Molecular characterization of two genes encoding plastidic ATP/ADP transport proteins in cassava

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Abstract

The import of ATP into plastids is facilitated by members of the plastidic ATP/ADP transporter (AATP) family. Our results indicate that the cassava (*Manihot esculenta* Crantz) genome possesses two genes encoding for putative ATP/ADP translocases, which we have designated as *MeAATP1* and *MeAATP2*. Their deduced products are 92 % identical, and phylogenetic reconstructions of plant AATP sequences suggest that *MeAATP1* and *MeAATP2* are the result of a relatively recent duplication event. Both genes were found to be expressed in a wide range of plant organs *via* RT-PCR, including young and mature leaves, fibrous and tuberous roots, and green stems. Neither *MeAATP1* nor *MeAATP2* showed evidence of increased transcription in the presence of exogenous sucrose. Interestingly, the transcriptional activity of *MeAATP1* in leaves appeared to be upregulated after wounding, potentially indicating its involvement in the wound response mechanism of cassava.

Additional key words: amyloplasts, *Manihot esculenta*, starch biosynthesis, wounding.

Introduction

Plastids are a set of developmentally-interrelated organelles which host several metabolic processes essential for the proper growth and development of plants, including carbon and nitrogen assimilation, and the synthesis of amino acids, fatty acids and starch. Though possessing their own genome, most plastid proteins are encoded by nuclear genes and are targeted to plastids posttranslationally. These include nuclear-encoded transmembrane proteins involved in the movement of metabolites across the plastidic double-membrane system. The outer membrane is freely permeable to molecules of 10 kDa or less *via* porins (Flügge and Benz 1983, Fischer *et al.* 1994), while the movement of specific molecules across the inner membrane is facilitated by specialized transporters (reviewed in Emes and Neuhaus 1997).

ATP is the principle energy carrier of living organisms. Although chloroplasts are capable of generating ATP *via* photosynthesis, non-photosynthetic plastids must rely upon the import of ATP from the cytosol. Movement of ATP across the plastid inner membrane is mediated by the members of at least two distinct transport protein families. Members of the plastidic ATP/ADP transport protein (AATP) family share significant sequence homology to a class of nucleotide transporters

Received 16 April 2007, *accepted* 6 December 2007.
Abbreviations: AATP - ATP/ADP transporter; ADP-Glc - ADP-glucose; AGPase - ADP-glucose pyrophosphorylase; CS - transit peptide cleavage sites; RACE - rapid amplification of cDNA ends; TM - transmembrane.
Acknowledgements: Cassava samples were graciously provided by Dr. Opas Boonseng of the Rayong Field Crops Research Center (Rayong, Thailand). We are grateful to Dr. Malinee Suksangpanomrung and Ms. Apaporn Rattanakitti for establishing and maintaining cassava plants at Thailand Science Park (Pathumthani, Thailand), and for advice and assistance with cassava nucleic acid extraction. We are also thankful to the members of the Starch Biosynthesis laboratory (BIOTEC Central Research Unit) and the Center for Cassava Molecular Biotechnology, Faculty of Science, Mahidol University for their helpful comments and suggestions. This research was supported by National Center for Genetic Engineering and Biotechnology grant BT-B-02-PM-BC-4706. C.Y.L. Yuen and O. Leelapon contributed equally to the paper.
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found in obligate intracellular parasites (Kampfenkel *et al*. 1995), and function as antiporters, translocating ATP in counter exchange for ADP (Tjaden *et al*. 1998b). More recently, it has been shown that *Solanum tuberosum* Brittle-1 (StBT1), a member of the mitochondrial carrier family, localizes specifically to the inner membrane of plastids rather than mitochondria, and possesses roughly equivalent affinities for the substrates ATP, ADP and AMP (Leroch *et al*. 2005). StBT1 is a homolog of *Zea mays* Brittle-1, the probable plastidic ADP-glucose (ADP-Glc) transporter in maize (Shannon *et al*. 1998), although StBT1 itself has no affinity to ADP-Glc (Leroch *et al*. 2005).

In the starch biosynthetic pathway, ADP-Glc serves as the direct precursor to the formation of nascent starch polymers, and is synthesized from glucose-1-phosphate and ATP *via* a reaction catalized by ADP-Glc pyrophosphorylase (AGPase). In general, AGPase occurs largely in cytosol in monocots, but in plastids in dicots (Emes and Neuhaus 1997). In consequence, the ability to mobilize ATP into amyloplasts is a requirement for starch production in the latter. Indeed, ATP/ADP transporter activity has a transport control coefficient of 0.78 over the starch biosynthesis pathway in potato. In the same plant species, the overexpression or antisense suppression of *AATP* transcript levels leads to increased or decreased tuber starch yield, respectively (Tjaden *et al*. 1998a).

Since the plastid interior hosts a wide range of metabolic processes, it is perhaps not surprising that the impairment of ATP import into plastids leads to a diverse spectrum of phenotypes. Antisense suppression of *StAATP1* in potato has been demonstrated to result in not only decreased starch content in tubers, but also altered tuber morphology and the occurrence of adventitious budding (Tjaden *et al*. 1998a). Antisense plants also exhibit enhanced resistance to *Alternaria solani* and *Erwinia carotovora* in tubers, while their leaves have increased resistance to *Phytophthora infestans* (Linke *et al*. 2002; Conrath *et al*. 2003). The basis for this improved pathogenic resistance remains unclear, but may be related to an increased capacity to release H_2O_2 (Conrath *et al*. 2003), a known trigger of plant defense genes (Orozco-Cárdenas *et al*. 2001). In the model plant system *A. thaliana*, which is known to possess only two *AATP* genes, double mutants harboring T-DNA disruptions in both *AtAATP1* and *AtAATP2* also exhibit an array of phenotypes, including diminished root growth, delayed chlorophyll accumulation in leaves, and reduced lipid content in seeds (Reiser *et al*. 2004). *AtAATP1* and *AtAATP2* possess partially overlapping, yet distinct, expression patterns, with the former exhibiting increased levels of expression in the presence of exogenous glucose or sucrose (Reiser *et al*. 2004).

In this report we describe the isolation and preliminary characterization of two putative ATP/ADP translocase genes from the tuberous root crop cassava (*Manihot esculenta* Crantz). In addition to assessing their expression across various plant organs, we also investigated whether either gene exhibited evidence of being sucrose-inducible. Although neither *MeAATP1* nor *MeAATP2* showed signs of significant upregulation in the presence of exogenous sucrose, we observed that *MeAATP1* appears to exhibit higher expression levels in leaves after mechanical damage, potentially suggesting that increased uptake of ATP by plastids facilitates wound response in cassava.

Materials and methods

Plants: Cassava (*Manihot esculenta* Crantz) cv. Kasetsart University 50 (KU50) plants were kindly provided by Dr. Opas Boonseng (Rayong Field Crops Research Center, Rayong, Thailand). Newly emerging (young) leaf and fully expanded (mature) leaf samples used in sucrose and wounding experiments were collected from 2-month-old potted plants grown at Thailand Science Park (Pathumthani, Thailand); all other samples were obtained from plants grown in the field at the Rayong Field Crops Research Center.

Isolation of cDNA sequences: Rapid amplification of cDNA ends (RACE) was performed with the *FirstChoice RLM-RACE* kit (*Ambion,* Foster City, CA, USA), in conjunction with the *BD Advantage 2* polymerase mix (*BD Biosciences,* San Jose, CA, USA). Total RNA was isolated from 6-month-old cassava tubers, essentially as described by Miyazawa *et al.* (1999), except that the composition of the extraction buffer was 100 mM Tris (pH 7.5), 100 mM NaCl, 20 mM EDTA, 1 % sarkosyl, and 50 mM β-mercaptoethanol. Gene-specific primers were guessmers derived from highly conserved segments within the peptide sequences of ATP/ADP translocases [3'-RACE primer: 5'-TCT GGA GTT TCT GCT TGT TCT ATG TCA TG-3'; 5'RACE outer primer: 5'-CAC ACT CCT CCA CAA TTC AGC CAT-3'; 5'RACE inner primer: 5'-AAT CCA ATA GCC ATA GGC ARR TTC ACC CA-3']. All PCR amplifications were performed under the following thermocycling conditions: 1 min at 95 °C; 35 cycles of 30 s at 95 °C, 1 min at 60 °C, and 2 min at 68 °C; and 10 min at 70 °C.

PCR amplification of the entire coding region of *MeAATP1* was achieved using primers AATP[β].F (5'-GAA TTT ATA TTT GCT AAG ATT CTT CTG TCT CTC-3') and AATP1.R (5'-GAA GTG GAC TTG AAA GAA GAG TAG A-3'); *MeAATP2* was amplified with the primers AATP[α].F (5'-AGA AAA CAA TCT CCT TTA GCC AC-3') and AATP2.R (5'-TGC CAT GGT TGG AAT GAT GGA AGA-3'). The template was the same cDNA preparation used in 3'-RACE experiments.

Southern blot analysis: Genomic DNA was isolated from the young leaves of cassava, essentially as described

by Rout *et al*. (1998). 20 µg of cassava genomic DNA were digested with various commercial restriction enzymes (*Fermentas,* Hanover, MD, USA), separated by agarose gel electrophoresis, and blotted to *Hybond-N* nylon membranes (*Amersham Pharmacia Biotech,* Piscataway, NJ, USA) via capillary transfer according to the manufacturer's instructions. ^{32}P-dCTP-labeled probes were generated *via* the *Rediprime* random primer labeling system (*Amersham Pharmacia Biotech*), using the full-length cDNA fragment of *AATP1* was as template. Hybridization of the probe to the Southern blots was performed overnight at low stringency (55 °C), using the hybridization buffer described by Sambrook *et al*. (1989). The membranes were washed twice with a 1× SSC; 0.1 % SDS solution at 53 °C, and subsequently examined by standard autoradiography.

Sequence analysis: The deduced products of *MeAATP1* and *MeAATP2* were compared to those of *AtAATP1* (GenBank accession number Q39002) and *AtAATP2* (P92935) from *Arabidopsis thaliana*, *OsAATP1* (AK069624) and *OsAATP2* (XM_464574) from *Oryza sativa*, *StAATP1* from *Solanum tuberosum* (T07420), and *McANT1* from *Mesembryanthemum crystallinum* (AB190777). Amino acid sequences were aligned with *ClustalW v1.4* (Thompson *et al*. 1994), and phylogenetic relationships between the aligned sequences analyzed through the program *Phylo_Win* (Galtier *et al*. 1996). For both Neighbor-Joining (NJ) and Maximum Parsimony (MP) methods, 1000 bootstrap replicates were performed. NJ analysis was conducted with global gap removal and Poisson-correction distance. Chloroplast localization and cleavage site (CS) predictions were performed using the neural network-based algorithm *ChloroP v1.1* (Emanuelsson *et al*. 1999). The proposed cleavage sites are the positions with the highest CS-score values within first 100 residues of each peptide sequence.

Gene expression studies: Samples used for tissue-specific analyses of gene expression patterns were obtained from 3-month-old cassava plants. Total RNA was prepared from leaves and tubers using the combined CTAB/RNeasy protocol described by Kim *et al*. (2004), while RNA from stems and fibrous roots was isolated using the *RNeasy Plant Mini Kit* (*Qiagen,* Valencia, CA, USA), with 1 % (m/v) polyvinylpyrrolidone-40 (*Sigma,* St. Louis, MO, USA) added to extraction buffer RLC immediately prior to usage. All samples were treated with DNase in-column, as recommended in the RNeasy kit manual.

The relative expression levels of *MeAATP1* and *MeAATP2* were compared *via* multiplex RT-PCR. First-strand cDNA synthesis was performed at 42 °C for 1.5 h, using 1 µg total RNA template, 4 µM of an oligo(dT)18-adapter primer (5'-GCG AGC ACA GAA TTA ATA CGA CT18-3'), 1× enzyme buffer, 1 mM dNTPs (each), 2 U mm^{-3} RNase inhibitor (*Fermentas*), and 10 U mm^{-3} *RevertAid H Minus* reverse transcriptase (*Fermentas*), in a total reaction volume of 20 mm3. Primers AATP1.F (5'-ACT TTT CAA CTC CCA CTG GCA TG-3') and AATP1.R were used to amplify *MeAATP1*, and primers AATP2.F (5'-GGT GAT TTC TCA ACT GCT ACT GGA-3') and AATP2.R for *MeAATP2*. Internal control primers MeEF1.F (5'-ATG GGT AAG GAG AAG GTT CAC) and MeEF1.R (5'-CAT CTT GTT ACA GCA GCA AAT CAT) were designed based on the cDNA sequence of *M. esculenta* elongation factor 1α (*MeEF1α*; GenBank accession number AF041463).

PCR reactions initially consisted of 0.5 mm^3 cDNA template, 1× PCR buffer, 4 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 U mm^{-3} Taq DNA polymerase (*Fermentas*), and 2 µM of either the *MeAATP1* or *MeAATP2* primer pair, in a total volume of 25 mm^3. After an initial 3 min denaturation at 94 °C and 6 cycles of PCR (30 s at 94 °C, 45 s at 60 °C, and 1 min at 72 °C), the reactions were placed on ice, and 25 mm^3 of a secondary reaction mix (similar to the initial, but replacing the *AATP*-specific primers for that of *MeEF1α*) was added to each tube. Amplification was then resumed for an additional 22 cycles. For subsequent experiments examining the effects of wounding or exogenous sucrose on gene expression levels in leaves, and only 4 cycles of PCR were performed prior to the addition of the *MeEF1α* primer mix. In the case of the wound-induction experiments, the amount of total RNA template used for reverse transcription was decreased to 0.5 µg per reaction.

Results

Cloning and sequence analysis of *MeAATP1* and *MeAATP2* cDNA: To isolate cDNAs encoding ATP/ADP transport proteins (AATP), we initially performed 5'- and 3'-RACE to obtain sequence information corresponding to the untranslated regions at both ends of putative *AATP* genes. After sequencing multiple RACE clones, gene-specific forward and reverse primers were developed, and tested in pairwise combinations to determine which primer pairings would yield amplicons of the desired size (~ 2 kb) when utilized in RT-PCR. Using this approach, we successfully cloned multiple cDNAs corresponding to two distinct tuber-expressed putative ATP/ADP translocase genes, which we designated as *MeAATP1* and *MeAATP2* (GenBank accession numbers DQ071875 and DQ071876, respectively).

Southern blot analysis was performed to determine if additional putative *AATP* homologs were present within the cassava genome (Fig. 1). Of the restriction enzymes utilized in our analysis, only *Bam*HI and *Eco*RV restriction sites are absent from the cDNA sequences of both genes. *Eco*RI, *Nco*I and *Xba*I all cut once within the coding region of *MeAATP1*, whereas the *MeAATP2* coding sequence contains but a single *Nco*I site. Since our

probe detects only two bands in *Eco*RV-digested genomic DNA, and three bands when digested with *Xba*I, the simplest interpretation of our data is that there are only two *AATP* homologs in cassava, although we cannot exclude the possibility of more distantly-related genes which are undetectable by our probe.

The deduced amino acid sequences of MeAATP1 and MeAATP2 are 92 % identical to each other, and share 81 - 82 % identity to *Solanum tuberosum* AATP1. Of the two cassava proteins, MeAATP2 is slightly larger due to an extension of 4 amino acids near its C-terminus. Multiple sequence alignment of MeAATP1 and MeAATP2 to the full-length sequences of previously characterized plastidic ATP/ADP transporters from *S. tuberosum* (StAATP1; Tjaden *et al*. 1998a) and *Arabidopsis thaliana* (AtAATP1 and AtAATP2; Tjaden *et al*. 1998b, Möhlmann *et al*. 1998), as well as putative homologs from *Oryza sativa* (OsAATP1 and OsAATP2) and *Mesembryanthemum crystallinum* (McANT1, for adenine nucleotide transporter) revealed an extremely high degree of sequence identity in the region spanning predicted transmembrane (TM) domains 1 through 12 (Fig. 2). Weaker sequence conservation was observed among the dicotyledonous proteins in the regions N-terminal to TM1 or C-terminal to TM12.

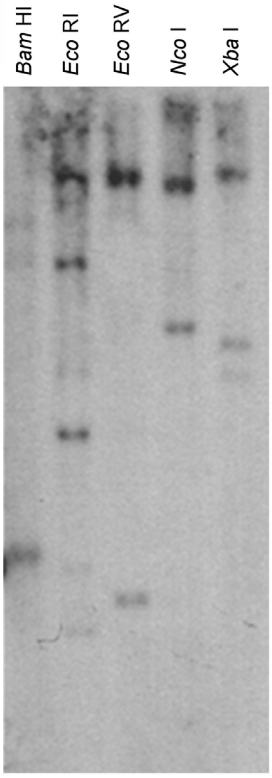

Fig 1. Southern blot analysis. Cassava genomic DNA was digested with *Bam*HI, *Eco*RI, *Eco*RV, *Nco*I or *Xba*I, as indicated.

Prediction of transit peptide cleavage sites: The precursor peptides of plastidic ATP/ADP translocases are expected to possess removable N-terminal transit peptides to facilitate their localization to the inner plastid membrane. All eight examined sequences (both monocot and dicot) were predicted by *ChloroP v1.1* to localize to plastids (Table 1). In the case of the dicot precursor peptides, the predicted transit peptide cleavage sites (CS) fell within an alanine-rich conserved motif [Ile-Cys-(Arg/Lys)↓Ala-Glu-Ala-Ala-Ala-Ala] (Table 1). Although neither OsAATP1 nor OsAATP2 possess this motif, their *ChloroP*-predicted cleavage sites are also associated with an enrichment of alanine residues.

Phylogenetic analysis of plastidic ATP/ADP transport proteins: Phylogenetic trees were constructed to examine the evolutionary relationships between MeAATP1 and MeAATP2 with ATP/ADP translocases from other plant species. Both Neighbor-Joining (NJ) and Maximum Parsimony (MP) methods were employed, with monocot proteins OsAATP1 and OsAATP2 defined as the

Table 1. Chloroplast cleavage site predictions. Chloroplast localization scores and transit peptide cleavage sites (cTP) were predicted using the neural network program *ChloroP v1.1*. The sequence flanking the potential cleavage site is presented, with the underlined residue being immediately C-terminal (+1) to the position. The chloroplast cleavage site prediction (CS) scores shown below are for the residue at this position, with higher values indicating a greater level of confidence. Alanine residues in the sequence are underlined for emphasis. *Arabidopsis thaliana* (At), *Solanum tuberosum* (St), *Oryza sativa* (Os) and *Manihot esculenta (*Me) ATP/ADP transporter (AATP)*, Mesembryanthemum crystallinum* (Mc) adenine nucleotide transporter (ANT).

Protein	Localization score	cTP length	CS score	Sequence
AtAATP1	0.503	79	8.083	Ile-Cys-Lys-Ala-Glu-Ala-Ala-Ala-Ala
AtAATP2	0.503	76	8.083	Ile-Cys-Lys-Ala-Glu-Ala-Ala-Ala-Ala
StAATP1	0.542	79	8.083	Ile-Cys-Lys-Ala-Glu-Ala-Ala-Ala-Ala
MeAATP1	0.556	75	11.216	Ile-Cys-Arg-Ala-Glu-Ala-Ala-Ala-Ala
MeAATP2	0.555	75	11.216	Ile-Cys-Arg-Ala-Glu-Ala-Ala-Ala-Ala
McANT1	0.570	78	11.216	Ile-Cys-Arg-Ala-Glu-Ala-Ala-Ala-Ala
OsAATP1	0.569	93	6.946	Ala-Gln-Pro-Ala-Ala-Ala-Ala-Ala-Ala
OsAATP2	0.507	71	11.302	Pro-Leu-Arg-Ala-Ala-Ala-Ala-Ser-Ala

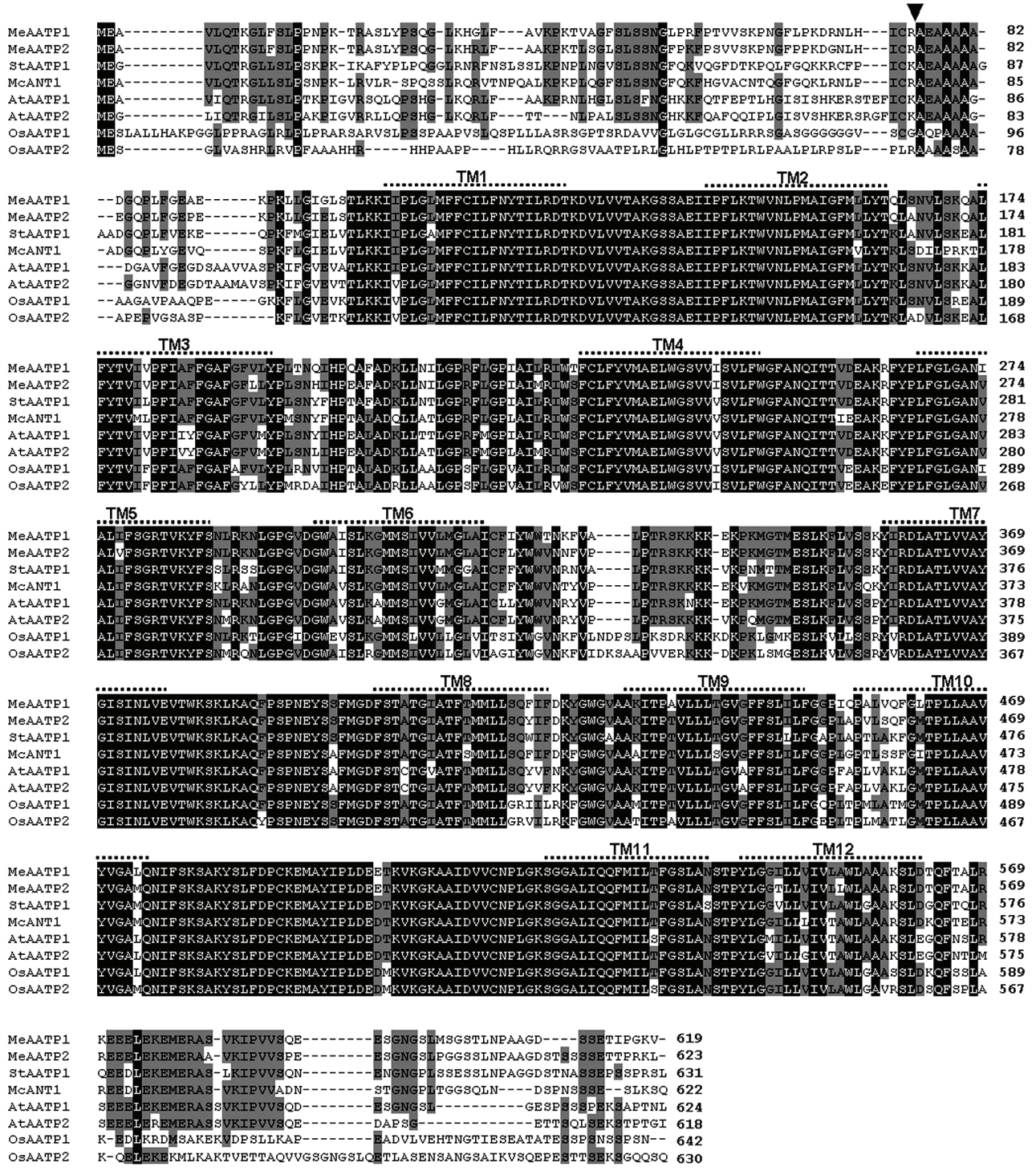

Fig 2. Multiple alignment of plastidic ATP/ADP transporters. *Black shading* is used to denote residues strictly conserved in all sequences, while residues matching the consensus (5 or more sequences) are *shaded in gray*. The positions of the 12 predicted transmembrane domains of AtAATP1, as reported by Trentmann *et al.* (2000), are indicated above the aligned sequences. The predicted transit peptide cleavage site for MeAATP1 and MeAATP2 is marked with a *triangle*.

outgroup for purposes of tree-rooting. Essentially similar trees were created by the NJ and MP algorithms, although MP did not provide bootstrap support for the node linking the two *Arabidopsis thaliana* isoforms to the remaining four dicot homologs (Fig. 3). Based on our phylogenetic reconstruction, the duplication event responsible for the

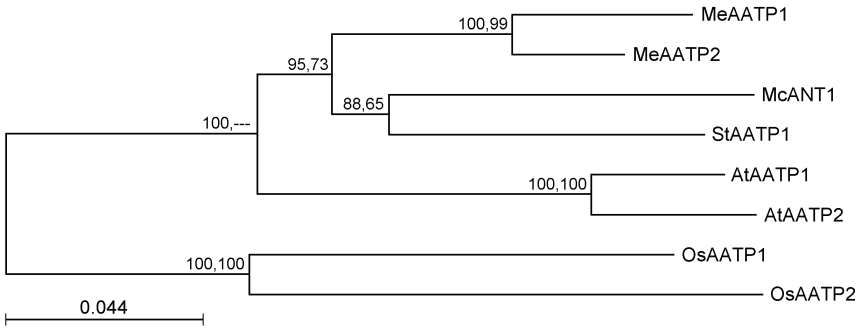

Fig 3. Phylogenetic reconstruction of the adenylate transporter family in plants. The Neighbor-Joining (NJ) tree was generated using deduced amino acid sequences, with global gap removal and Poisson-correction distance. Similar sequence groupings were obtained by Maximum Parsimony (MP). Numbers at each node represent bootstrap values (NJ, MP) for 1000 replicates.

formation of MeAATP1 and MeAATP2 appears to have occurred after the divergence of cassava from its most recent common ancestors to *Arabidopsis*, potato and rice.

Analysis of *MeAATP1* and *MeAATP2* gene expression patterns: Duplicated genes can become functionally divergent not only through alterations in the properties of their encoded products, but also through the temporal and/or spatial differentiation of their expression patterns. To determine the extent to which the expression patterns of *MeAATP1* and *MeAATP2* overlap, we generated primer pairs specific to each gene and analyzed their relative expression levels across various cassava tissues *via* RT-PCR. Primers for the amplification of the housekeeping gene *ELONGATION FACTOR 1α* (*MeEF1α*) were added to each reaction to serve as an internal control standard (*i.e.* multiplex PCR). For our analyses, we obtained tissue samples from 3-month-old cassava cv. KU50 plants grown under field conditions. Samples were collected from fibrous and tuberous roots, mature (fully-expanded, sink) and young (newly-emerging, source) leaves, and green (non-woody) stems. Floral expression was not assessed due to the weak flowering phenotype of this particular cultivar. Both *MeAATP1* and *MeAATP2* transcripts were detected in all tissue types examined (Fig. 4*A*).

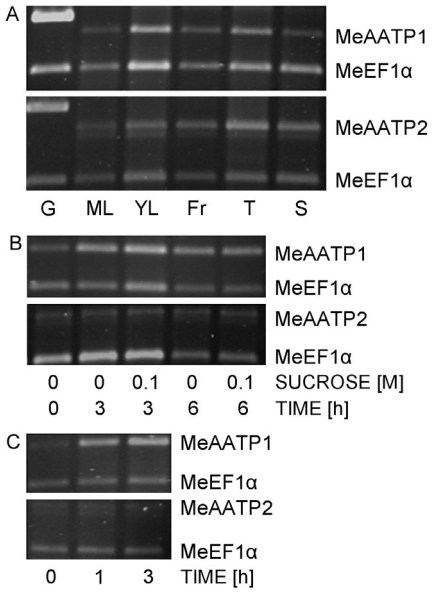

Fig 4. Analysis of *MeAATP1* and *MeAATP2* expression patterns. Multiplex RT-PCR was used to compare the relative expression levels of *MeAATP1* and *MeAATP2* in various cassava tissues (*A*), in response to exogenous sucrose (*B*), or after wounding (*C*). Tissues examined included mature leaves (ML), young leaves (YL), fibrous roots (Fr), tuberous roots (T), and green stems (S). A control PCR reaction using 100 ng genomic DNA as template was loaded in *lane G*. The larger size of the *MeAATP1* and *MeAATP2* amplicons in genomic DNA control reactions is presumably due to the presence of one or more introns.

As the main form of carbon transported through the vascular system, sucrose is the principle starting point for the synthesis of a large number of important organic compounds within sink tissues. It has recently been demonstrated that *AtAATP1* is upregulated in *Arabidopsis* leaves incubated in the presence of exogenous sucrose, whereas *AtAATP2* is not (Reiser *et al*. 2004). To test if *MeAATP1* and/or *MeAATP2* transcript levels are also increased by sucrose, we placed roughly equivalent-sized (~1cm-wide) strips of green cassava leaves in either water or a 100 mM sucrose solution. After 3 or 6 h of incubation, the relative expression levels of *MeAATP1* and *MeAATP2* in sucrose-treated samples did not differ appreciably from that of untreated controls incubated over the same time interval (Fig. 4*B*), indicating that neither gene appears to be sucrose-inducible in leaves.

Unexpectedly, we observed that the expression level of *MeAATP1* after 3 h or 6 h of incubation (with or without sucrose) was consistently higher than that of 0 h control leaves (Fig. 4b). This led us to question whether *MeAATP1* was being upregulated in response to mechanical wounding. To test this hypothesis, we again cut cassava leaves into equal-sized strips, and either froze them immediately in liquid N_2, or kept them at room temperature for 1 or 3 h on a dry paper towel. The relative expression levels of both *MeAATP1* and *MeAATP2* were again assessed through multiplex RT-PCR. Consistent with our earlier results, the relative abundance

of *MeAATP1* was found to be higher 1 or 3 h after wounding, whereas the expression level of *MeAATP2* did not appear to be altered appreciably over a similar time interval (Fig. 4*C*).

Discussion

The movement of cytosolic ATP across the plastid inner membrane is facilitated by members of the plastidic ATP/ADP transport protein family. In this study we isolated two tuber-expressed cDNAs encoding for putative plastidic adenylate transporters in cassava. The deduced amino acid sequence for the products encoded by *MeAATP1* and *MeAATP2* show strong sequence identity to other plastidic ATP/ADP transporter homologs, particularly in the region encompassing their predicted membrane spanning helices (Fig. 2). In addition, all dicot members of the AATP family possessed a conserved N-terminal motif, Ile-Cys-(Arg/Lys)-Ala-Glu-Ala-Ala-Ala-Ala, which was associated with the predicted transit peptide cleavage sites for MeAATP1 and MeAATP2 (Table 1). Interestingly, although the precursor peptide sequences of OsAATP1 and OsAATP2 do not contain this motif, they are also predicted to localize to plastids, and their predicted transit peptide cleavage sites are also associated with a short alanine-rich stretch of amino acids.

The genomes of *Arabidopsis* and rice both possess two plastidic ATP/ADP transporter genes, and Southern blot analysis suggests that there are also only two putative *AATP* homologs in cassava (Fig. 1). Since cassava is potentially an allotetraploid (Umanah and Hartmann 1973), it is possible that *MeAATP1* and *MeAATP2* are homologous genes originating from the hybridization of *M. esculenta*'s hypothetical diploid progenitors, although alternative events leading to gene duplication cannot be excluded. Phylogenetic reconstructions indicate that the *MeAATP1*/*MeAATP2* duplication event is distinct from the duplications that gave rise to the homologous pairs in *Arabidopsis* or rice, and thus the differential expression patterns reported by Reiser *et al*. (2004) for *AtAATP1* and *AtAATP2* are unlikely to be shared by the isoform pairs in either rice or cassava.

In *Arabidopsis*, *AtAATP1* and *AtAATP2* exhibit partially overlapping, yet distinct, expression patterns, and *AtAATP1* is upregulated in the presence of exogenous glucose or sucrose. Sucrose is the main starting point of the starch biosynthesis pathway, and thus the association of *AtAATP1* activity with the presence of sucrose may serve as a means of modulating the rate of starch biosynthesis to reflect the availability of substrates, or to reprogram chloroplasts into starch-accumulating, ATP-importing storage plastids (Reiser *et al*. 2004). Considering the high flux control coefficient of the plastidic ATP/ADP transporter on the starch biosynthetic pathway in potato, the ability to couple the rate of plastidic ATP import to cytosolic sucrose levels may serve to increase the efficiency of starch biosynthesis in sink tissues. Expression analyses of *MeAATP1* and *MeAATP2* revealed that both genes were expressed in leaves, stems, and tuberous and fibrous roots (Fig. 4*A*), but neither *MeAATP1* nor *MeAATP2* appeared to be sucrose-inducible (Fig. 4*B*). Thus, one potential means of increasing the efficiency of starch biosynthesis in cassava would be to introduce a plastidic ATP/ATP transporter gene under the control of a sucrose-inducible promoter such as that of *AtAATP1*.

Interestingly, we observed that *MeAATP1* showed signs of significant upregulation in leaves after mechanical wounding, although it remains to be determined what role, if any, the increased expression of *MeAATP1* has on the wound-response mechanism of cassava. Plants respond to wounding by increasing the production of various compounds related to wound repair and the defense against pathogens, as well as proteins involved with basic cell metabolism and upkeep. Some of these processes are known to occur within plastids: fatty acid signaling in plastids, for example, has been shown to modulate both jasmonic acid- and salicylic acid-related defense pathways in *Arabidopsis* (Kachroo *et al*. 2003). More recently, it has been reported that tomato leaves damaged by fire accumulate transcripts corresponding to a variety of chloroplast-targeted proteins, including putative homologs of *A. thaliana* acyl carrier protein 4 (ACP4), a participant in fatty acid synthesis (Branen *et al*. 2003), adenosine 5'-phosphosulfate reductase 1 (APR1), involved in cysteine and glutathione synthesis (Bick *et al.* 2001), PS 2 oxygen-evolving complex protein 3 (PsbQ), and ribosomal protein S30 (Coker *et al*. 2005). Thus, multiple plastid-bound processes are elicited in response to wounding, and it is conceivable that increased ATP/ADP transporter activity assists in one or more of these pathways by supplying biochemical energy in the form of ATP. To our knowledge, the effects of mechanical damage on the transcriptional activity of *AATP* genes has not been assessed in other plants, and thus it is unclear if wound-induction of AATP genes is common to the plant kingdom or a novel adaptation in cassava.

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