PLANT SIGNALING & BEHAVIOR
2017, VOL. 12, NO. 1, e1276688 (4 pages)
http://dx.doi.org/10.1080/15592324.2016.1276688

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SHORT COMMUNICATION

Regulating cytoplasmic oxalate homeostasis by Acyl activating enzyme3 is critical for plant Al tolerance

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ABSTRACT

Oxalic acid is the simplest of the dicarboxylic acids. In addition to its role in biological and metabolic processes, oxalate has been implicated in biotic and abiotic stresses. Being a strong chelator of Al, oxalate also has pivotal role in Al resistance mechanisms. However, we demonstrated that cytoplasmic oxalate accumulation is a critical event leading to root growth inhibition under Al stress. Transcriptome analysis from three crop plants identified *Acyl Activating Enzyme3* (*AAE3*) genes to be upregulated by Al stress. These AAE3 proteins display high sequence identity to known AAE3 proteins, suggesting they are oxalyl-CoA synthetases specifically involved in oxalate degradation. However, phylogenetic analysis revealed divergence of AAE3 between monocots and dicots, pointing to the necessity for functional characterization of AAE3 proteins from other plant species with respect to Al stress.

ARTICLE HISTORY
Received 9 December 2016
Revised 20 December 2016
Accepted 20 December 2016

KEYWORDS
Acyl activating enzyme; aluminum toxicity; acid soil; organic acids; oxalate; transcriptome

Aluminum toxicity constraints severely crop production on acid soils which comprise approximately 50% of world potentially arable lands.[1] However, many native plants and some crops thrive on these acidic soils, implicating that they have evolved sophisticated mechanisms to deal with Al toxicity. In general, plant Al resistance mechanisms can be classified into external exclusion mechanism or internal tolerance mechanism.[2,3] The external exclusion mechanisms are those that prevent Al from entering the root apex (both apoplasm and symplasm), and internal tolerance mechanisms refer to those that detoxify and sequester Al once it enters the plant.[2,3] Having strong binding capacity to Al and being ubiquitous in plant cells, organic acid anions, mainly citrate, malate and oxalate, are key component of both mechanisms.[3,4,5]

Before the cloning and characterization of genes encoding transporters that facilitate malate and citrate exudation, many studies have focused on improvement of plant Al resistance through genetic manipulation of malate and citrate biosynthesis.[6] Although some reports remain uncertain,[7] others believe that increasing OA content will improve Al resistance either through increased exudation or internal detoxification.[8,9,10,11,12] However, the information on the relationship between oxalate content and Al resistance is still lacking.

Intuitively, increasing oxalate content in root cells will be beneficial for Al resistance. However, we previously demonstrated that oxalate accumulation is harmful for root growth in response to Al stress in rice bean (*Vigna umbellata*).[13] We further identified a gene *VuAAE3* (*Vigna umbellata Acyl Activating Enzyme3*) that converts oxalate to oxalyl-CoA, thereby preventing oxalate toxicity induced by Al stress.[13] Although *in silico* analysis revealed that AAE3 proteins seem to be conserved among plant species, more evidence is required to support the viewpoint that AAE3 proteins-dependent Al resistance mechanism is conserved among plant species. In the present study, we provided more compelling evidence that regulating of cytoplasmic oxalate homeostasis by AAE3 proteins provides additional layer of Al resistance mechanisms in plants.

We have carried out transcriptome analysis of Al-responsive genes from crops including rice bean, common buckwheat (*Fagopyrum esculentum*), and amaranth (*Amaranthus hypochondriacus*) (unpublished data). From upregulated genes, one of consistently identified were *AAE3* genes (Fig. 1). In a previous study, we found that VuAAE3 transcripts were frequently identified in the upregulated suppression subtractive hybridization libraries both under low and high Al stress conditions.[14] To date, AAE3 proteins from rice bean, *Medicago truncatula*, and Arabidopsis (*Arabidopsis thaliana*) have been characterized as oxalyl-CoA synthetases.[13,15,16] Here, amino acid sequence alignment revealed that AAE3 proteins from common buckwheat and amaranth display high identity with known AAE3 proteins, all having a conserved AMP binding domain and acetyl-CoA synthetase domain (Fig. 2). Although the biochemical and molecular biological characterization of FeAAE3 and AhAAE3 proteins has to be investigated, it is reasonable to suggest that they also function as oxalyl-CoA synthetases. Given that AAE3 protein is characterized by having activities specific to oxalate,[13,15,16] the upregulation of AAE3 genes suggests that oxalate accumulates under Al stress.

It is worth to note that common buckwheat and amaranth plants are oxalate accumulators.[17] However, both plant species have evolved AAE3-dependent regulation of cytoplasmic

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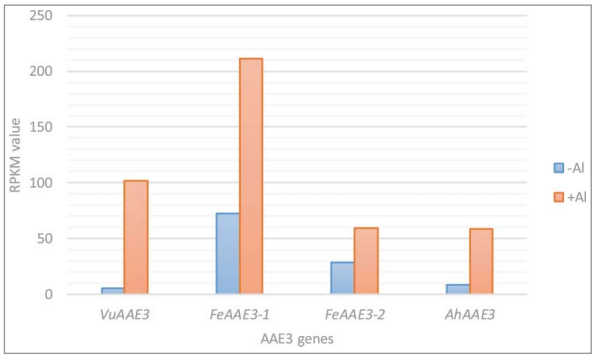

Figure 1. AAE3 gene transcription in response to Al stress in rice bean (*VuAAE3*), buckwheat (*FeAAE3-1* and *FeAAE3-2*), and amaranth (*AhAAE3*). The data was based on the transcriptome analysis of roots under Al stress (25 μM, 6 h for rice bean, 20 μM, 6 h for buckwheat, and 10 μM, 6 h for amaranth). The expression level expressed as RPKM (the Reads Per kb Million reads) value.

oxalate content under Al stress, suggesting that cytoplasmic oxalate content must be tightly controlled. Clearly, free oxalate in cytosol is toxic, because it represents a strong acid, reductant, and chelator.[18] In line with our expectations, transgenic Arabidopsis plants overexpressing a bacterial oxalic acid biosynthetic enzyme gene displayed not only significant increase in oxalate content, but also a reduction in plant stature as well as a pronounced delay in bolting and seed set.[19]

In addition to AAE3-dependent degradation of oxalate, oxalate can be oxidized into CO_2 and H_2O_2 by oxalate oxidase that belongs to germin protein family. However, it seems that oxalate oxidase is only present in monocots. Thus, question arises as to whether AAE3 proteins from monocots also play important role in regulating cytoplasmic oxalate homeostasis, because phylogenetic analysis clearly indicated that AAE3 proteins are evolutionally separated between dicots and

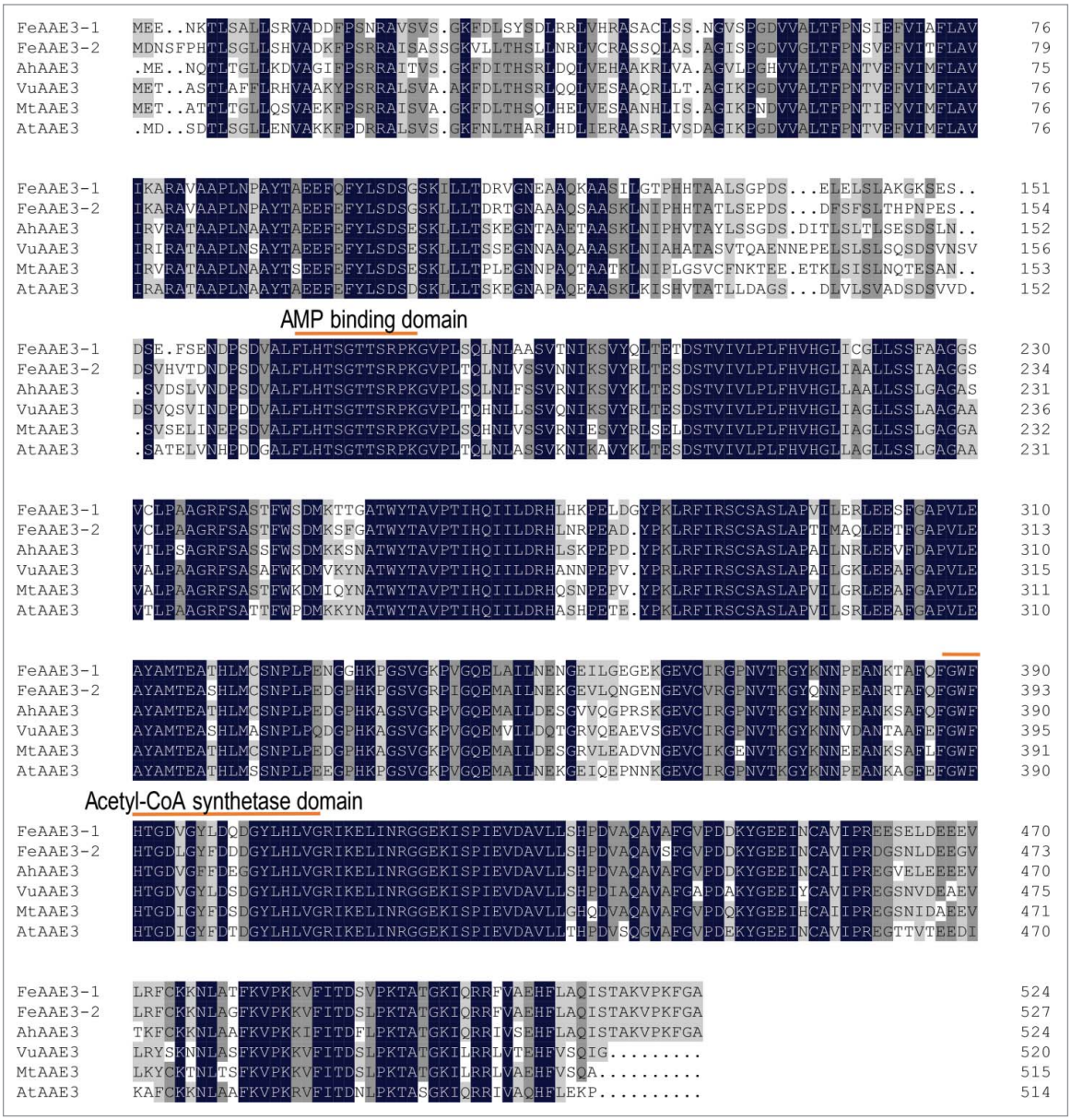

Figure 2. Amino acid sequence alignment of AAE3 proteins from rice bean (VuAAE3), Arabidopsis (AtAAE3), *Medicago truncatula* (MtAAE3), *Amaranthus hypochondriacus* (AhAAE3), and *Fagopyrum esculentum* (FeAAE3-1 and FeAAE3-2). The conserved AMP binding domain and acetyl-CoA synthetase domain are indicated.

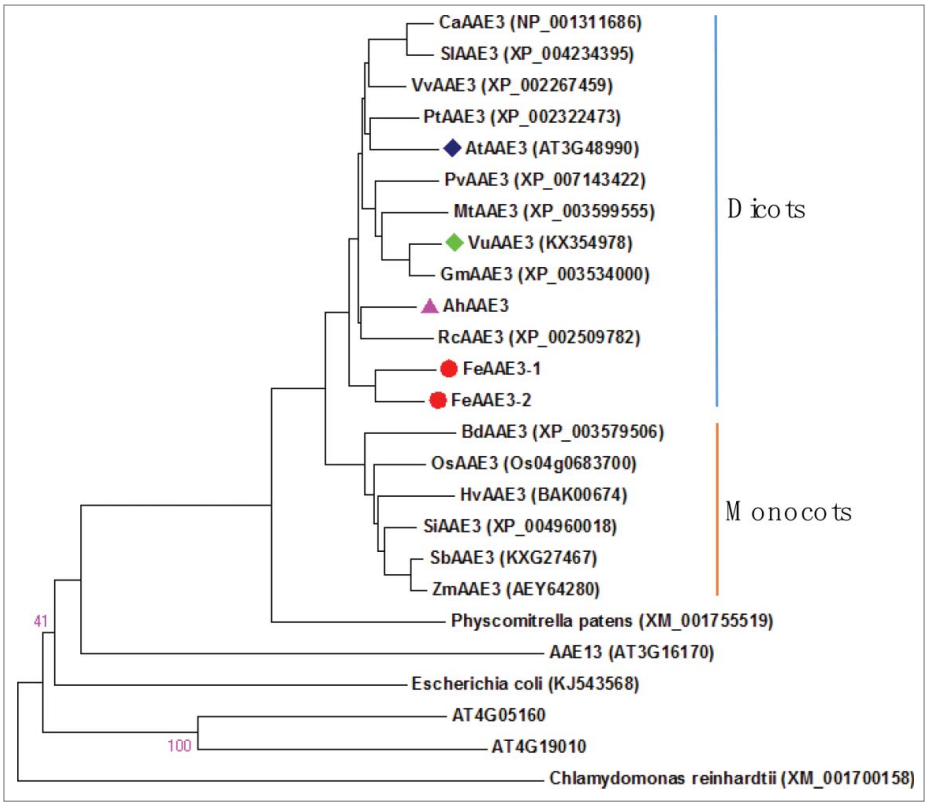

Figure 3. Evolutional relationship of AAE3 proteins. AAE3 proteins are derived from dicots: *Capsicum annuum* (CaAAE3), *Solanum lycopersicum* (SlAAE3), *Vitis vinifera* (VvAAE3), *Amaranthus hypochondriacus* (AhAAE3), *Populus trichocarpa* (PtAAE3), *Arabidopsis thaliana* (AtAAE3, AtAAE13, At4g05160, and At4g19010), *Phaseolus vulgaris* (PvAAE3), *Ricinus communis* (RcAAE3), *Medicago truncatula* (MtAAE3), *Vigna umbellate* (VuAAE3), *Glycine max* (GmAAE3), *Fagopyrum esculentum* (FeAAE3-1 and FeAAE3-2); monocots: *Brachypodium distachyon* (BdAAE3), *Oryza sativa* (OsAAE3), *Setaria italic* (0SiAAE3), *Hordeum vulgare* (HvAAE3), *Sorghum bicolor* (SbAAE3), *Zea mays* (ZmAAE3); Embrophyte (*Physcomitrella patens*); Bacteria (*Escherichia coli*); Chlorophyte (*Chlamydomonas reinhardtii*).

monocots (Fig. 3). In the future, it would be necessary to characterize the role of AAE3 proteins from buckwheat and amaranth as well as those from monocots in Al resistance.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by grant from the 973 Project (2014CB441002), the Natural Science Foundation of China (31572193; 31222049; 31601765), and China Scholarship Council.

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