not always economically or physically possible because of the enormous amount of limestone required for the treatments, in addition to being dependent on soil pH, texture and acidity of the lower layers (GARCIA-OLIVEIRA et al., 2015). No-till farming, commonly used for wheat, soybeans (*Glycine max* L.) and corn (*Zea mays* L.), makes it difficult to incorporate limestone through soil rotation, limiting the amount that can be applied. In such cases, a pH gradient develops in the soil that in turn limits root depth (CAIRES et al., 2006). Large applications of limestone can also cause adverse effects on plants such as deficiency of various nutrients or even the favoring of diseases such as root rot caused by *Gaeumannomyces graminis* (Sacc.) Arx & D.L. Olivier (LEBRETON et al., 2014). Therefore, many farmers today have resorted to the use of Al-tolerant wheat varieties, as they are environmentally safe alternatives that do not require substantial expenditures for soil pH correction.

Mechanisms of Al^{3+} tolerance

In tropical and subtropical regions, approximately 60% of arable land is acidified (KOCHIAN et al., 2015). In Brazil, the soils used for wheat farming are predominantly acidic. As a consequence, most wheat genotypes developed in Brazil are globally recognized as good sources of Al tolerance (AGUILHERA et al., 2016). Tolerance to Al^{3+} is a relatively common feature of Brazilian genotypes, as opposed to genotypes from other countries (PEREIRA et al., 2015).

Historically, several Al-tolerant wheat cultivars, including Fronteira, Surpresa, Minuano, Jesuíta, Guarani, BH1146, Carazinho and Toropi, were derived from the Brazilian genotypes Alfredo Chaves 6-21 and Polyssu. Modern wheat cultivars that have some tolerance to Al, developed in various countries, have Brazilian genetic material in their pedigrees (GARCIA-OLIVEIRA et al., 2015).

Plants deal with toxic Al in various ways. Tolerance-related genes may be involved in several metabolic processes, including cell division and elongation, cell wall formation, oxidative stress, iron metabolism, signal transduction and other cellular mechanisms (MA et al., 2016; CHANDRAN et al., 2008; SUN et al., 2018). However, they act principally through exclusion mechanisms based on the exudation of organic acids (OAs) responsible for chelating Al^{3+}

ions in the rhizosphere, preventing Al^{3+} access to the root apex and/or by tolerance mechanisms, where Al^{3+} enters the plant and is detoxified and sequestered (KOCHIAN et al., 2015).

One of the most studied mechanisms of tolerance to date is the efflux of OAs through the apex of tolerant plant roots, via transmembrane transporters (HE et al., 2015; SASAKI et al., 2004). The type of OA varies according to plant species and; although, most plants secrete only one type, it is not uncommon to find species that release more than one OA (PEREIRA et al., 2015). Several low molecular weight OAs were exuded from wheat roots, including malate, citrate, oxalate, succinate, tartaric acid and fumarate (GARCIA-OLIVEIRA et al., 2015; HE et al., 2015). Nevertheless, in wheat, the most important OAs for Al detoxification are malate, associated with the gene *TaALMT1* (*Aluminum-activated malate transporter 1*) (SASAKI et al., 2004) and citrate, associated with *TaMATE1B* (*Multi-drug and toxin extrusion 1*).

Malate chelates toxic Al^{3+} ions to form 2:2 complexes. Malate coats these ions by leaving them in a form that is non-toxic to the plants, protecting the root apex and reducing Al's contact with the roots. This mechanism prevents Al from binding to the negatively-charged sites on the cell wall and plasma membrane (GARCIA-OLIVEIRA et al., 2015), allowing tolerant wheat genotypes to have larger roots and to be more productive than sensitive ones. The pattern of secretion of OAs can be identified based on their release, and can be divided into two patterns: Pattern I, where secretion occurs almost immediately after contact with Al, suggesting that Al activates an anion channel in the pre-existing plasma membrane and gene induction is not required; and Pattern II, occurring hours after exposure to Al, suggesting that gene induction is required for activation of the responses (KOCHIAN et al., 2015; CHEN et al., 2013; FONTECHA et al., 2007). Response time is directly linked to the degree of intrinsic tolerance of the species tested. HE et al. (2015) observed that tolerant wheat strains responded rapidly to Al stress and release OAs, on average, 10 min after contact, by activating anion channels present in the plasma membrane. Therefore, most studies of wheat tolerance mechanisms have focused on responses that occur rapidly after exposure to toxic Al (Figure 1).

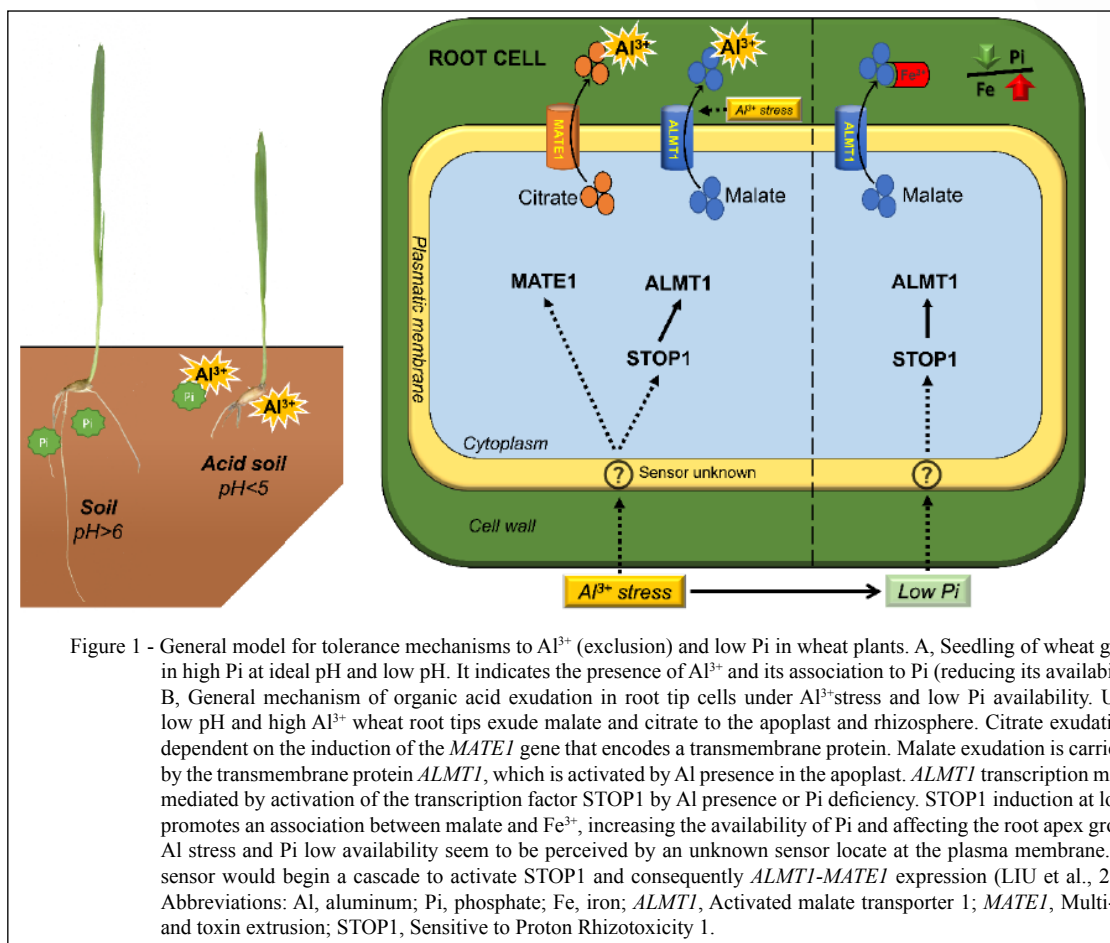*The principal gene involved in tolerance to toxic Al^{3+}, TaALMT1*

The first identified member of the *ALMT* family (*Aluminum-activated malate transporter*) was *TaALMT1*, discovered in the tips of the roots of wheat expressing a constitutively expressed transmembrane protein (SASAKI et al., 2004). Genes of this family have a high degree of similarity and basically consist of a highly-conserved N-terminal region with six transmembrane domains, followed by a long, variable and hydrophilic C-terminal region that may contain one or more transmembrane domains (KOCHIAN et al., 2015).

TaALMT1 is located on the 4DL chromosome of wheat and is responsible for encoding a malate carrier protein, that; although, functional in the absence of extracellular Al^{3+}, has increased basal activity in the presence of Al^{3+} (KOBAYASHI et al., 2007; SASAKI et al., 2004). Their expression levels are strongly correlated with tandem repeats located in the promoter region. These repeats define alleles of the *TaALMT1* gene promoter, named according to the number of copies, ranging from I to VII (Figure 2) (AGUILHERA et al., 2016; PEREIRA et al., 2015). Promoters containing a large number of replicates, i.e., from V to VII, present high levels of gene expression; and are therefore, more tolerant to Al^{3+} than are the promoters with lower numbers of replicates, from I to II; these have very low rates of gene expression and consequently greater sensitivity to Al^{3+} (AGUILHERA et al., 2016). Evidence further suggests that direct phosphorylation of *AtALMT1* protein by a protein kinase C (PKC) is one of the pre requisites for the activation of *AtALMT1* (KOBAYASHI et al., 2007).

The origin of the block repeats in the *TaALMT1* promoter is not yet clear. Nevertheless, these replicates have often appeared in *T. aestivum* over the last 10,000 years, and alleles with various patterns of replications, for the most part, have independent origins (RYAN et al., 2010). Since the discovery of *TaALMT1*, several orthologs of this gene have been identified in diverse species (Table 1), indicating that the malate release mechanism is shared by a wide range of species. FONTECHA et al. (2007) reported that the *ScALMT1* gene has a structure very similar to that of *TaALMT1*, with at least two alleles of *ScALMT1* (*ScALMT1-1* and *ScALMT1-2*) present in rye.

Unlike *TaALMT1*, *AtALMT1* is not constitutively expressed in *Arabidopsis thaliana* L. *AtALMT1* has Al-induced expression (KOBAYASHI et al., 2007), being mediated by the transcription factors STOP1, STOP2 (Sensitive to Proton Rhizotoxicity 1 and 2) and WRKY46 (KOCHIAN et al., 2015; CHEN et al., 2013) that are also responsible for regulating other genes critical for tolerance to acid soils. It has recently been reported that *Arabidopsis* mutants in the

Figure 1 - General model for tolerance mechanisms to Al^{3+} (exclusion) and low Pi in wheat plants. A, Seedling of wheat grown in high Pi at ideal pH and low pH. It indicates the presence of Al^{3+} and its association to Pi (reducing its availability). B, General mechanism of organic acid exudation in root tip cells under Al^{3+} stress and low Pi availability. Under low pH and high Al^{3+} wheat root tips exude malate and citrate to the apoplast and rhizosphere. Citrate exudation is dependent on the induction of the *MATE1* gene that encodes a transmembrane protein. Malate exudation is carried on by the transmembrane protein *ALMT1*, which is activated by Al presence in the apoplast. *ALMT1* transcription may be mediated by activation of the transcription factor STOP1 by Al presence or Pi deficiency. STOP1 induction at low Pi promotes an association between malate and Fe^{3+}, increasing the availability of Pi and affecting the root apex growth. Al stress and Pi low availability seem to be perceived by an unknown sensor locate at the plasma membrane. This sensor would begin a cascade to activate STOP1 and consequently *ALMT1-MATE1* expression (LIU et al., 2009). Abbreviations: Al, aluminum; Pi, phosphate; Fe, iron; *ALMT1*, Activated malate transporter 1; *MATE1*, Multi-drug and toxin extrusion; STOP1, Sensitive to Proton Rhizotoxicity 1.

stop1 gene did not express detectable *ALMT1* levels, suggesting that STOP1 was essential for the expression of *ALMT1* (MORA-MACÍAS et al., 2017).
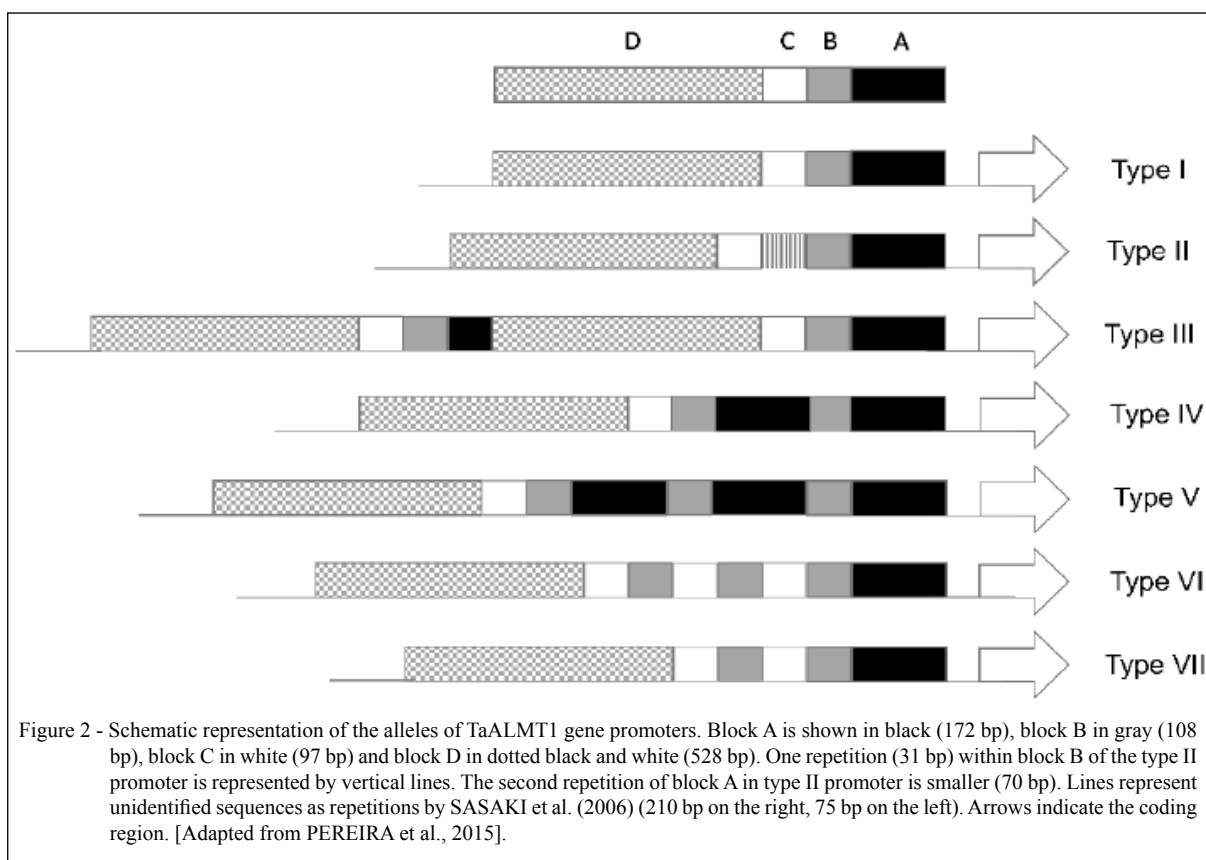
Exudation of malate was increased 8- to 10-fold in transgenic tobacco cells that possessed the *BnALMT1* and *BnALMT2* genes under toxic Al stress (LIGABA et al., 2006). CHEN et al. (2013) reported similar results, in which *MsALMT1* overexpression showed superior malate exudation to that of wild-type plants both under normal and Al-stressed conditions.

Rye (*Secale cereale* L.) may be slower to achieve high exudation rates of OAs after exposure to Al, most likely because *ScALMT1* proteins are present at low levels in the absence of Al and only increase after Al exposure to post-transcriptional regulation (COLLINS et al., 2008). Conversely, various tolerant wheat cultivars respond faster to Al because *TaALMT1* is expressed at high levels even before exposure to Al (SASAKI et al., 2004).

To verify the specificity of *ALMT1* to Al, *A. thaliana* roots were exposed to cadmium (Cd), copper (Cu), erbium (Er), lanthanum (La), sodium (Na)

and low pH. Only Al induced malate release above levels observed in control plants (MAGALHAES et al., 2007), suggesting that the mechanism employed by this gene increased gene expression only in the presence of Al and was not significantly influenced by other elements. Recently, it was reported that P limitation induced *ALMT1* in *Arabidopsis* (MORA-MACÍAS et al., 2017). This possible association with P may justify the existence of an Al tolerance mechanism in wheat that originated in non-toxic environments. The mechanism would originally be involved in the response to P limitation.

Exudation of malate in *Arabidopsis* is essential for root length reduction in P deficiency mediated by Fe accumulation in the apoplast (MORA-MACÍAS et al., 2017; BALZERGUE et al., 2017). P limitation induced both ALMT1 and MATE, with only the former being expressed at the root apex in *Arabidopsis*. This suggested that malate, by retaining Fe^{3+}, increases the action of LOW PHOSPHATE ROOT 1 (LPR1) on Fe^{2+} reduction, triggering callose synthesis and consequent inhibition

Figure 2 - Schematic representation of the alleles of TaALMT1 gene promoters. Block A is shown in black (172 bp), block B in gray (108 bp), block C in white (97 bp) and block D in dotted black and white (528 bp). One repetition (31 bp) within block B of the type II promoter is represented by vertical lines. The second repetition of block A in type II promoter is smaller (70 bp). Lines represent unidentified sequences as repetitions by SASAKI et al. (2006) (210 bp on the right, 75 bp on the left). Arrows indicate the coding region. [Adapted from PEREIRA et al., 2015].

of cell division (MORA-MACÍAS et al., 2017), as well as stimulating peroxidases that cause cell wall stiffening, reducing elongation (BALZERGUE et al., 2017). These responses have not yet been confirmed in Poaceae, in which the Fe absorption system does not involve the reduction of Fe to Fe^{2+}.

Significant differences in functions can be reported between orthologous genes with highly similar sequences. *ZmALMT1* was the first member of the *ALMT* family that did not have Al tolerance activity (PIÑEROS et al., 2008). This gene product is located on the plasma membrane of maize cells, but it is less permeable to OAs and is probably involved in inorganic anion homeostasis and mineral nutrition (PIÑEROS et al., 2008). The *HvALMT1* gene from barley is another characteristic example; it encodes a malate channel expressed in guard cells, in the zone of root elongation, in floral tissues and in seeds (GRUBER et al., 2010). Although, it has high similarity to the TaALMT1 sequence and its product is localized in the plasma membrane, interestingly, *HvALMT1* is not involved in Al tolerance.

TaMATE1B

Multi-drug and toxin extrusion (MATE) proteins utilize the electrochemical Na^+/proton exchange gradient to export a wide variety of substrates, including secondary metabolites and xenobiotics (HE et al., 2015). The family of *MATE* genes was initially identified in response to toxic Al in sorghum (Alt_{SB}) and barley. In sorghum, Al-activated citrate exudation is controlled by the Alt_{SB} locus and accounts for more than 80% of the phenotypic variation in Al tolerance in the mapping populations studied (MAGALHAES et al., 2007). Therefore, pioneering studies in this family come from these two species.

There are at least 40 *MATE* orthologous genes in the rice genome (*Oryza sativa* L.) (YOKOSHO et al., 2009); however, only a few members of this family have been functionally characterized to date. Barley is the most sensitive cereal to toxic Al (FURUKAWA et al., 2007); nevertheless, it presents significant variation among genotypes (MA et al., 2016; BIAN et al., 2015). The presence of a significant number of genes encoding MATE proteins and carrying a variety of substrates

Table 1 - Orthologous genes of TaALMT1 reported in various plant species.

Genes	Species	References
BnALMT1	Oilseed rape (Brassica napus)	LIGABA et al., 2006
AtALMT1	Arabidopsis (Arabidopsis thaliana)	KOBAYASHI et al., 2007
ScALMT1	Rye (Secale cereale)	COLLINS et al., 2008; FONTECHA et al., 2007
HvALMT1	Barley (Hordeum vulgare)	GRUBER et al., 2010
ZmALMT1	Corn (Zea mays)	PIÑEROSET al., 2008
MsALMT1	Alfalfa (Medicago sativa)	CHEN et al., 2013
HlALMT1	Common velvet grass (Holcus lanatus)	CHEN et al., 2013

suggests that transporters of this family must perform a number of biological roles in plants.

The *TaMATE1B* gene is located on the 4BL wheat chromosome, encoding a citrate-carrying transmembrane protein. RYAN et al. (2009) identified the Xce_c locus in the Brazilian cultivar Carazinho as being responsible for more than 50% of the total phenotypic variation of citrate efflux. The MATE family is important in various species, as demonstrated by its orthologues that have been described in recent years (Table 2).

According to LIU et al. (2009) the coding sequences of citrate transporters for monocotyledons (*SbMATE* and *HvMATE*) and for dicotyledons (*AtFRD3* and *AtMATE*) are highly conserved at the amino acid sequence level. In addition, they share a single topological structure, i.e., a large cytoplasmic loop in the N-terminal portion of the protein that distinguishes them from the rest of the members of the MATE family (LIU et al., 2009).

Some members of the MATE family have unique mechanisms. The *OsFRDL1* genes (*Ferric reductase defective-like 1*) and *GmMATE* encode proteins involved in iron translocation from the roots towards the aerial parts of rice and soybean, respectively (YOKOSHO et al., 2011; 2009). These proteins are located in pericycle cells and mediate the release of citrate to the xylem (YOKOSHO et al., 2009). When it comes into contact with iron, citrate forms a complex that is translocated towards the aerial part of the plants. In *Arabidopsis*, *AtFRD3* is also a citrate transporter, required for the translocation of Fe to the shoot; mutants in these transporters form Fe precipitates in the root (DURRETT et al., 2007).

The *LaMATE* gene derived from the lupine (*Lupinus albus* L.) was highly expressed under conditions of P deficiency and may play a role in citrate transport as an adaptive response to increase P availability in soils with low nutrient content (UHDE-STONE et al., 2005). Considering that toxic Al and P deficiency are two of the most important agricultural constraints in acid soils, the MATE family may have a broader role in adapting plants to soils with low pH (MAGALHAES et al., 2007).

The relationship between TaALMT1 and TaMATE1B

The *TaALMT1* and *TaMATE1B* genes confer tolerance to toxic Al through the release of OAs by the roots; this appears to be a striking example of functional co-evolution of Al tolerance by two carriers that are structurally but functionally quite different (KOCHIAN et al., 2015). Tandem repeats in the TaALMT1 promoter, as well as the insertion of a transposon in the *TaMATE1B* promoter region, have been related to levels of gene expression, efflux of OAs and Al^{3+} tolerance in several Brazilian wheat cultivars (PEREIRA et al., 2015). Among 300 Brazilian wheat cultivars tested, the highest relative root growth in response to Al was observed in the genotypes that showed promoters of *TaALMT1* type V and VI and the insertion of the *TaMATE1B* promoter (PEREIRA et al., 2015). ZHENG et al. (1998) observed that the release of malate by *TaALMT1* had a greater impact than did the release of citrate by *TaMATE1B*; although, citrate is eight times more efficient than malate in terms of Al^{3+} chelation. One reason is the amount of malate released is 10 times greater than that of citrate in tolerant wheat roots (RYAN et al., 2009).

Studies related to wheat *TaALMT1* genes (SASAKI et al., 2004), *SbMATE* in sorghum (MAGALHAES et al., 2007) and *HvMATE/HvAACT1*

Table 2 - Orthologous *TaMATE1B* genes reported in various plant species.

Genes	Species	References
ZmMATE1	Corn *(Zea mays)*	MARON et al., 2010
AtMATE1	Arabidopsis (*A. thaliana*)	LIU et al., 2009
VuMATE1	Bean (*Vignaum bellata*)	YANG et al., 2011
OsFRDL1	Rice (*Oryza sativa*)	YOKOSHO et al., 2011
SbMATE	Sorghum (*Sorgum bicolor*)	MAGALHAES et al., 2007
HvMATE (*HvAACT1*)	Barley (*Hordeum vulgare*)	FURUKAWA et al., 2007; BIAN et al., 2015
ScAACT1	Rye (*Secale cereale*)	SILVA-NAVAS et al., 2012
GmMATE	Soybean (*Glycine max*)	YOKOSHO et al., 2009
MtMATE	Barrelmedic (*Medicago truncatula*)	CHANDRAN et al., 2008
LaMATE	Lupine (*Lupinus albus*)	UHDE-STONE et al., 2005

in barley (BIAN et al., 2015; FURUKAWA et al., 2007) suggested that these loci present a greater effect on Al^{3+} tolerance and that this has a monogenic inheritance in these species. Nevertheless, there are several cases where an apparent transgressive segregation can be observed (FONTECHA et al., 2007), raising the possibility that this type of inheritance can be much more complex and that other important genes may be yet unidentified in these species (GARCIA-OLIVEIRA et al., 2015; KOCHIAN et al., 2015).

Other regions of interest in the wheat genome

Technology directed to studies in plant biotechnology has evolved exponentially. The availability and ease of obtaining various mapping populations associated with more robust molecular markers not only facilitates the identification of various genetic loci involved in complex features, but also helps to understand how genes act on a particular characteristic. The QTL (Quantitative trait loci) mapping has helped many geneticists to identify regions of interest within the genome of several plant species. Numerous studies have been carried out using various types of mapping populations, derived from several parental genotypes (MA et al., 2016; BIAN et al., 2015; YOKOSHO et al., 2011; RYAN et al., 2009). Such studies demonstrate that there are one to three QTLs involved in wheat tolerance to Al among the studied populations.

Identification of QTLs without the need for bi parental mapping populations has become possible due to the use of Genome-wide association mapping (GWAS), representing a complementary approach to linkage mapping studies. GWAS is based on binding disequilibrium in genetically different germplasms. Using this technology, RAMAN et al. (2010) identified at least 16 genetic loci for tolerance to toxic Al in wheat chromosomes 1A, 1B, 2A, 2B, 2D, 3A, 3B, 4A, 4B, 4D, 5B, 6A, 6B, 7A and 7B. These data demonstrated the genetic diversity among genotypes in relation to tolerance to toxic Al^{3+} and suggested the existence of other genes in addition to *TaALMT1* and *TaMATE1B* acting in the wheat genome.

CONCLUSION

The primary agricultural regions in southern Brazil naturally present soils with acidic pH and high concentrations of Al^{3+}. Therefore, OA transporters play key roles in tolerance to toxic Al^{3+} in various species of agricultural interest, especially in winter cereals such as wheat. Transmembrane proteins *TaALMT1* and *TaMATE1B*, carriers of OAs such as malate and citrate, respectively, are the main sources of Al tolerance in wheat genotypes. Induction of these by P limitation and its subsequent involvement in the reduction of root elongation still requires demonstration in wheat. The use of molecular tools associated with the phenotype allows the rapid identification and selection of genotypes with various levels of Al tolerance.

DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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