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Ammonium and urea transporter inventory of the *Selaginella* and *Physcomitrella* genomes

Roberto De Michele*[1,2]*, Dominique Loqué*[3]*, Sylvie Lalonde*[1] *and Wolf B. Frommer*[1]**

[1] *Department of Plant Biology, Carnegie Institution for Science, Stanford, CA, USA*
[2] *Plant Genetics Institute, National Research Council of Italy, Palermo, Italy*
[3] *Joint BioEnergy Institute, Emeryville, CA, USA*

Edited by:
Amy Litt, The New York Botanical Garden, USA

Reviewed by:
Biao Ding, The Ohio State University, USA
Stefan A. Rensing, University of Freiburg, Germany

****Correspondence:***
Wolf B. Frommer, Department of Plant Biology, Carnegie Institution for Science, 260 Panama Street, Stanford, CA 94305, USA.
e-mail: wfrommer@carnegiescience.edu

Ammonium and urea are important nitrogen sources for autotrophic organisms. Plant genomes encode several families of specific transporters for these molecules, plus other uptake mechanisms such as aquaporins and ABC transporters. *Selaginella* and *Physcomitrella* are representatives of lycophytes and bryophytes, respectively, and the recent completion of their genome sequences provided us with an opportunity for comparative genome studies, with special emphasis on the adaptive processes that accompanied the conquest of dry land and the evolution of a vascular system. Our phylogenetic analysis revealed that the number of genes encoding urea transporters underwent a progressive reduction during evolution, eventually down to a single copy in vascular plants. Conversely, no clear evolutionary pattern was found for ammonium transporters, and their number and distribution in families varies between species. In particular *Selaginella*, similar to rice, favors the AMT2/MEP family of ammonium transporters over the plant-specific AMT1 type. In comparison, *Physcomitrella* presents several members belonging to both families.

Keywords: *Selaginella, Physcomitrella*, ammonium, urea, transporter, uptake

INTRODUCTION

Of all mineral elements required by plants, nitrogen is quantitatively the most important and thus often growth-limiting for many plants. Nitrogen is found in many organic compounds such as amino acids, nucleic acid, and consequently in proteins, as well as in nucleic acids. In most soils, nitrogen is heterogeneously distributed and found in various forms such as ammonium, nitrate, urea, amino acids, peptides, and even in water-insoluble forms (Jackson et al., 1990). Among the different nitrogen sources, ammonium is often preferred by plants, since its assimilation requires less energy. Moreover, ammonium is the predominant nitrogen form in anoxic soils, such as paddy rice fields, and it is abundantly used as fertilizer.

Due to the impermeability or poor permeability of the lipid bilayer of the plasma membrane to most nutrients including nitrogen compounds, uni and multicellular organisms developed a suite of nitrogen transporters. In 1979, electrophysiological studies in the unicellular algae *Chara australis* showed a positive inward current across the plasma membrane induced by ammonium addition (Walker et al., 1979). Fifteen years later, the first ammonium transporter, *AtAMT1;1* from *Arabidopsis*, was cloned by yeast complementation (Ninnemann et al., 1994), in parallel with the yeast MEP orthologs (Marini et al., 1994). Since then, many para and orthologs were isolated either by yeast complementation or via homology cloning (Lauter et al., 1996; Gazzarrini et al., 1999; Simon-Rosin et al., 2003; Sonoda et al., 2003). Homologs have been found in bacteria, fungi, algae, plants, and animals (Marini et al., 1997, 2000; Gonzalez-Ballester et al., 2004) and the transport of the charged form of ammonium (NH_4^+) was confirmed for the plant ammonium transporter AMT1;1 by electrophysiological characterization in *Xenopus* oocytes (Ludewig et al., 2002; Wood et al., 2006; Loqué et al., 2009).

Ammonium transporters fall into three subfamilies, Rhesus, Mep/AMTB (also called AMT2), and AMT1. Plant ammonium transporters belong to the two subclasses: AMT1 and AMT2 (Sohlenkamp et al., 2002). The AMT2 members are sequence-wise more closely related to fungal and bacterial ammonium transporters (Mep and AMTB) forming the Mep/AMTB/AMT2 subfamily (Ludewig et al., 2001). Bacterial AMTs, from *Escherichia coli* and from the archaebacterium *Archaeoglobus fulgidus*, have been crystallized, revealing a trimeric structure (Khademi et al., 2004; Andrade et al., 2005; Conroy et al., 2007).

In both Eubacteria and Archaea, AMT activity is regulated by the interaction with GlnK proteins (Coutts et al., 2002). Importantly, at least the bacterial AMTs, similar to the plant nitrate transporter CHL1 and the yeast glucose and amino acid transporters RGT2, SNF1, and GAP1 have a dual function as sensors and transporters, and thus can be defined as transceptors (Hyde et al., 2007; Ho et al., 2009; Thevelein and Voordeckers, 2009). Therefore, at least the bacterial AMTs link energy and nitrogen status of the cell with ammonium uptake to control ammonium-dependent transcription (Tremblay and Hallenbeck, 2009). Plants also contain GlnK homologs, however these are confined to the chloroplast (Hsieh et al., 1998). In plants, plasma membrane AMT activity is regulated by an allosteric auto-inhibition driven by the conserved cytosolic C-terminal tail, which connects the adjacent members in the trimeric complex (Loqué et al., 2007, 2009; Neuhauser et al., 2007; Lanquar et al., 2009; Lanquar and Frommer, 2010). An increase in the concentration of extracellular ammonium leads to the phosphorylation of a specific threonine residue in the cytosolic C-terminus of AtAMT1;1, resulting in closure of the pores of the transporter complex (Loqué et al., 2007; Lanquar et al., 2009). Since the C-terminus, and this regulatory threonine in particular, is highly conserved in plant AMT1s, it is conceivable that this feedback mechanism represents a common feature of plant ammonium transporters. We recently demonstrated that the *Archaeglobus* AMT1 also uses a *trans*-activation allosteric mechanism involving the conserved C-terminus, indicating that this unique regulatory system was developed early in evolution and has been maintained. This was surprising, because point mutations in the pore region were found that release the transporter from the strict dependence on *trans*-activation (Loqué et al., 2009). Such point mutations must have occurred during evolution, but apparently have not been fixed, suggesting a strong pressure to retain this regulatory mechanism, potentially to protect against accumulation of toxic levels of ammonium.

Urea is another nitrogen source used by plants that is commonly available in nature. Urea is mainly derived from decomposition of nitrogen-containing molecules and from urine secretion by animals. Additionally, urea is widely used in agriculture as a fertilizer due to its stability, low toxicity and cost–effective production.

Urea transporters are present in most organisms and belong to different classes, some functioning as passive channels, others as secondary active transporters. Interestingly, urea transporters commonly found in animals and bacteria (UT, UreI, Yut) are absent in plant and fungal genomes (Wang et al., 2008). Plant and fungal genomes encode a different family of urea transporters, called DUR3. In 1993 the first plant urea transporter (*Dur3*) was cloned from *Arabidopsis* by homology to the yeast transporter *DUR3* (Elberry et al., 1993). DUR3 proteins are secondary active transporters, structurally belonging to the sodium solute symporter (SSS) superfamily (Wang et al., 2008). Electrophysiological studies showed that a proton, rather than sodium, is co-transported with urea by AtDUR3 (Liu et al., 2003). *In planta* characterization showed that *DUR3* genes from *Arabidopsis* and rice are induced by nitrogen deficiency (Liu et al., 2003; Wang et al., 2011). DUR3s have an important role in high affinity urea transport under nitrogen limiting growth conditions (Kojima et al., 2007; Wang et al., 2011). DUR3 is not the only mechanism for urea uptake, since several aquaporins in the TIP and NOD26 families have been shown to facilitate urea import through the plasma membrane (for review, see Kojima et al., 2006). Additionally, ABC transporters are known to transport urea in cyanobacteria (Valladares et al., 2002). Typically, plant genomes contain > 100 ABC transporter homologs; whether any of these transport urea remains an open question.

The genome sequence of the lycophyte *Selaginella moellendorffii* (Banks et al., 2011) provides a valuable tool for comparative genomic studies in plants. The lineage of this species arose ∼400 millions years ago early after vascular plants had evolved, and shares many primitive traits with bryophytes. This species adds to the increasing list of plants for which genome sequences have become available, including the moss *Physcomitrella patens* (Rensing et al., 2008).

Here, we compared the ammonium and urea transporter homologs of two dicots (*Arabidopsis* and poplar), one monocot (rice) a lycophyte (*Selaginella*), a bryophyte (*Physcomitrella*), and a unicellular alga (*Chlamydomonas reinhardtii*) with the aim of gaining more insight into the evolution of these important transporters. For *Arabidopsis*, poplar, rice and *Chlamydomonas*, *AMT* and *DUR3* members have already been annotated and characterized elsewhere, although in the case of poplar annotation is still not complete (**Table 1**; Loqué and von Wirén, 2004; Cuturier et al., 2006; Fernandez and Galvan, 2007; Wang et al., 2008; Li et al., 2009). Our results indicate a progressive reduction of the number of genes encoding urea transporters during higher plant evolution, possibly suggesting a shift in nutrient form preference of angiosperms for other nitrogen sources. Analysis of number and distribution of ammonium transporters between the AMT1 and AMT2 families revealed that most species, with the exception of *Physcomitrella*, present a bias for one or the other subfamily (*Arabidopsis*: AMT1, *Selaginella* and rice: AMT2).

MATERIALS AND METHODS

Protein sequences of the six members of *Arabidopsis* AMTs and AtDUR3 were used to retrieve homologs of the ammonium and

Table 1 | Comparison of number of homologs for ammonium and urea transporters in various plant and algal species.

			Number of putative homologs					
Gene function	**Gene family**	**Protein used as query**	***Arabidopsis***	**Poplar***	**Rice**	***Selaginella***	***Physcomitrella***	***Chlamydomonas***
Ammonium transport	AMT1	AtAMT1;1	5	7 + 1 pseudo	3	1	6	8
Ammonium transport	AMT2	AtAMT2	1	8 + 3 putative + 11 pseudo	9 + 1 pseudo	3	7	0
Ammonium transport	Rh	HsRhAG	0	0	0	0	0	2
Urea transport	Dur3	AtDUR3	1	1	1	1	2	3

**The genome of poplar contains several short pseudogenes for AMTs and three full length putative AMT2 genes which have not been annotated yet.*

urea transporter families in *Selaginella*, *Physcomitrella*, rice, poplar, and *Chlamydomonas* (**Table 1**). For poplar, only the paralogs already annotated as AMT (Cuturier et al., 2006) were further considered in the phylogenic analysis. Sequences were blasted against a translated-nucleotide sequence database in the Ensembl Genome browser (www.ensemblgenomes.org). Searches for the urea transporter classes UT-A, -B, -C retrieved no hits in the plant databases. Within a species, coding and flanking sequences of the paralogs were carefully analyzed to distinguish between gene duplications and alleles. Protein sequences were aligned by ClustalW and poorly conserved N- and C-terminal domains (corresponding to amino acids 1–39 and 475–501 for AtAMT1;1 and 1–27 and 660–704 for AtDUR3) were trimmed (**Figures A1** and **A2** in Appendix). Phylogenetic trees were created on the basis of these alignments by the Mega5.05 program (Tamura et al., 2011) using a Neighbor-Joining, Poisson-corrected method, with pairwise deletion option and 1000 bootstraps. Additional trees were generated by Maximum-Likelihood method, Poisson-corrected, with 1000 bootstraps (**Figures A3** and **A4** in Appendix).

RESULTS AND DISCUSSION

Analysis of the *S. moellendorffii* genome indicates the presence of four ammonium transporters, with little variation between the two haplotypes sequenced (**Tables 1** and **2**). One AMT (*SmAMT1*) belongs to the AMT1 family and are closely related to the *Physcomitrella* AMTs (**Figure 1**; **Figure A3** in Appendix). The three other *Selaginella* AMTs fall into the AMT2 subfamily.

The genome of the moss *P. patens* revealed a higher and more diversified number of ammonium transporters, with six members of the AMT1 subfamily and seven members of the AMT2 subfamily (**Table 1**). AMT paralogs often differ with respect to affinities for ammonium and regulation. The existence of multiple paralogs may either help to increase transport capacity, ensure flexibility for regulation, and help cover a wider range of ammonium levels. In both AMT1 and AMT2 clades, the members of the *Physcomitrella* AMTs group closely together, suggesting a recent origin of the paralogs within the group (**Figure 1**; **Figure A3** in Appendix). Three of the AMT1 members (named *PpAMT1;1*, *PpAMT1;2*, and *PpAMT1;3*), highly similar, correspond to recent gene duplications as they occur in tandem at the same genetic locus. The persistence of several paralogs in *Physcomitrella* is not limited to the AMT family, and in fact it seems to be a common feature derived from genome duplication events (Lang et al., 2005; Rensing et al., 2007).

The unicellular alga *C. reinhardtii* has eight AMTs, all belonging to the AMT1 family, and sharing only 31–46% identity with AMT1s of *Arabidopsis* or *Selaginella*. Two of them (*CrAMT-E* and *-G*) also show alternative splicing (Fernandez and Galvan, 2007). Regarding phylogeny, they fall into three subclades, separated from other plant genes. This divergence is emphasized also by the lack of the conserved regulatory threonine in the C-terminus (Loqué et al., 2007). Other residues, usually conserved in AMT1s but missing or misplaced in some *Chlamydomonas* AMTs are the N-terminal cysteines, which have been shown to be important to promote interactions among monomers in the trimeric complex

Table 2 | List of predicted ammonium and urea transporters in *Selaginella* and *Physcomitrella*.

Species	Gene and allele name	Locus identifier in Ensembl Genome	Protein (aa)
Selaginella	*SmAMT1*	SELMODRAFT_163770 SELMODRAFT_169094	513
	SmAMT2.1	SELMODRAFT_93278 SELMODRAFT_108685	443
	SmAMT2.2	SELMODRAFT_120477 SELMODRAFT_84585	486
	SmAMT2.3	SELMODRAFT_150679 SELMODRAFT_173475	483
Physcomitrella	*PpAMT1.1*	XM_001758548	504
	PpAMT1.2	XM_00178547	503
	PpAMT1.3	XM_001758551	505
	PpAMT1.4	XM_001785448	505
	PpAMT1.5	XM_001786003	495
	PpAMT1.6	XM_001758559	511
	PpAMT2.1	XM_001762751	475
	PpAMT2.2	XM_001752462	490
	PpAMT2.3	XM_001754186	493
	PpAMT2.4	XM_001770002	498
	PpAMT2.5	XM_001754764	494
	PpAMT2.6	XM_001781152	495
	PpAMT2.7	XM_001778469	461
Selaginella	*SmDUR3*	SELMODRAFT_135570 SELMODRAFT_172808	678/679
Physcomitrella	*PpDUR3A*	XM_001779344	713
	PpDUR3B	XM_001784492	678

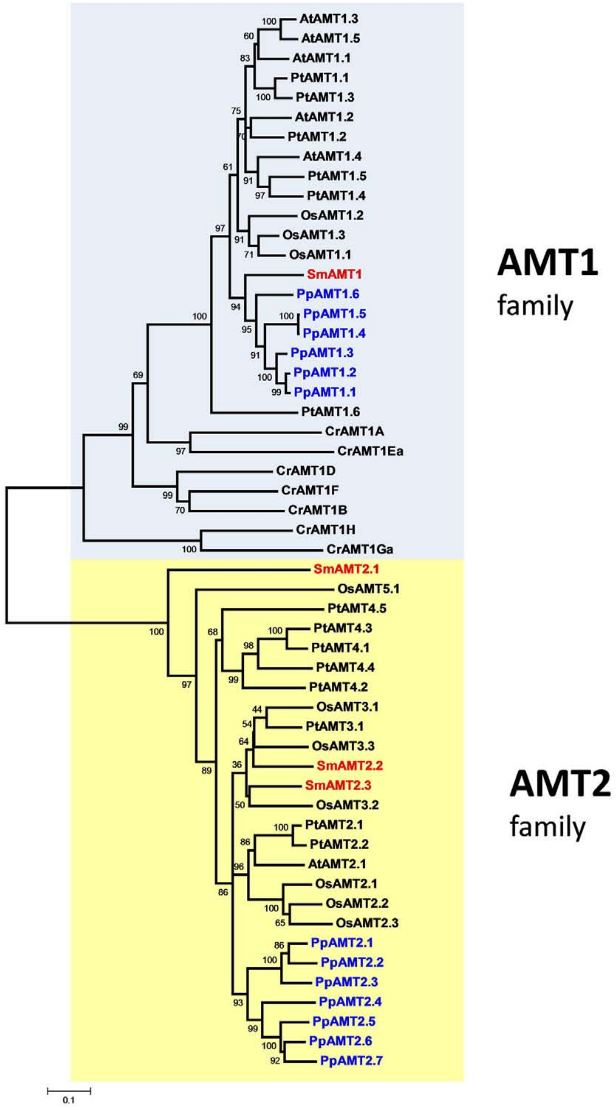

FIGURE 1 | Unrooted phylogenetic tree of the ammonium transporters in *Arabidopsis thaliana* (At), *Populus trichocarpa* (Pt), *Oryza sativa* (Os), *Selaginella moellendorffii* (Sm), *Physcomitrella patens* (Pp), and *Chlamydomonas reinhardtii* (Cr). Red indicates *Selaginella* AMTs, blue *Physcomitrella.* The tree was constructed by aligning the protein sequences by ClustalW and generated with the Neighborhood-Joining method.

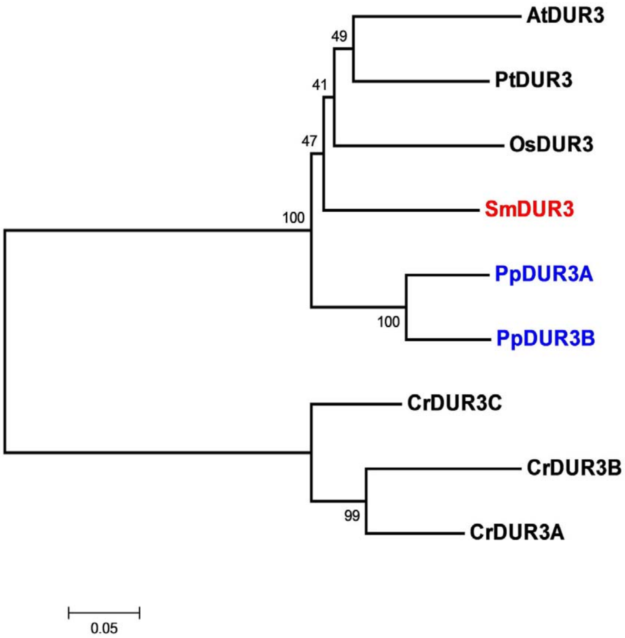

FIGURE 2 | Unrooted phylogenetic tree of the urea transporters in *Arabidopsis thaliana* (At), *Populus trichocarpa* (Pt), *Oryza sativa* (Os), *Selaginella moellendorffii* (Sm), *Physcomitrella patens* (Pp), and *Chlamydomonas reinhardtii* (Cr). Red indicates *Selaginella* DUR3, blue *Physcomitrella.* The tree was constructed by aligning the protein sequences by ClustalW and generated with the Neighborhood-Joining method.

(Graff et al., 2011). It will be interesting to study how AMTs in this organism assemble and are regulated. Apart from the AMT genes, the genome of *Chlamydomonas* also contains homologs of the animal Rhesus ammonia transporters (Rh), that are distantly related to the AMT family (Soupene et al., 2004). Rh genes are however absent in *Selaginella*, *Physcomitrella*, and in any plant species sequenced so far.

An interesting observation is that the proportion of AMT1 and AMT2 genes in the various species is different (**Table 1**). The *Arabidopsis* genome possesses five AMT1 members and one AMT2, but in rice the proportion is inverted (three AMT1s and nine AMT2s). Similarly, the *Selaginella* genome is more enriched in AMT2s (three members) and a single AMT1. Similar to poplar, *Physcomitrella* is more balanced, with six AMT1 and seven AMT2 types; finally, *Chlamydomonas* is so far the species containing more AMT1 members (eight) and totally lacking the AMT2 type. It is interesting to observe that the C-terminus of the AMT1 family is highly conserved also in *Selaginella* and *Physcomitrella* (but not *Chlamydomonas*) as is the equivalent of threonine-460 in the C-terminus AtAMT1;1, which is involved in ammonium-dependent allosteric regulation (Loqué et al., 2007; Lanquar et al., 2009), suggesting that the regulatory mechanism via phosphorylation of AMT1 members is maintained in all multicellular plants.

UREA TRANSPORTER GENE FAMILY

Analysis of the *Selaginella* genome suggests the presence of a unique *DUR3* homolog (named *SmDUR3*), similar to the one found in *Arabidopsis,* poplar, or rice genomes (**Table 1**). Whereas vascular plants have a single urea transporter, the moss *Physcomitrella* possesses two DUR3 homologs (*PpDUR3A* and *B*), with 85% identity between each other. Going further back in the evolutionary tree, *Chlamydomonas* has three different copies, named *DUR3A, B* and *C,* more closely related to each other (about 80% identity) than with the DUR3 members of multicellular plants (**Figure 2**; **Figure A4** in Appendix). Other algae, as well as fungi, are known to have additional copies of *DUR3* genes (Kakinuma et al., 2008; Morel et al., 2008).

The progressive loss of genes for urea transporters could indicate that the evolution of the vascular system shifted the preference for other nitrogen sources, such as ammonium, nitrate, and amino acids, all present in the xylem sap of vascular plants (Smirnoff and Stewart, 1985; Atkins, 2000; Schjoerring et al., 2002).

CONCLUSION

The comparative analysis of ammonium transporters revealed that different lineages of plants vary in the number of AMTs belonging to either family, and in many cases they show a bias toward one of the two AMT subfamilies, with some organisms particularly enriched in the plant-specific AMT1 clade (*Arabidopsis*, *Chlamydomonas*), while others rely more on the AMT2 family, typical for bacteria and fungi. The physiological and evolutionary relevance of these shifts remains to be determined. The number of urea transporters decreased during evolution, maybe indicating a preference of vascular plants for other nitrogen sources or the evolution of yet uncharacterized urea transporters.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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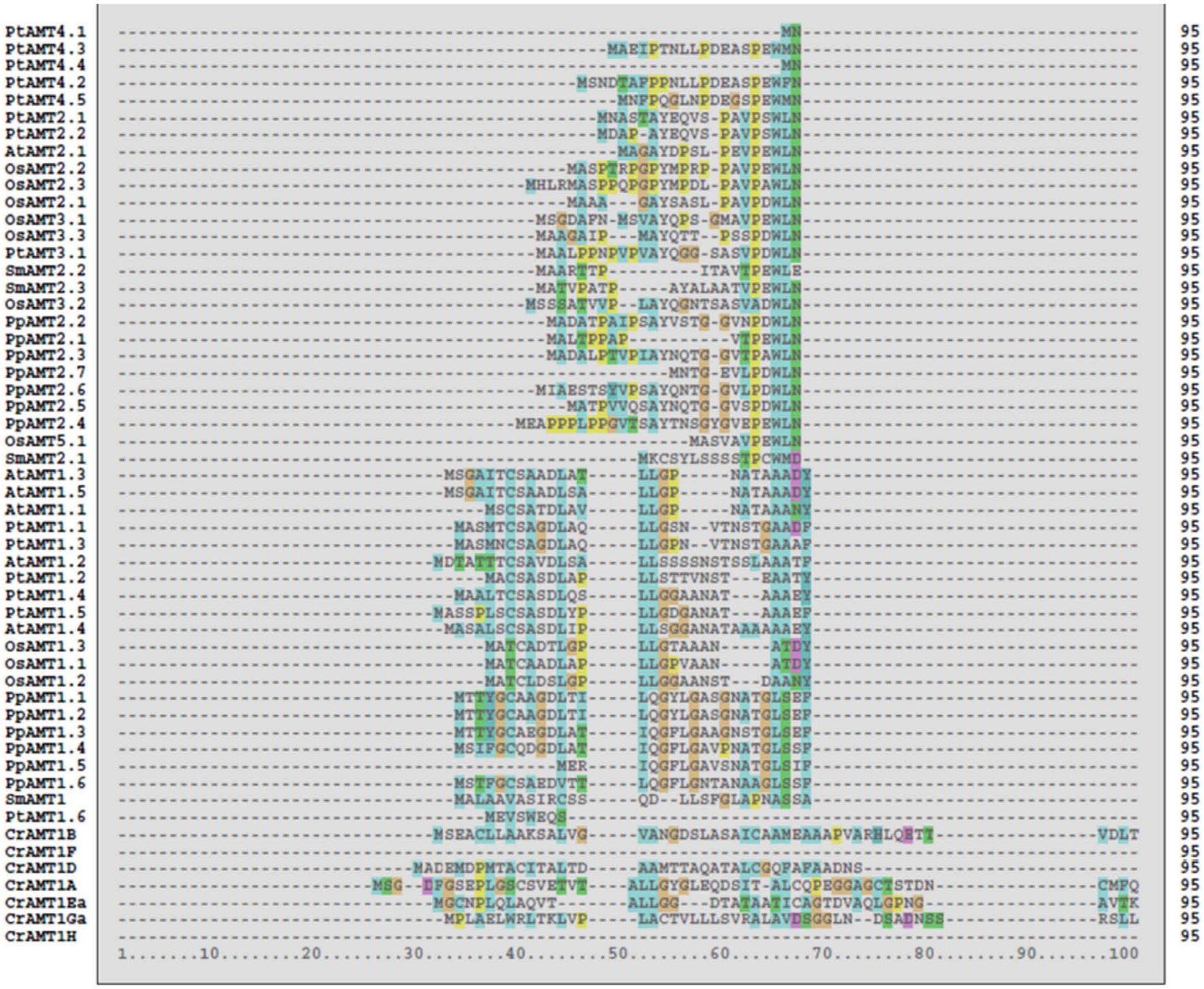
APPENDIX



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PtAMT4.5    -------------------------------------------------MNFPQGLNPDEGSPEWMN--------------------------------- 95
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AtAMT2.1    -------------------------------------------------MAGAYDPSL-PEVPEWLN--------------------------------- 95
OsAMT2.2    --------------------------------------------MASPTRPGPYMPRP-PAVPEWLN--------------------------------- 95
OsAMT2.3    ----------------------------------------MHLRMASPPQPGPYMPDL-PAVPAWLN--------------------------------- 95
OsAMT2.1    --------------------------------------------MAAA---GAYSASL-PAVPDWLN--------------------------------- 95
OsAMT3.1    -----------------------------------------MSGDAFN-MSVAYQPS-GMAVPEWLN--------------------------------- 95
OsAMT3.3    -----------------------------------------MAAGAIP---MAYQTT--PSSPDWLN--------------------------------- 95
PtAMT3.1    -----------------------------------------MAALPPNPVPVAYQGG-SASVPDWLN--------------------------------- 95
SmAMT2.2    -----------------------------------------MAARTTP---------ITAVTPEWLE--------------------------------- 95
SmAMT2.3    -----------------------------------------MATVPATP-----AYALAATVPEWLN--------------------------------- 95
OsAMT3.2    ----------------------------------------MSSSATVVP--LAYQGNTSASVADWLN--------------------------------- 95
PpAMT2.2    ------------------------------------------MADATPAIPSAYVSTG-GVNPDWLN--------------------------------- 95
PpAMT2.1    ------------------------------------------MALTPPAP----------VTPEWLN--------------------------------- 95
PpAMT2.3    ------------------------------------------MADALPTVPIAYNQTG-GVTPAWLN--------------------------------- 95
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PpAMT2.5    --------------------------------------------MATPVVQSAYNQTG-GVSPDWLN--------------------------------- 95
PpAMT2.4    ---------------------------------------MEAPPPLPPGVTSAYTNSGYGVEPEWLN--------------------------------- 95
OsAMT5.1    --------------------------------------------------------MASVAVPEWLN--------------------------------- 95
SmAMT2.1    ---------------------------------------------------MKCSYLSSSSTPCWMD--------------------------------- 95
AtAMT1.3    --------------------------------MSGAITCSAADLAT-----LLGP-----NATAAADY-------------------------------- 95
AtAMT1.5    --------------------------------MSGAITCSAADLSA-----LLGP-----NATAAADY-------------------------------- 95
AtAMT1.1    ------------------------------------MSCSATDLAV-----LLGP-----NATAAANY-------------------------------- 95
PtAMT1.1    ---------------------------------MASMTCSAGDLAQ-----LLGSN--VTNSTGAADF-------------------------------- 95
PtAMT1.3    ---------------------------------MASMNCSAGDLAQ-----LLGPN--VTNSTGAAAF-------------------------------- 95
AtAMT1.2    -------------------------------MDTATTTCSAVDLSA-----LLSSSSNSTSSLAAATF-------------------------------- 95
PtAMT1.2    ------------------------------------MACSASDLAP-----LLSTTVNST---EAATY-------------------------------- 95
PtAMT1.4    ---------------------------------MAALTCSASDLQS-----LLGGAANAT---AAAEY-------------------------------- 95
PtAMT1.5    -------------------------------MASSPLSCSASDLYP-----LLGDGANAT---AAAEF-------------------------------- 95
AtAMT1.4    --------------------------------MASALSCSASDLIP-----LLSGGANATAAAAAAEY-------------------------------- 95
OsAMT1.3    ------------------------------------MATCADTLGP-----LLGTAAAN-----ATDY-------------------------------- 95
OsAMT1.1    ------------------------------------MATCAADLAP-----LLGPVAAN-----ATDY-------------------------------- 95
OsAMT1.2    ------------------------------------MATCLDSLGP-----LLGGAANST---DAANY-------------------------------- 95
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PpAMT1.2    ---------------------------------MTTYGCAAGDLTI-----LQGYLGASGNATGLSEF-------------------------------- 95
PpAMT1.3    ---------------------------------MTTYGCAEGDLAT-----IQGFLGAAGNSTGLSEF-------------------------------- 95
PpAMT1.4    ---------------------------------MSIFGCQDGDLAT-----IQGFLGAVPNATGLSSF-------------------------------- 95
PpAMT1.5    -------------------------------------------MER-----IQGFLGAVSNATGLSIF-------------------------------- 95
PpAMT1.6    ---------------------------------MSTFGCSAEDVTT-----LQGFLGNTANAAGLSSF-------------------------------- 95
SmAMT1      ---------------------------------MALAAVASIRCSS-----QD--LLSFGLAPNASSA-------------------------------- 95
PtAMT1.6    ------------------------------------MEVSWEQS-------------------------------------------------------- 95
CrAMT1B     -------------------------------MSEACLLAAKSALVG-----VANGDSLASAICAAMEAAAPVARHLQETT----------------VDLT 95
CrAMT1F     ---------------------------------------------------------------------------------------------------- 95
CrAMT1D     ----------------------------MADEMDPMTACITALTD-----AAMTTAQATALCGQFAFAADNS--------------------------- 95
CrAMT1A     ------------------------MSG--DFGSEPLGSCSVETVT----ALLGYGLEQDSIT-ALCQPEGGAGCTSTDN----------------CMFQ 95
CrAMT1Ea    ------------------------------MGCNPLQLAQVT-------ALLGG---DTATAATICAGTDVAQLGPNG-----------------AVTK 95
CrAMT1Ga    -------------------------------MPLAELWRLTKLVP-----LACTVLLLSVRALAVDSGGLN--DSADNSS---------------RSLL 95
CrAMT1H     ---------------------------------------------------------------------------------------------------- 95
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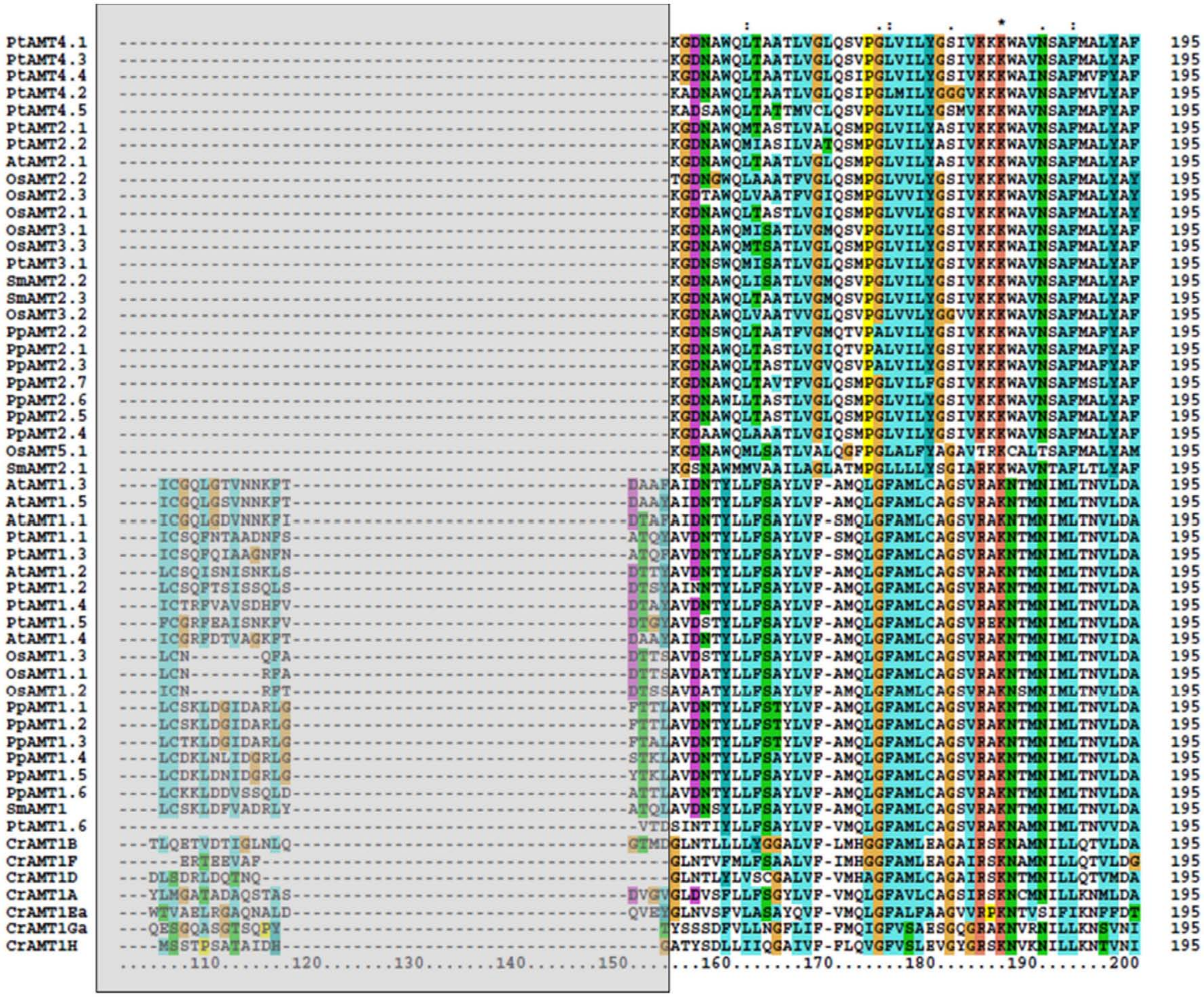
FIGURE A1 | Continued



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OsAMT2.1    ------------------------------------------------------KGDNAWQLTASTLVGIQSMPGLVVLYGSIVKKKWAVNSAFMALYAY 195
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OsAMT3.3    ------------------------------------------------------KGDNAWQMTSATLVGLQSMPGLVILYGSIVKKKWAINSAFMALYAF 195
PtAMT3.1    ------------------------------------------------------KGDNSWQMISATLVGLQSMPGLVILYGSIVKKKWAVNSAFMALYAF 195
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SmAMT2.3    ------------------------------------------------------KGDNAWQLTAATLVGMQSVPGLVILYGSIVKKKWAVNSAFMALYAF 195
OsAMT3.2    ------------------------------------------------------KGDNAWQLVAATVVGLQSVPGLVVLYGGVVKKKWAVNSAFMALYAF 195
PpAMT2.2    ------------------------------------------------------KGDNSWQLTAATFVGMQTVPALVILYGSIVKKKWAVNSAFMAFYAF 195
PpAMT2.1    ------------------------------------------------------KGDNAWQLTASTLVGIQTVPALVILYGSIVKKKWAVNSAFMAFYAF 195
PpAMT2.3    ------------------------------------------------------KGDNAWQLTASTLVGVQSVPALVILYGSIVKKKWAVNSAFMAFYAF 195
PpAMT2.7    ------------------------------------------------------KGDNAWQLTAVTFVGLQSMPGLVILFGSIVKKKWAVNSAFMSLYAF 195
PpAMT2.6    ------------------------------------------------------KGDNAWLLTASTLVGLQSMPGLVILYGSIVKKKWAVNSAFMALYAF 195
PpAMT2.5    ------------------------------------------------------KGDNAWQLTASTLVGLQSMPGLVILYGSIVKKKWAVNSAFMALYAF 195
PpAMT2.4    ------------------------------------------------------KGDAAWQLAAATLVGIQSMPGLVILYGSIVKKKWAVNSAFMALYAF 195
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AtAMT1.5    ----ICGQLGSVNNKFT---------------------------------DAAYAIDNTYLLFSAYLVF-AMQLGFAMLCAGSVRAKNTMNIMLTNVLDA 195
AtAMT1.1    ----ICGQLGDVNNKFI---------------------------------DTAFAIDNTYLLFSAYLVF-SMQLGFAMLCAGSVRAKNTMNIMLTNVLDA 195
PtAMT1.1    ----ICSQFNTAADNFS---------------------------------ATQYAVDNTYLLFSAYLVF-SMQLGFAMLCAGSVRAKNTMNIMLTNVLDA 195
PtAMT1.3    ----ICSQFQIAAGNFN---------------------------------ATQFAVDNTYLLFSAYLVF-SMQLGFAMLCAGSVRAKNTMNIMLTNVLDA 195
AtAMT1.2    ----LCSQISNISNKLS---------------------------------DTTYAVDNTYLLFSAYLVF-AMQLGFAMLCAGSVRAKNTMNIMLTNVLDA 195
PtAMT1.2    ----LCSQFTSISSQLS---------------------------------DTSYAINNTYLLFSAYLVF-AMQLGFAMLCAGSVRAKNTMNIMLTNVLDA 195
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PpAMT1.4    ----LCDKLNLIDGRLG---------------------------------STKLAVDNTYLLFSAYLVF-AMQLGFAMLCAGSVRAKNTMNIMLTNVLDA 195
PpAMT1.5    ----LCDKLDNIDGRLG---------------------------------YTKLAVDNTYLLFSAYLVF-AMQLGFAMLCAGSVRAKNTMNIMLTNVLDA 195
PpAMT1.6    ----LCKKLDDVSSQLD---------------------------------ATTLAVDNTYLLFSAYLVF-AMQLGFAMLCAGSVRAKNTMNIMLTNVLDA 195
SmAMT1      ----LCSKLDFVADRLY---------------------------------ATQLAVDNSYLLFSAYLVF-AMQLGFAMLCAGSVRAKNTMNIMLTNVLDA 195
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CrAMT1B     ---TLQETVDTIGLNLQ---------------------------------GTMDGLNTLLLLYGGALVF-LMHGGFAMLEAGAIRSKNAMNILLQTVLDA 195
CrAMT1F     ------ERTEEVAF----------------------------------------GLNTVFMLFSAALVF-IMHGGFAMLEAGAIRSKNAMNILLQTVLDG 195
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CrAMT1A     ---YLMGATADAQSTAS---------------------------------DVGVGLDVSFLLFSGYLVF-VMQLGFAVLCAGSIRSKNCMNILLKNMLDA 195
CrAMT1Ea    ---WTVAELRGAQNALD---------------------------------QVEYGLNVSFVLASAYQVF-VMQLGFALFAAGVVRPKNTVSIFIKNFFDT 195
CrAMT1Ga    ---QESGQASGTSQPY-------------------------------------TYSSSDFVLLNGFLIF-FMQIGFVSAESGQGRAKNVRNILLKNSVNI 195
CrAMT1H     ----MSSTPSATAIDH-------------------------------------GATYSDLLIIQGAIVF-FLQVGFVSLEVGYGRSKNVKNILLKNTVNI 195
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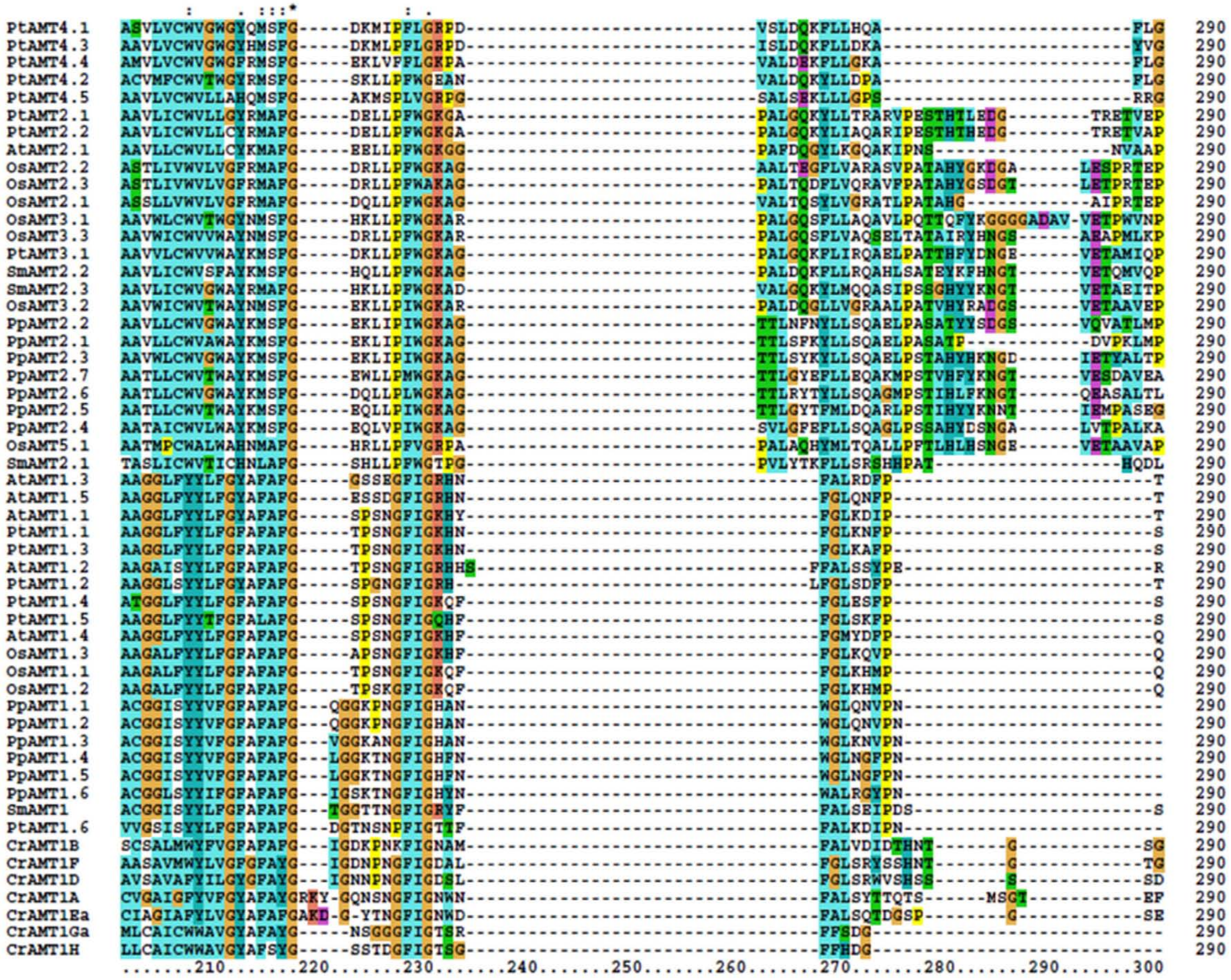

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OsAMT2.1    ASSLLVWVLVGFRMAFG-----DQLLPFWGKAG----------------------------VALTQSYLVGRATLPATAHG------------AIPRTEP    290
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PtAMT1.1    AAGGLFYYLFGFAFAFG-----TPSNGFIGKHN----------------------------------FGLKNFP-------------------------S    290
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OsAMT1.3    AAGALFYYLFGFAFAFG-----APSNGFIGKHF----------------------------------FGLKQVP-------------------------Q    290
OsAMT1.1    AAGALFYYLFGFAFAFG-----TPSNGFIGKQF----------------------------------FGLKHMP-------------------------Q    290
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PpAMT1.3    ACGGISYYVFGFAFAFG---VGGKANGFIGHAN----------------------------------WGLKNVPN-------------------------    290
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PpAMT1.5    ACGGISYYVFGFAFAFG---LGGKTNGFIGHFN----------------------------------WGLNGFPN-------------------------    290
PpAMT1.6    ACGGLSYYIFGFAFAFG---IGSKTNGFIGHYN----------------------------------WALRGYPN-------------------------    290
SmAMT1      ACGGISYYLFGFAFAFG---TGGTTNGFIGRYF----------------------------------FALSEIPDS-----------------------S    290
PtAMT1.6    VVGSISYYLFGFAFAFG---DGTNSNPFIGTTF----------------------------------FALKDIPN-------------------------    290
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CrAMT1D     AVSAVAFYILGYGFAYG---IGNNPNGFIGDSL----------------------------------FGLSRWVSHSS-------S------------SD    290
CrAMT1A     CVGAIGFYVFGYAFAYGRKY-GQNSNGFIGNWN----------------------------------FALSYTTQTS------MSGT-----------EF    290
CrAMT1Ea    CIAGIAFYLVGYAFAFGAKD-G-YTNGFIGNWD----------------------------------FALSQTDGSP--------G------------SE    290
CrAMT1Ga    MLCAICWWAVGYAFAYG-----NSGGGFIGTSR----------------------------------FFSDG----------------------------    290
CrAMT1H     LLCAICWWAVGYAFSYG-----SSTDGFIGTSG----------------------------------FFHDG----------------------------    290
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FIGURE A1 | Continued

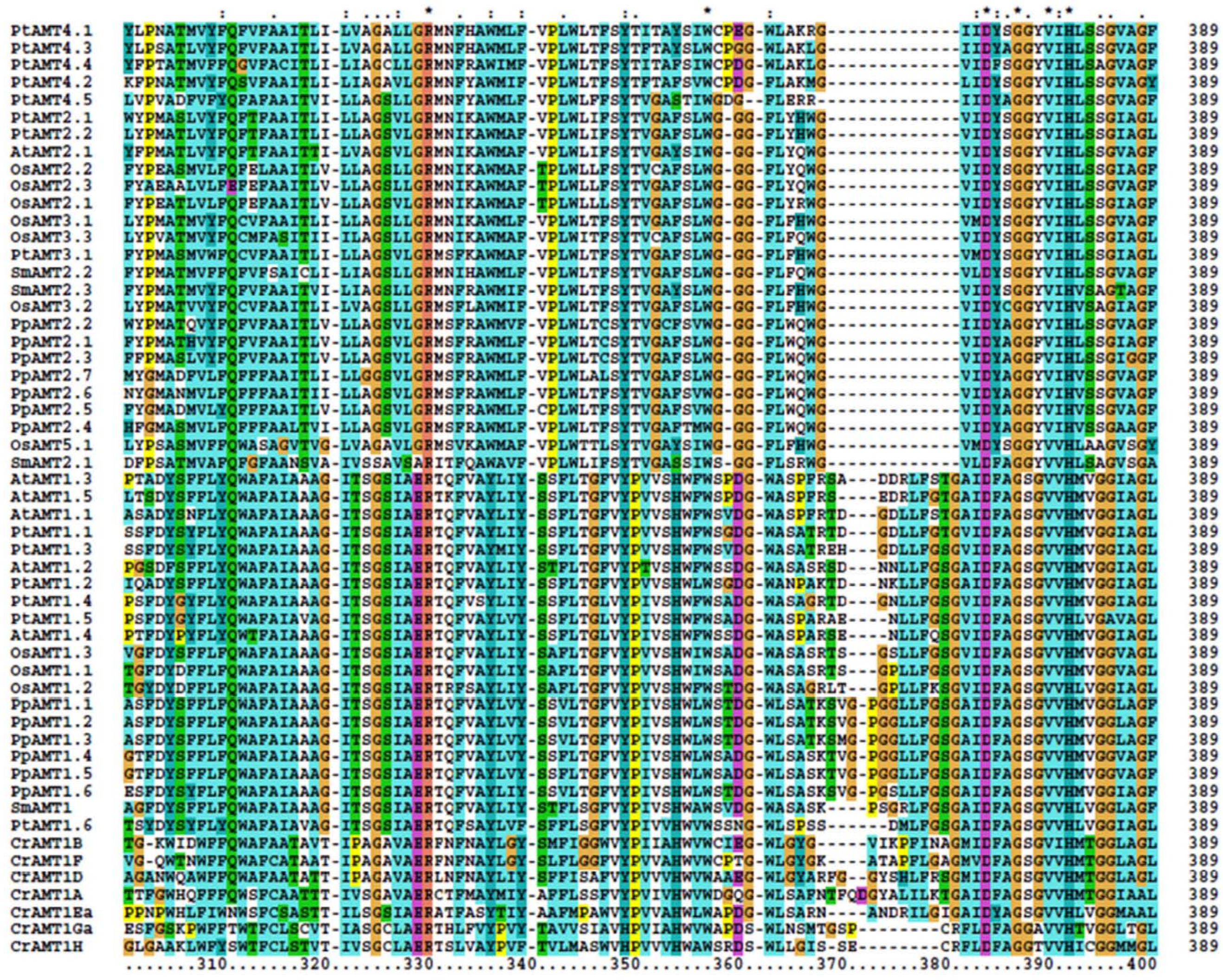
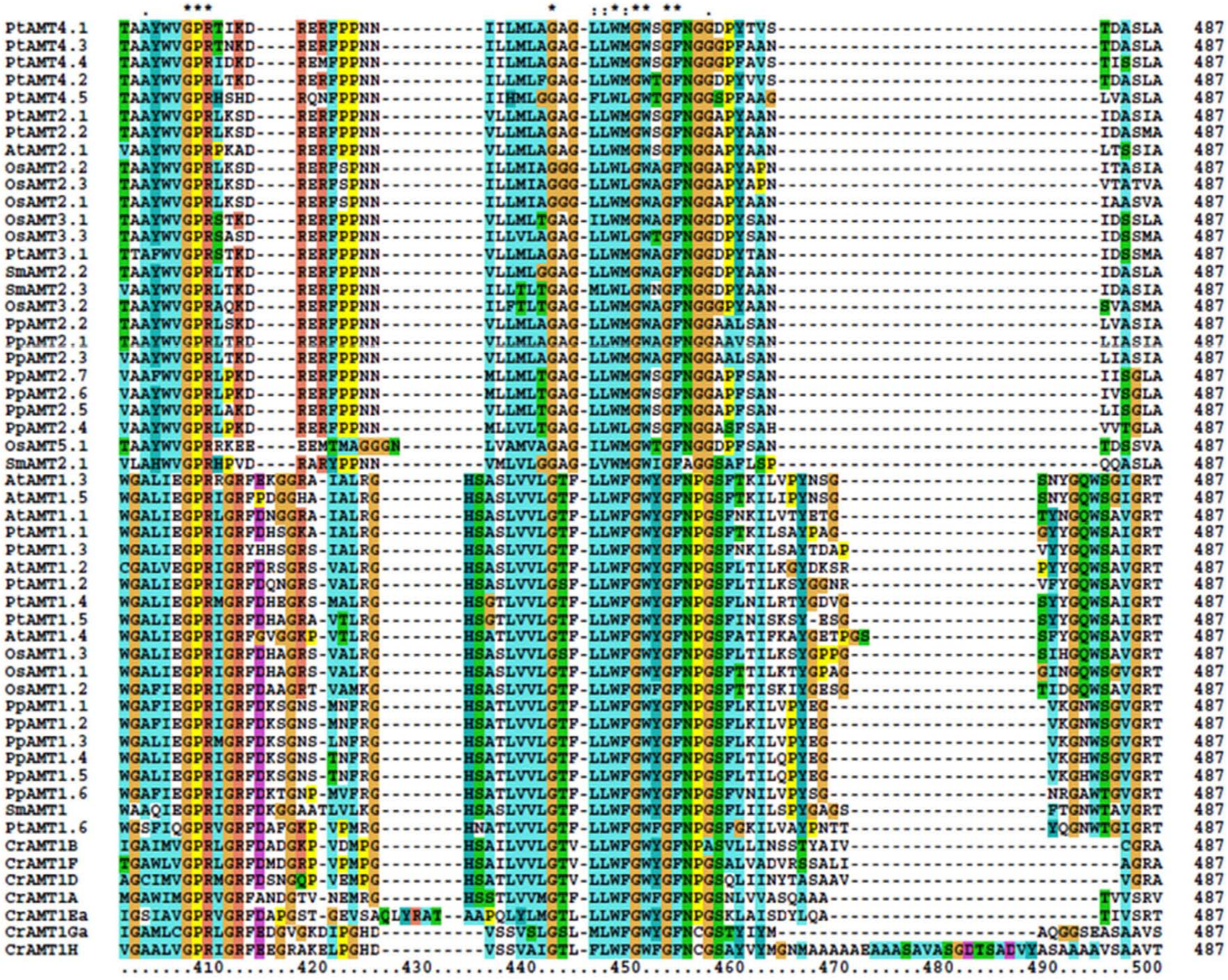


FIGURE A1 | Continued



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PtAMT4.3    TAAYWVGPRTNKD----RERFPPNN----------ILLMLAGAG-LLWMGWSGFNGGGPFAAN-------------------------------TDASLA   487
PtAMT4.4    TAAYWVGPRIDKD----REMFPPNN----------IILMLAGAG-LLWMGWSGFNGGGPFAVS-------------------------------TISSLA   487
PtAMT4.2    TAAYWVGPRLTKD----RERFPPNN----------ILLMLFGAG-LLWMGWTGFNGGDPYVVS-------------------------------TDASLA   487
PtAMT4.5    TAAYWVGPRHSHD----RQNFPPNN----------IIHMLGGAG-FLWLGWTGFNGGSPFAAG-------------------------------LVASLA   487
PtAMT2.1    TAAYWVGPRLKSD----RERFPPNN----------VLLMLAGAG-LLWMGWSGFNGGAPYAAN-------------------------------IDASIA   487
PtAMT2.2    TAAYWVGPRLKSD----RERFPPNN----------VLLMLAGAG-LLWMGWSGFNGGAPYAAN-------------------------------IDASMA   487
AtAMT2.1    VAAYWVGPRPKAD----RERFPPNN----------VLLMLAGAG-LLWMGWSGFNGGAPYAAN-------------------------------LTSSIA   487
OsAMT2.2    TAAYWVGPRLKSD----RERFSPNN----------ILLMIAGGG-LLWLGWAGFNGGAPYAPN-------------------------------ITASIA   487
OsAMT2.3    TAAYWVGPRLKSD----RERFSPNN----------ILLMIAGGG-LLWLGWAGFNGGAPYAPN-------------------------------VTATVA   487
OsAMT2.1    TAAYWVGPRLKSD----RERFSPNN----------ILLMIAGGG-LLWMGWAGFNGGAPYAAN-------------------------------IAASVA   487
OsAMT3.1    TAAYWVGPRSTKD----RERFPPNN----------VLLMLTGAG-ILWMGWAGFNGGDPYSAN-------------------------------IDSSLA   487
OsAMT3.3    TAAYWVGPRSASD----RERFPPNN----------ILLVLAGAG-LLWLGWTGFNGGDPYSAN-------------------------------IDSSMA   487
PtAMT3.1    TTAFWVGPRSTKD----RERFPPNN----------VLLMLAGAG-LLWMGWAGFNGGDPYTAN-------------------------------IDSSMA   487
SmAMT2.2    TAAYWVGPRLTKD----RERFPPNN----------VLLMLGGAG-LLWMGWAGFNGGDPYAAN-------------------------------IDASLA   487
SmAMT2.3    VAAYWVGPRLTKD----RERFPPNN----------ILLTLTGAG-MLWLGWNGFNGGDPYAAN-------------------------------IDASIA   487
OsAMT3.2    TAAYWVGPRAQKD----RERFPPNN----------ILFTLTGAG-LLWMGWAGFNGGGPYAAN-------------------------------SVASMA   487
PpAMT2.2    TAAYWVGPRLSKD----RERFPPNN----------VLLMLAGAG-LLWMGWAGFNGGAALSAN-------------------------------LVASIA   487
PpAMT2.1    TAAYWVGPRLTRD----RERFPPNN----------VLLMLAGAG-LLWMGWAGFNGGAAVSAN-------------------------------LIASIA   487
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PpAMT2.7    VAAFWVGPRLPKD----RERFPPNN----------MLLMLTGAG-LLWMGWSGFNGGAPFSAN-------------------------------IISGLA   487
PpAMT2.6    VAAYWVGPRLPKD----RERFPPNN----------MLLMLTGAG-LLWMGWSGFNGGAPFSAN-------------------------------IVSGLA   487
PpAMT2.5    VAAYWVGPRLAKD----RERFPPNN----------VLLMLTGAG-LLWMGWSGFNGGAPFSAN-------------------------------LISGLA   487
PpAMT2.4    VAAYWVGPRLPKD----RERFPPNN----------MLLVLTGAG-LLWLGWSGFNGGASFSAH-------------------------------VVTGLA   487
OsAMT5.1    TAAYWVGPRRKEE----EEMTMAGGGN--------LVAMVAGAG-ILWMGWTGFNGGDPFSAN-------------------------------TDSSVA   487
SmAMT2.1    VLAHWVGPRHPVD----RARYPPNN----------VMLVLGGAG-LVWMGWIGFAGGSAFLSP-------------------------------QQASLA   487
AtAMT1.3    WGALIEGPRRGRFEKGGRA-IALRG--------HSASLVVLGTF-LLWFGWYGFNPGSFTKILVPYNSG-------------------SNYGQWSGIGRT   487
AtAMT1.5    WGALIEGPRIGRFPDGGHA-IALRG--------HSASLVVLGTP-LLWFGWYGFNPGSFTKILIPYNSG-------------------SNYGQWSGIGRT   487
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AtAMT1.4    WGALIEGPRIGRFGVGGKP-VTLRG--------HSATLVVLGTF-LLWFGWYGFNPGSFATIFKAYGETPGS----------------SFYGQWSAVGRT   487
OsAMT1.3    WGALIEGPRIGRFDHAGRS-VALRG--------HSASLVVLGSF-LLWFGWYGFNPGSFLTILKSYGPPG------------------SIHGQWSAVGRT   487
OsAMT1.1    WGALIEGPRIGRFDHAGRS-VALKG--------HSASLVVLGTF-LLWFGWYGFNPGSFTTILKTYGPAG------------------GINGQWSGVGRT   487
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PpAMT1.1    WGAFIEGPRIGRFDKSGNS-MNFRG--------HSATLVVLGTF-LLWFGWYGFNPGSFLKILVPYEG---------------------VKGNWSGVGRT   487
PpAMT1.2    WGAFIEGPRIGRFDKSGNS-MNFRG--------HSATLVVLGTF-LLWFGWYGFNPGSFLKILVPYEG---------------------VKGNWSGVGRT   487
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PpAMT1.5    WGALIEGPRIGRFDKSGNS-TNFRG--------HSATLVVLGTF-LLWFGWYGFNPGSFLTILQPYEG---------------------VKGHWSGVGRT   487
PpAMT1.6    WGAFIEGPRIGRFDKTGNP-MVFRG--------HSATLVVLGTF-LLWFGWYGFNPGSFVNILVPYSG---------------------NRGAWTGVGRT   487
SmAMT1      WAAQIEGPRIGRFDKGGAATLVLKG--------HSASLVVLGTF-LLWFGWYGFNPGSFLIILSPYGAGS-------------------FTGNWTAVGRT   487
PtAMT1.6    WGSFIQGPRVGRFDAFGKP-VPMRG--------HNATLVVLGTF-LLWFGWFGFNPGSFGKILVAYPNTT-------------------YQGNWTGIGRT   487
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CrAMT1F     TGAWLVGPRLGRFDMDGRP-VPMPG--------HSAILVVLGTV-LLWFGWYGFNPGSALVADVRSSALI--------------------------AGRA   487
CrAMT1D     AGCIMVGPRMGRFDSNGQP-VEMPG--------HSATLVVLGTV-LLWFGWYGFNPGSQLIINYTASAAV--------------------------VGRA   487
CrAMT1A     MGAWIMGPRVGRFANDGTV-NEMRG--------HSSTLVVMGTF-LLWFGWFGFNPGSNLVVASQAAA--------------------------TVVSRV   487
CrAMT1Ea    IGSIAVGPRVGRFDAPGST-GEVSAQLYRAT--AAPQLYLMGTL-LLWFGWYGFNPGSKLAISDYLQA--------------------------TIVSRT   487
CrAMT1Ga    IGAMLCGPRLGRFEDGVGKDIPGHD----------VSSVSLGSL-MLWFGWYGFNCGSTYIYM-------------------------AQGGSEASAAVS   487
CrAMT1H     VGAALVGPRIGRFEEGRAKELPGHD----------VSSVAIGTL-FLWFGWFGFNCGSAYVYMGNMAAAAAEAAASAVASGDTSADVYASAAAAVSAAVT   487
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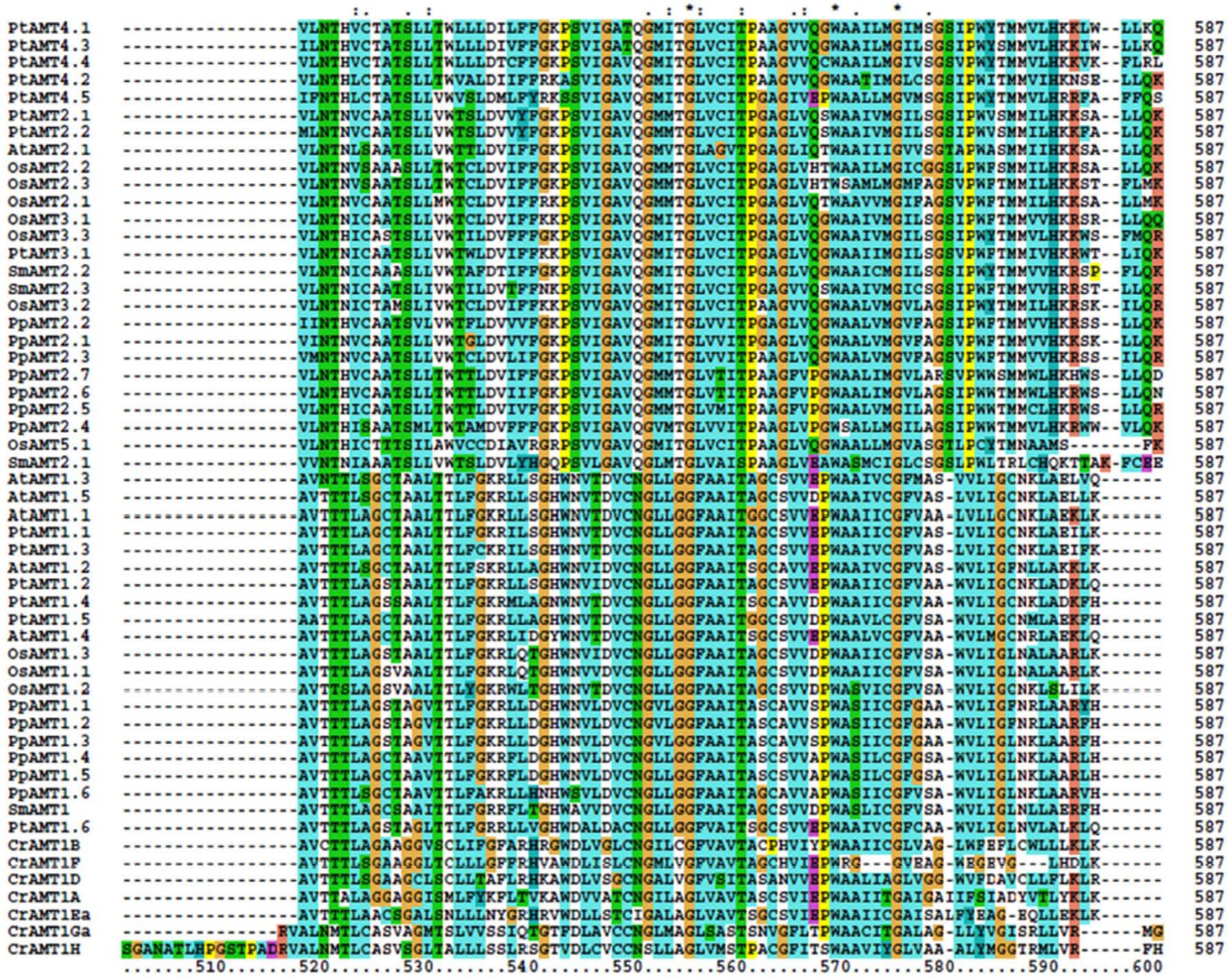
FIGURE A1 | Continued



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PpAMT2.5    -----------------VLNTHICAATSLLTWTTLDVIVFGKPSVIGAVQGMMTGLVMITPAAGFVPGWAALVMGILAGSIPWWTMMCLHKRWS--LLQR  587
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PtAMT1.1    -----------------AVTTTLAGCTAALTTLFGKRILSGHWNVTDVCNGLLGGFAAITAGCSVVEPWAAIICGFVAS-LVLIGCNKLAEILK------  587
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OsAMT1.1    -----------------AVTTTLAGSVAALTTLFGKRLQTGHWNVVDVCNGLLGGFAAITAGCSVVDPWAAIICGFVSA-WVLIGLNALAARLK------  587
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PpAMT1.1    -----------------AVTTTLAGSTAGVTTLFGKRLLDGHWNVLDVCNGVLGGFAAITASCAVVSPWASIICGFGAA-WVLIGFNRLAARYH------  587
PpAMT1.2    -----------------AVTTTLAGSTAGVTTLFGKRLLDGHWNVLDVCNGVLGGFAAITASCAVVSPWASIICGFGAA-WVLIGFNRLAARFH------  587
PpAMT1.3    -----------------AVTTTLAGSTAGVTTLFGKRLLDGHWNVLDVCNGVLGGFAAITASCAVVSPWASIICGFGAA-WVLIGLNKLAARFH------  587
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PpAMT1.5    -----------------AVTTTLAGCTAAVTTLFGKRFLDGHWNVLDVCNGLLGGFAAITASCSVVAPWASILCGFGSA-WVLIGLNKLAARLH------  587
PpAMT1.6    -----------------AVTTTLSGCTAAVTTLFAKRLLHNHWSVLDVCNGLLGGFAAITAGCAVVAPWASIICGFVSA-WVLIGLNKLAARVH------  587
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PtAMT1.6    -----------------AVTTTLAGSTAGLTTLFGRRLLVGHWDALDACNGLLGGFVAITSGCSVVEPWAAIVCGFCAA-WVLIGLNVLALKLQ------  587
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CrAMT1F     -----------------AVTTTLSGAAGGLTCLLLGFFRHVAWDLISLCNGMLVGFVAVTAGCHVIEPWRG---GVEAG-WEGEVG---LHDLK------  587
CrAMT1D     -----------------AVTTTLSGAAGCLSCLLTAFLRHKAWDLVSGCNGALVGFVSITASANVVEPWAALIAGLVGG-WVFDAVCLLFLKLR------  587
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CrAMT1Ga    ---------------RVALNMTLCASVAGMTSLVVSSIQTGTFDLAVCCNGLMAGLSASTSNVGFLTPWAACITGALAG-LLYVGISRLLVR------MG  587
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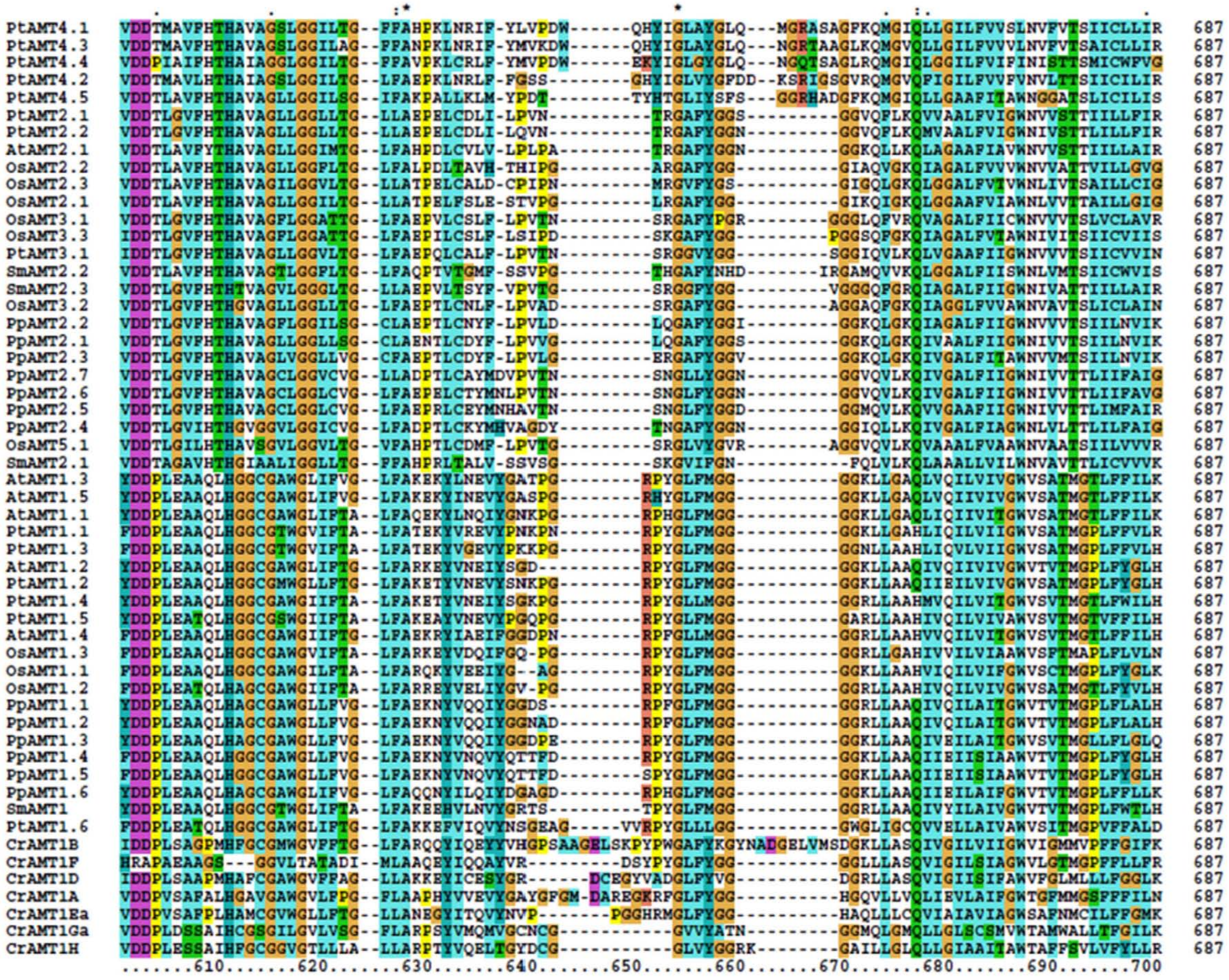
FIGURE A1 | Continued



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PpAMT2.4    VDDTLGVIHTHGVGGVLGGICVG--LFADPTLCKYMHVAGDY---------TNGAFYGGN---------GGIQLLKQIVGALFIAGWNLVLTTLILFAIG   687
OsAMT5.1    VDDTLGILHTHAVSGVLGGVLTG--VFAHPTLCDMF-LPVTG---------SRGLVYGVR--------AGGVQVLKQVAAALFVAAWNVAATSIILVVVR   687
SmAMT2.1    VDDTAGAVHTHGIAALIGGLLTG--FFAHPRLTALV-SSVSG---------SKGVIFGN-----------FQLVLKQLAAALLVILWNVAVTTLICVVVK   687
AtAMT1.3    YDDPLEAAQLHGGCGAWGLIFVG--LFAKEKYLNEVYGATPG--------RPYGLFMGG----------GGKLLGAQLVQILVIVGWVSATMGTLFFILK   687
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AtAMT1.1    YDDPLEAAQLHGGCGAWGLIFTA--LFAQEKYLNQIYGNKPG--------RPHGLFMGG----------GGKLLGAQLIQIIVITGWVSATMGTLFFILK   687
PtAMT1.1    FDDPLEAAQLHGGCGTWGVIFTA--LFATEKYVREVYPNKPN--------RPYGLFMGG----------GGKLLGAHLIQILVIIGWVSATMGPLFFVLR   687
PtAMT1.3    FDDPLEAAQLHGGCGTWGVIFTA--LFATEKYVGEVYPKKPG--------RPYGLFMGG----------GGNLLAAHLIQVLVIIGWVSATMGPLFFVLH   687
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PtAMT1.2    YDDPLEAAQLHGGCGMWGLLFTG--LFAKETYVNEVYSNKPG--------RPYGLFMGG----------GGKLLAAQIIEILVIVGWVSATMGPLFYGLH   687
PtAMT1.4    YDDPLEAAQLHGGCGAWGIIFTA--LFAKETYVNEIYSGKPG--------RPYGLLMGG----------GGRLLAAHMVQILVITGWVSVTMGTLFWILH   687
PtAMT1.5    YDDPLEATQLHGGCGSWGIIFTA--LFAKEAYVNEVYPGQPG--------RPYGLFMGG----------GARLLAAHIVQILVIVAWVSVTMGTVFFILH   687
AtAMT1.4    FDDPLEAAQLHGGCGAWGIIFTG--LFAEKRYIAEIFGGDPN--------RPFGLLMGG----------GGRLLAAHVVQILVITGWVSVTMGTLFFILH   687
OsAMT1.3    FDDPLEAAQLHGGCGAWGVIFTA--LFARKEYVDQIFGQ-PG--------RPYGLFMGG----------GGRLLGAHIVVILVIAAWVSFTMAPLFLVLN   687
OsAMT1.1    FDDPLEAAQLHGGCGAWGILFTA--LFARQKYVEEIYG--AG--------RPYGLFMGG----------GGKLLAAHVIQILVIFGWVSCTMGPLFYGLK   687
OsAMT1.2    FDDPLEATQLHAGCGAWGIIFTA--LFARREYVELIYGV-PG--------RPYGLFMGG----------GGRLLAAHIVQILVIVGWVSATMGTLFYVLH   687
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SmAMT1      YDDPLEAAQLHGGCGTWGLIFTA--LFAKEEHVLNVYGRTS---------TPYGLFMGG----------GGRLLAAQIVYILAIVGWVTVTMGPLFWTLH   687
PtAMT1.6    FDDPLEATQLHGGCGAWGLIFTG--LFAKKEFVIQVYNSGEAG-----VVRPYGLLLGG----------GWGLIGCQVVELLAIVAWVSITMGPVFFALD   687
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CrAMT1F     HRAPAEAAGS---GGVLTATADI--MLAAQEYIQQAYVR---------DSYPYGLFYGG----------GGLLLASQVIGILSIAGWVLGTMGPFFLLFR   687
CrAMT1D     IDDPLSAAPMHAFCGAWGVFFAG--LLAKKEYICESYGR------DCEGYVADGLFYVG----------DGRLLASQVIGIISIFAWVFGLMLLLFGGLK   687
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CrAMT1Ea    VDDPVSAFPLHAMCGVWGLLFTG--LLANEGYITQVYNVP-------PGGHRMGLFYGG----------HAQLLLCQVIAIAVIAGWSAFNMCILFFGMK   687
CrAMT1Ga    VDDPLDSSAIHCGSGILGVLVSG--FLARPSYVMQMVGCNCG-----------GVVYATN---------GGMQLGMQLLGLSCSMVWTAMWALLTFGILK   687
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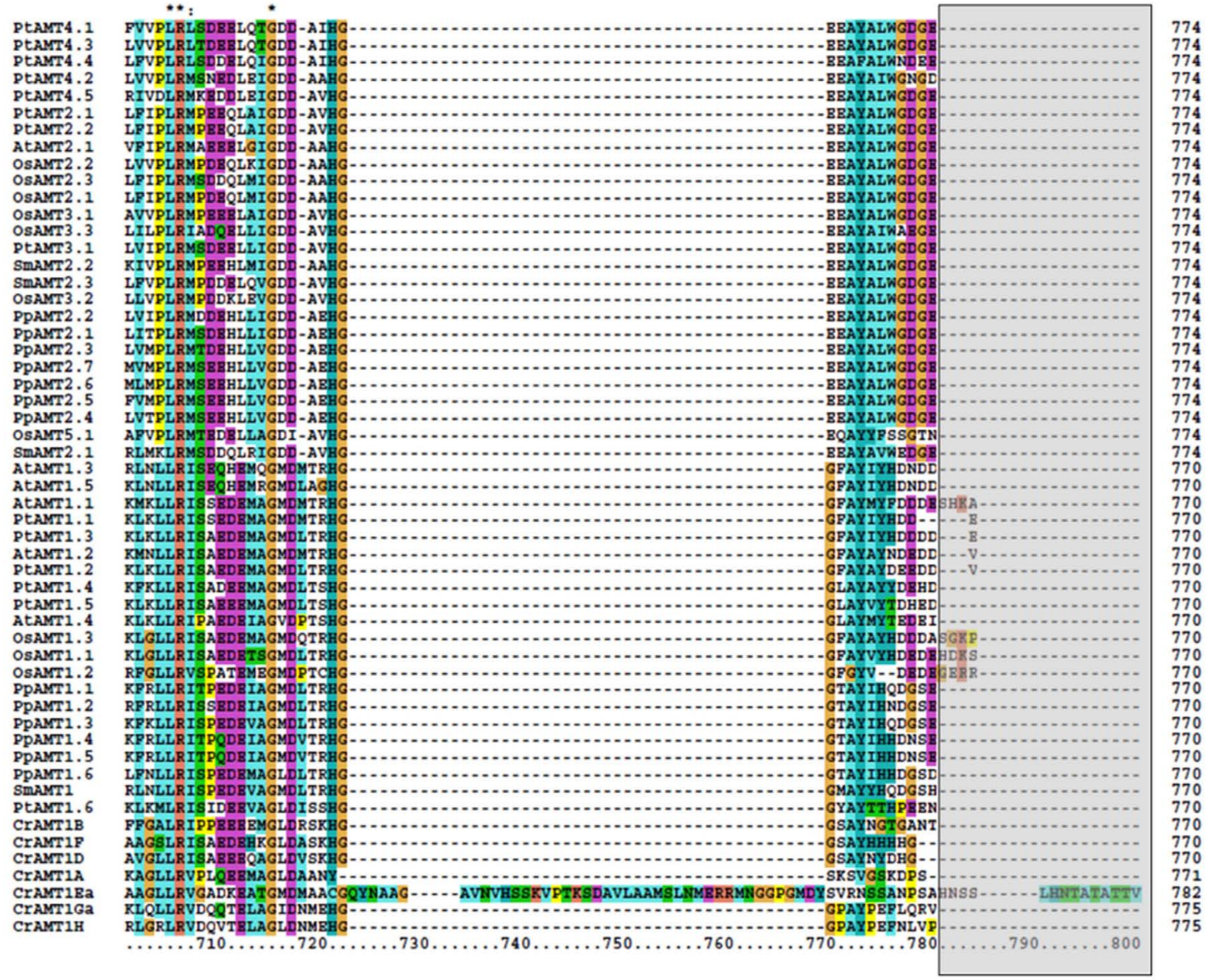
FIGURE A1 | Continued



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PtAMT2.2    LFIPLRMPEEQLAIGDD-AVHG-----------------------------------------------EEAYALWGDGE-------------------- 774
AtAMT2.1    VFIPLRMAEEELGIGDD-AAHG-----------------------------------------------EEAYALWGDGE-------------------- 774
OsAMT2.2    LVVPLRMPDEQLKIGDD-AAHG-----------------------------------------------EEAYALWGDGE-------------------- 774
OsAMT2.3    LFIPLRMSDDQLMIGDD-AAHG-----------------------------------------------EEAYALWGDGE-------------------- 774
OsAMT2.1    LFIPLRMPDEQLMIGDD-AAHG-----------------------------------------------EEAYALWGDGE-------------------- 774
OsAMT3.1    AVVPLRMPEEELAIGDD-AVHG-----------------------------------------------EEAYALWGDGE-------------------- 774
OsAMT3.3    LILPLRIADQELLIGDD-AVHG-----------------------------------------------EEAYAIWAEGE-------------------- 774
PtAMT3.1    LVIPLRMSDEELLIGDD-AVHG-----------------------------------------------EEAYALWGDGE-------------------- 774
SmAMT2.2    KIVPLRMPEEHLMIGDD-AAHG-----------------------------------------------EEAYALWGDGE-------------------- 774
SmAMT2.3    LFVPLRMPDDELQVGDD-AVHG-----------------------------------------------EEAYALWGDGE-------------------- 774
OsAMT3.2    LLVPLRMPDDKLEVGDD-AVHG-----------------------------------------------EEAYALWGDGE-------------------- 774
PpAMT2.2    LVIPLRMDDEHLLIGDD-AEHG-----------------------------------------------EEAYALWGDGE-------------------- 774
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PpAMT2.7    MVMPLRMSEEHLLVGDD-AEHG-----------------------------------------------EEAYALWGDGE-------------------- 774
PpAMT2.6    MLMPLRMSEEHLLVGDD-AEHG-----------------------------------------------EEAYALWGDGE-------------------- 774
PpAMT2.5    FVMPLRMSEEHLLVGDD-AEHG-----------------------------------------------EEAYALWGDGE-------------------- 774
PpAMT2.4    LVTPLRMSEEHLLVGDD-AEHG-----------------------------------------------EEAYALWGDGE-------------------- 774
OsAMT5.1    AFVPLRMTEDELLAGDI-AVHG-----------------------------------------------EQAYYFSSGTN-------------------- 774
SmAMT2.1    RLMKLRMSDDQLRIGDD-AVHG-----------------------------------------------EEAYAVWEDGE-------------------- 774
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AtAMT1.1    KMKLLRISSEDEMAGMDMTRHG-----------------------------------------------GFAYMYFDDDESHKA---------------- 770
PtAMT1.1    KLKLLRISSEDEMAGMDMTRHG-----------------------------------------------GFAYIYHDD-----E---------------- 770
PtAMT1.3    KLKLLRISAEDEMAGMDLTRHG-----------------------------------------------GFAYIYHDDDD---E---------------- 770
AtAMT1.2    KMNLLRISAEDEMAGMDMTRHG-----------------------------------------------GFAYAYNDEDD---V---------------- 770
PtAMT1.2    KLKLLRISAEDEMAGMDLTRHG-----------------------------------------------GFAYAYDEEDD---V---------------- 770
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PtAMT1.5    KLKLLRISAEEEMAGMDLTSHG-----------------------------------------------GLAYVYTDHED-------------------- 770
AtAMT1.4    KLKLLRIPAEDEIAGVDPTSHG-----------------------------------------------GLAYMYTEDEI-------------------- 770
OsAMT1.3    KLGLLRISAEDEMAGMDQTRHG-----------------------------------------------GFAYAYHDDDASGKP---------------- 770
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CrAMT1F     AAGSLRISAEDEHKGLDASKHG-----------------------------------------------GSAYHHHHG---------------------- 770
CrAMT1D     AVGLLRISAEEEQAGLDVSKHG-----------------------------------------------GSAYNYDHG---------------------- 770
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CrAMT1Ea    AAGLLRVGADKEATGMDMAACGQYNAAG-----AVNVHSSKVPTKSDAVLAAMSLNMERRMNGGPGMDYSVRNSSANPSAHNSS------LHNTATATTV 782
CrAMT1Ga    KLQLLRVDQQTELAGIDNMEHG-----------------------------------------------GPAYPEFLQRV-------------------- 775
CrAMT1H     RLGRLRVDQVTELAGLDNMEHG-----------------------------------------------GPAYPEFNLVP-------------------- 775
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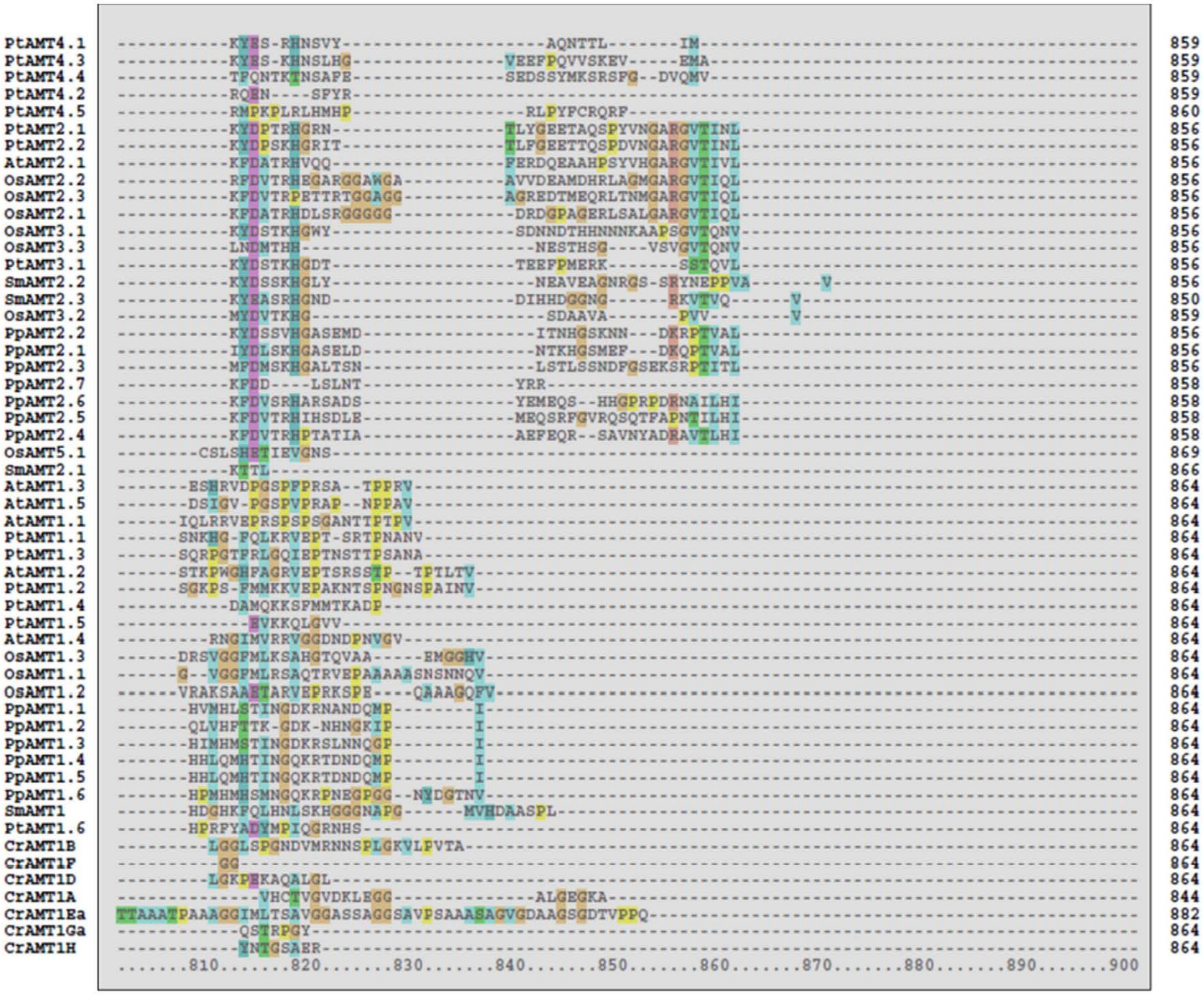
FIGURE A1 | Continued



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PtAMT4.2  -----------RQEN----SFYR-----------------------------------------------------------------------------  859
PtAMT4.5  -----------RMPKPLRLHMHP-----------------RLPYFCRQRF--------------------------------------------------  860
PtAMT2.1  -----------KYDPTRHGRN-----------------TLYGEETAQSPYVNGARGVTINL---------------------------------------  856
PtAMT2.2  -----------KYDPSKHGRIT----------------TLFGEETTQSPDVNGARGVTINL---------------------------------------  856
AtAMT2.1  -----------KFDATRHVQQ-----------------FERDQEAAHPSYVHGARGVTIVL---------------------------------------  856
OsAMT2.2  -----------RFDVTRHEGARGGAWGA----------AVVDEAMDHRLAGMGARGVTIQL---------------------------------------  856
OsAMT2.3  -----------KFDVTRPETTRTGGAGG----------AGREDTMEQRLTNMGARGVTIQL---------------------------------------  856
OsAMT2.1  -----------KFDATRHDLSRGGGGG------------DRDGPAGERLSALGARGVTIQL---------------------------------------  856
OsAMT3.1  -----------KYDSTKHGWY------------------SDNNDTHHNNNKAAPSGVTQNV---------------------------------------  856
OsAMT3.3  -----------LNDMTHH-----------------------NESTHSG----VSVGVTQNV---------------------------------------  856
PtAMT3.1  -----------KYDSTKHGDT------------------TEEFPMERK-------SSTQVL---------------------------------------  856
SmAMT2.2  -----------KYDSSKHGLY--------------------NEAVEAGNRGS-SRYNEPPVA-------V------------------------------  856
SmAMT2.3  -----------KYEASRHGND------------------DIHHDGGNG------RKVTVQ------V---------------------------------  850
OsAMT3.2  -----------MYDVTKHG-----------------------SDAAVA-------PVV--------V---------------------------------  859
PpAMT2.2  -----------KYDSSVHGASEMD-----------------ITNHGSKNN---DKRPTVAL---------------------------------------  856
PpAMT2.1  -----------IYDLSKHGASELD-----------------NTKHGSMEF---DKQPTVAL---------------------------------------  856
PpAMT2.3  -----------MFDMSKHGALTSN-----------------LSTLSSNDFGSEKSRPTITL---------------------------------------  856
PpAMT2.7  -----------KFDD----LSLNT---------------YRR----------------------------------------------------------  858
PpAMT2.6  -----------KFDVSRHARSADS---------------YEMEQS--HHGPRPDRNAILHI---------------------------------------  858
PpAMT2.5  -----------KFDVTRHIHSDLE---------------MEQSRFGVRQSQTFAPNTILHI---------------------------------------  858
PpAMT2.4  -----------KFDVTRHPTATIA---------------AEFEQR--SAVNYADRAVTLHI---------------------------------------  858
OsAMT5.1  --------CSLSHETIEVGNS-------------------------------------------------------------------------------  869
SmAMT2.1  -----------KTTL-------------------------------------------------------------------------------------  866
AtAMT1.3  -------ESHRVDPGSPFPRSA--TPPRV-----------------------------------------------------------------------  864
AtAMT1.5  -------DSIGV-PGSPVPRAP--NPPAV-----------------------------------------------------------------------  864
AtAMT1.1  ------IQLRRVEPRSPSPSGANTTPTPV-----------------------------------------------------------------------  864
PtAMT1.1  ------SNKHG-FQLKRVEPT-SRTPNANV----------------------------------------------------------------------  864
PtAMT1.3  ------SQRPGTFRLGQIEPTNSTTPSANA----------------------------------------------------------------------  864
AtAMT1.2  ------STKPWGHFAGRVEPTSRSSTP--TPTLTV-----------------------------------------------------------------  864
PtAMT1.2  ------SGKPS-FMMKKVEPAKNTSPNGNSPAINV-----------------------------------------------------------------  864
PtAMT1.4  -----------DAMQKKSFMMTKADP--------------------------------------------------------------------------  864
PtAMT1.5  -------------EVKKQLGVV------------------------------------------------------------------------------  864
AtAMT1.4  ---------RNGIMVRRVGGDNDPNVGV------------------------------------------------------------------------  864
OsAMT1.3  ------DRSVGGFMLKSAHGTQVAA-----EMGGHV----------------------------------------------------------------  864
OsAMT1.1  ------G--VGGFMLRSAQTRVEPAAAAASNSNNQV----------------------------------------------------------------  864
OsAMT1.2  ------VRAKSAAETARVEPRKSPE----QAAAGQFV---------------------------------------------------------------  864
PpAMT1.1  -------HVMHLSTINGDKRNANDQMP--------I----------------------------------------------------------------  864
PpAMT1.2  -------QLVHFTTK-GDK-NHNGKIP--------I----------------------------------------------------------------  864
PpAMT1.3  -------HIMHMSTINGDKRSLNNQGP--------I----------------------------------------------------------------  864
PpAMT1.4  -------HHLQMHTINGQKRTDNDQMP--------I----------------------------------------------------------------  864
PpAMT1.5  -------HHLQMHTINGQKRTDNDQMP--------I----------------------------------------------------------------  864
PpAMT1.6  -------HPMHMHSMNGQKRPNEGPGG--NYDGTNV----------------------------------------------------------------  864
SmAMT1    -------HDGHKFQLHNLSKHGGGNAPG------MVHDAASPL---------------------------------------------------------  864
PtAMT1.6  -------HPRFYADYMPIQGRNHS----------------------------------------------------------------------------  864
CrAMT1B   ---------LGGLSPGNDVMRNNSPLGKVLPVTA------------------------------------------------------------------  864
CrAMT1F   ----------GG----------------------------------------------------------------------------------------  864
CrAMT1D   ---------LGKPEKAQALGL-------------------------------------------------------------------------------  864
CrAMT1A   --------------VHCTVGVDKLEGG--------------ALGEGKA----------------------------------------------------  844
CrAMT1Ea  TTAAATPAAAGGIMLTSAVGGASSAGGSAVPSAAASAGVGDAAGSGDTVPPQ------------------------------------------------  882
CrAMT1Ga  ------------QSTRPGY---------------------------------------------------------------------------------  864
CrAMT1H   ------------YNTGSAER--------------------------------------------------------------------------------  864
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FIGURE A1 | Alignment of protein sequences of ammonium transporters by ClustalW. In gray squares are the regions excluded by analyses.

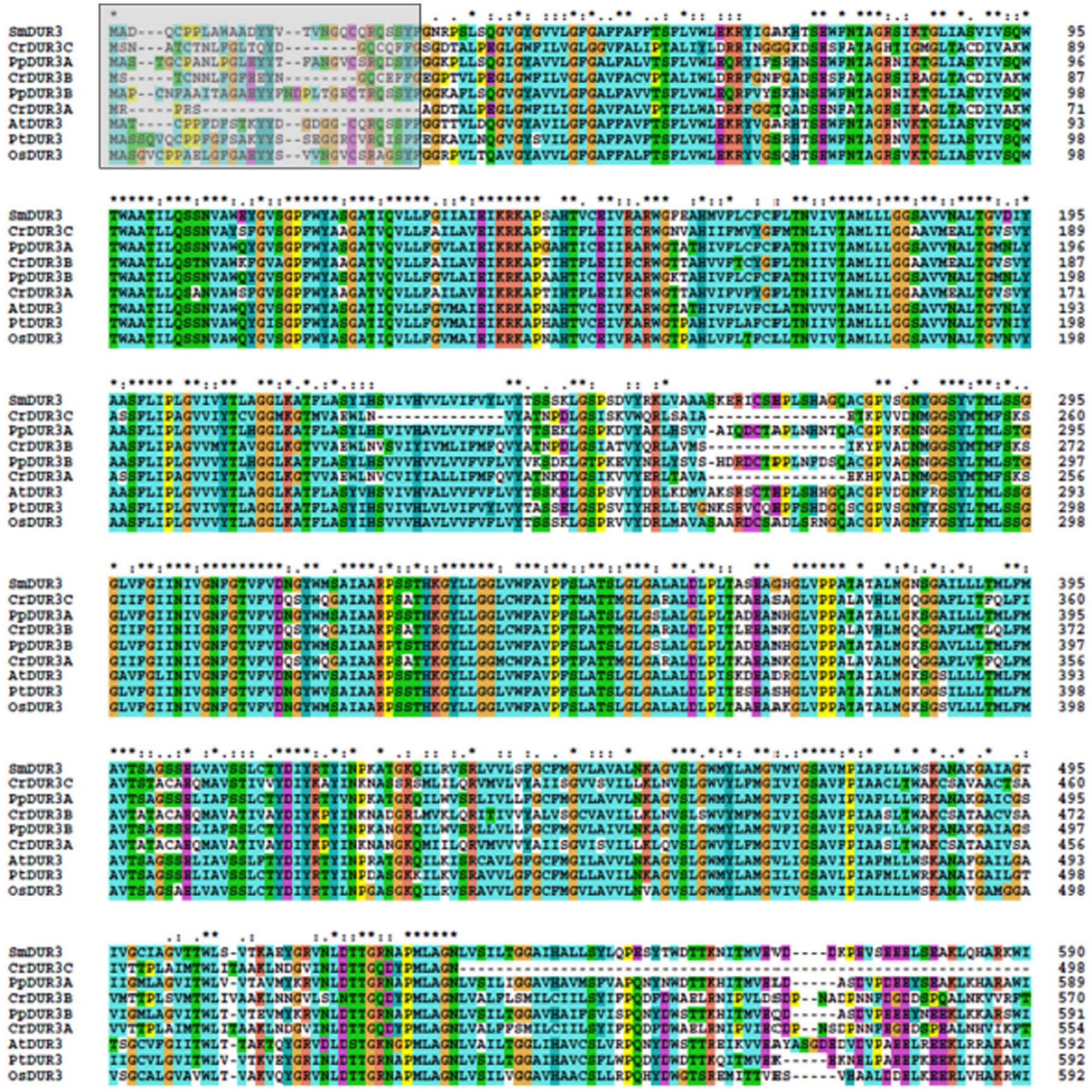

FIGURE A2 | Continued

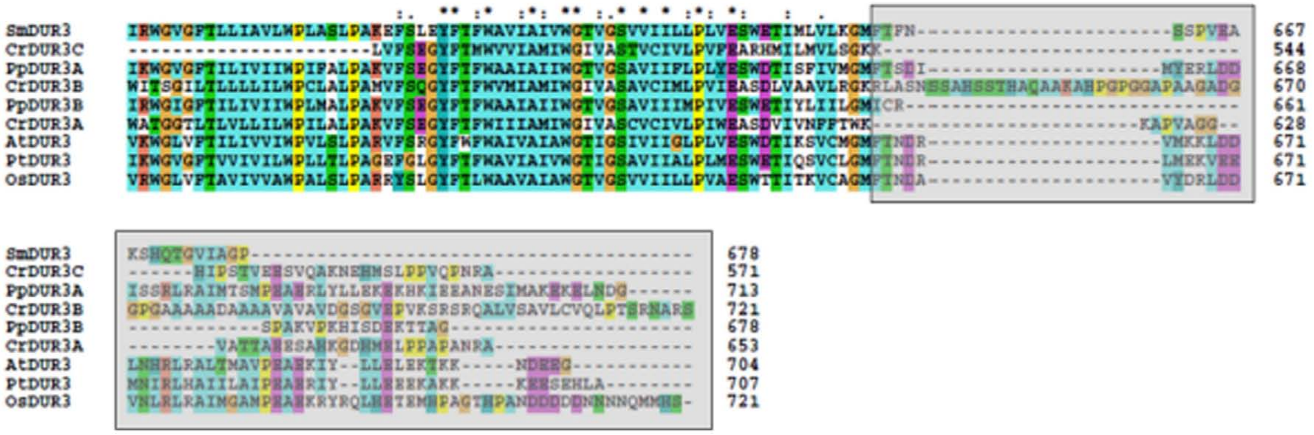

FIGURE A2 | Alignment of protein sequences of urea transporters by ClustalW. In gray squares are the regions excluded by analyses.

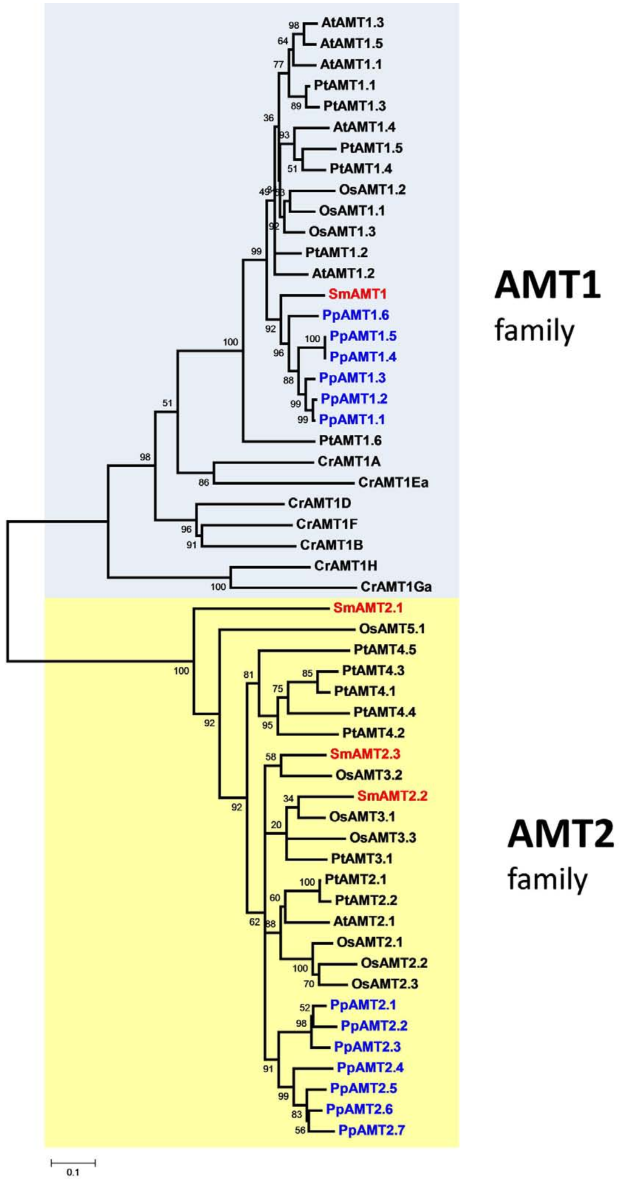

FIGURE A3 | Unrooted phylogenetic tree of the ammonium transporters in *Arabidopsis thaliana* (At), *Populus trichocarpa* (Pt), *Oryza sativa* (Os), *Selaginella moellendorffii* (Sm), *Physcomitrella patens* (Pp), and *Chlamydomonas reinhardtii* (Cr). Red indicates *Selaginella* AMTs, blue *Physcomitrella*. The tree was constructed by aligning the protein sequences by ClustalW and generated with the Maximum-Likelihood method.

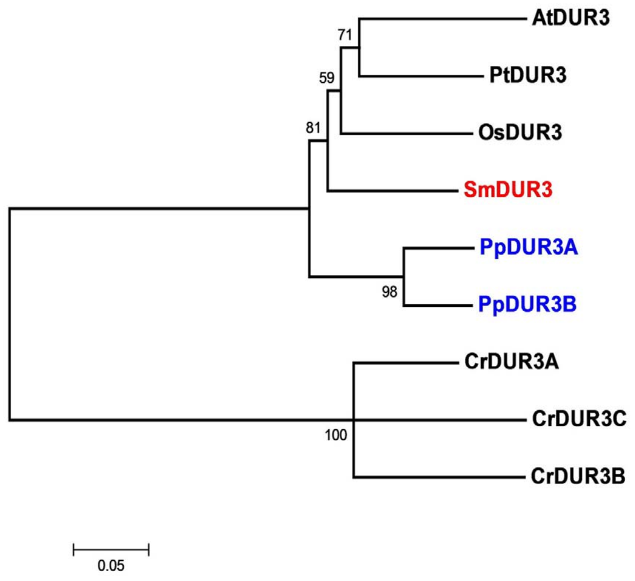

FIGURE A4 | Unrooted phylogenetic tree of the urea transporters in *Arabidopsis thaliana* (At), *Populus trichocarpa* (Pt), *Oryza sativa* (Os), *Selaginella moellendorffii* (Sm), *Physcomitrella patens* (Pp), and *Chlamydomonas reinhardtii* (Cr). Red indicates *Selaginella* DUR3, blue *Physcomitrella*. The tree was constructed by aligning the protein sequences by ClustalW and generated with the Maximum-Likelihood method.