Identification of genes encoding ALMT and MATE transporters as candidate aluminum tolerance genes from a typical acid soil plant, *Psychotria rubra* (Rubiaceae)

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ABSTRACT

To understand how tropical plants have adapted to acid soils, we analyzed the transcriptome of seedlings of *Psychotria rubra*, a typical species found on acid soils. Using RNA-seq, we identified 22,798 genes, including several encoding proteins of the Al^{3+}-activated malate transporter (ALMT) and multidrug and toxic compound extrusion (MATE) families. Molecular phylogenetic analysis of ALMTs and MATEs revealed the grouping of those from *P. rubra*, which may be useful to select targets for elucidating the molecular basis of *P. rubra* adaptation to acid soils in the future. The transcriptome datasets obtained in this study would help us to further understand the physiological and ecological aspects of soil adaptation of *Psychotria* species.

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INTRODUCTION

Understanding how plants adapt to various soils is essential in plant biology (*Hiradate, Ma & Matsumoto, 2007*) because plants are sessile and need to grow roots in settled soils. Adaptation to acid soils is an important issue because acid soils cover a considerable part of Earth's arable land and prevent agriculture of most plants (*Von Uexküll & Mutert, 1995*). In acid soils, aluminum is toxic to root tip growth, and various aspects, from molecular to physiological, of the mechanisms of aluminum toxicity have been explored in detail (reviewed in *Ma, 2007*).

Proteins of the Al^{3+}-activated malate transporter (ALMT) and multidrug and toxic compound extrusion (MATE) families are likely involved in plant adaptation to acid soils (*Delhaize, Gruber & Ryan, 2007*; *Delhaize, Ma & Ryan, 2012*; *Ma, 2007*; *Ma, Chen & Shen, 2014*). ALMTs and MATEs release organic acids (malate and citrate, respectively), which bind Al^{3+} and detoxify it. ALMTs and MATEs related to aluminum tolerance have been

Figure 1 *Psychotria rubra.* (A) Shrub on Mount Nago-dake, Okinawa, Japan. (B) Seedlings.

identified in model and agricultural plants (wheat: *Sasaki et al., 2004*; barley: *Delhaize et al., 2004*; *Furukawa et al., 2007*; maize: *Maron et al., 2010*; *Arabidopsis*: *Hoekenga et al., 2006*; *Liu et al., 2009*), but the composition of those families in non-model wild plants has hardly been explored.

Psychotria (Rubiaceae) is a highly diversified genus comprising more than 1,600 species distributed in all tropical and some subtropical regions (*Hamilton, 1989*; *Davis et al., 2001*; *Razafimandimbison et al., 2014*). Because *Psychotria* species adapt to several types of soils (e.g., soils with high concentrations of nickel; *Merlot et al., 2014*), the genus is an ideal target to use to understand how adaptation of wild plants to different types of soils has evolved. In this study, we report ALMTs and MATEs of *P. rubra*, which grows on acid soils (*Miyawaki, 1989*).

MATERIALS AND METHODS

Sampling, RNA extraction, RNA-seq library preparation, and sequencing

Seeds of *P. rubra* were collected on Mt. Nago-dake, in the north of Okinawa Island, and seedlings were grown in a greenhouse of the National Institute of Technology, Okinawa College (Fig. 1). RNA was extracted from the seedlings (three–five cm height) using an RNeasy Plant Mini kit (Qiagen, Hilden, Germany). An RNA-seq library was prepared using a TruSeq Stranded mRNA Sample Prep Kit (Illumina, San Diego, CA, USA). The library was sequenced (100-bp paired-end reads) on an Illumina HiSeq 2500 platform. The above procedures on RNA-seq were outsourced to Hokkaido System Science Corporation, Japan.

Table 1 Summary of de novo assembly.

Number of contigs	131,578
Total bases (bp)	161,583,381
Longest contig length (bp)	16,195
Shortest contig length (bp)	185
Average contig length (bp)	1,228
N50	2,007

De novo assembly and annotation of transcriptome sequences

FASTQ files were filtered, the reads with poor-quality bases ($Q < 20$) and those shorter than 20-bp were excluded, and adapter sequences were removed in cutadapt v. 1.9.1 software (*Martin, 2011*). PCR duplicates that arose during library preparation were removed by ConDeTri software (*Smeds & Künstner, 2011*). The remaining paired-end reads were assembled in Trinity v. 2.8.4 software (*Grabherr et al., 2011*) with the default options. Open reading frames of >150 amino acids were identified by using TransDecoder software (*Haas et al., 2013*) and the results of BLASTP searches within the Swiss-Prot database (e-value $<1e^{-5}$). Redundant amino acid sequences were removed in CD-HIT v. 4.7 software ($-c$ 0.95; *Li & Godzik, 2006*). The remaining sequences were used as queries in BLASTP searches (e-value $<1e^{-5}$) of *Arabidopsis thaliana* sequences (TAIR10; *Swarbreck et al., 2007*) and selected sequences assumed to be *P. rubra* itself. Basic information on sequences was obtained in SeqKit v. 0.9.3 software (*Shen et al., 2016*). Sequence data are accessible under the DNA Data Bank of Japan (DDBJ) Sequence Read Archive (accession: DRA008339). The raw data has been also available at Figshare (https://doi.org/10.6084/m9.figshare.7848425.v2).

Extraction of ALMTs and MATEs of *P. rubra* and molecular phylogenetic analysis

We searched for *P. rubra* ALMTs and MATEs by BLASTP (e-value $<1e^{-5}$) using sequences of TaALMT1 (UniProt database ID: Q76LB1) and HvAACT1 (UniProt ID: A7M6U2; this was the first MATE identified in barley (*Furukawa et al., 2007*) as queries). We then performed BLASTP searches against the Swiss-Prot database (e-value $<1e^{-5}$) with each ALMT and MATE of *P. rubra* and selected the top 10 hits for each. The amino acid sequences of ALMTs and MATEs from *P. rubra* and related sequences from Swiss-Prot and other studies (*Dreyer et al., 2012*; *Liu et al., 2016*) were aligned in MAFFT v. 7.407 software (*Katoh & Standley, 2013*). We excluded four *P. rubra* ALMT sequences (Prub_02169, Prub_02171, Prub_08405, Prub_08554) from the following analysis because of poor alignment. We selected only plant MATEs, including HvAACT1 and those from (*Liu et al., 2016*), for the following analysis. Neighbor-joining trees of ALMTs and MATEs were constructed in MEGA7 software (*Kumar, Stecher & Tamura, 2016*) with the following settings: Poisson model, Uniform rates, and Pairwise deletion. To evaluate the confidence of phylogenetic trees, bootstrap tests were performed with 1,000 replicates.

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Table 2 Summary of blastp of TaALMT1 and HvAACT1 against *P. rubra* amino acid sequences and annotation of *P. rubra* sequences from the Swiss-Prot database.

Gene name	Sequence ID	% identity	*e*-value	Bit score	Annotate description (Swiss-prot)	Accession no. (Swiss-prot)	*e*-value (Swiss-prot)
TaALMT1	Prub_08163	45.966	1.05E−110	334	Aluminum-activated malate transporter 2 (AtALMT2)	Q9SJE8	3.67E−166
TaALMT1	Prub_08164	49.477	4.78E−90	277	Aluminum-activated malate transporter 2 (AtALMT2)	Q9SJE8	1.12E−133
TaALMT1	Prub_10125	43.902	1.19E−77	251	Aluminum-activated malate transporter 12 (AtALMT12) (Quick anion channel 1)	O49696	0
TaALMT1	Prub_08553	36.111	3.39E−63	214	Aluminum-activated malate transporter 9 (AtALMT9)	Q9LS46	0
TaALMT1	Prub_16333	33.333	6.06E−57	197	Aluminum-activated malate transporter 9 (AtALMT9)	Q9LS46	0
TaALMT1	Prub_03305	51.19	5.72E−53	176	Aluminum-activated malate transporter 10 (AtALMT10)	O23086	3.21E−59
TaALMT1	Prub_02168	33.772	4.74E−23	98.6	Putative aluminum-activated malate transporter 3 (AtALMT3)	Q9LPQ8	1.74E−74
TaALMT1	Prub_02171	27.801	1.10E−22	98.6	Aluminum-activated malate transporter 9 (AtALMT9)	Q9LS46	1.99E−141
TaALMT1	Prub_16334	39.655	1.03E−19	86.3	Aluminum-activated malate transporter 9 (AtALMT9)	Q9LS46	6.62E−51
TaALMT1	Prub_08405	30.108	2.45E−19	88.6	Aluminum-activated malate transporter 9 (AtALMT9)	Q9LS46	9.01E−62
TaALMT1	Prub_08554	51.852	6.42E−12	63.2	Aluminum-activated malate transporter 9 (AtALMT9)	Q9LS46	3.20E−27
TaALMT1	Prub_02170	39.062	4.77E−11	62	Aluminum-activated malate transporter 9 (AtALMT9)	Q9LS46	7.28E−32
TaALMT1	Prub_02169	30	2.57E−07	51.6	Aluminum-activated malate transporter 4 (AtALMT4)	Q9C6L8	1.57E−73
TaALMT1	Prub_02167	38.129	3.26E−07	50.8	Aluminum-activated malate transporter 9 (AtALMT9)	Q9LS46	3.96E−26
HvAACT1	Prub_03062	57.752	0	537	Protein DETOXIFICATION 42 (AtDTX42) (Aluminum-activated citrate transporter) (AtMATE) (FRD-like protein) (Multidrug and toxic compound extrusion protein 42) (MATE protein 42)	Q9SYD6	0
HvAACT1	Prub_17943	55.955	0	536	Protein DETOXIFICATION 42 (AtDTX42) (Aluminum-activated citrate transporter) (AtMATE) (FRD-like protein) (Multidrug and toxic compound extrusion protein 42) (MATE protein 42)	Q9SYD6	0
HvAACT1	Prub_17944	59.432	0	531	Protein DETOXIFICATION 42 (AtDTX42) (Aluminum-activated citrate transporter) (AtMATE) (FRD-like protein) (Multidrug and toxic compound extrusion protein 42) (MATE protein 42)	Q9SYD6	0
HvAACT1	Prub_17945	55.894	3.61E−168	485	Protein DETOXIFICATION 42 (AtDTX42) (Aluminum-activated citrate transporter) (AtMATE) (FRD-like protein) (Multidrug and toxic compound extrusion protein 42) (MATE protein 42)	Q9SYD6	0
HvAACT1	Prub_03064	63.372	4.58E−147	429	Protein DETOXIFICATION 43 (AtDTX43) (Multidrug and toxic compound extrusion protein 43) (MATE protein 43) (Protein FERRIC REDUCTASE DEFECTIVE 3) (AtFRD3) (Protein MANGANESE ACCUMULATOR 1)	Q9SFB0	1.12E−160
HvAACT1	Prub_03541	41.322	1.43E−120	368	Protein DETOXIFICATION 45, chloroplastic (AtDTX45) (Multidrug and toxic compound extrusion protein 45) (MATE protein 45)	Q9SVE7	0

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Table 2 (*continued*)

Gene name	Sequence ID	% identity	*e*-value	Bit score	Annotate description (Swiss-prot)	Accession no. (Swiss-prot)	*e*-value (Swiss-prot)
HvAACT1	Prub_18165	39.506	2.75E−114	350	Protein DETOXIFICATION 45, chloroplastic (AtDTX45) (Multidrug and toxic compound extrusion protein 45) (MATE protein 45)	Q9SVE7	0
HvAACT1	Prub_03065	54.4	6.52E−55	186	Protein DETOXIFICATION 42 (AtDTX42) (Aluminum-activated citrate transporter) (AtMATE) (FRD-like protein) (Multidrug and toxic compound extrusion protein 42) (MATE protein 42)	Q9SYD6	3.65E−69
HvAACT1	Prub_03063	53.441	4.31E−52	179	Protein DETOXIFICATION 42 (AtDTX42) (Aluminum-activated citrate transporter) (AtMATE) (FRD-like protein) (Multidrug and toxic compound extrusion protein 42) (MATE protein 42)	Q9SYD6	1.00E−66
HvAACT1	Prub_14684	43.541	4.14E−37	137	Protein DETOXIFICATION 44, chloroplastic (AtDTX44) (Multidrug and toxic compound extrusion protein 44) (MATE protein 44)	Q84K71	4.41E−94
HvAACT1	Prub_14685	38.174	2.44E−22	96.7	Protein DETOXIFICATION 44, chloroplastic (AtDTX44) (Multidrug and toxic compound extrusion protein 44) (MATE protein 44)	Q84K71	4.59E−53
HvAACT1	Prub_18629	23.25	7.67E−14	73.6	Protein DETOXIFICATION 46, chloroplastic (AtDTX46) (Multidrug and toxic compound extrusion protein 46) (MATE protein 46) (Protein EDS5 HOMOLOGUE)	Q8W4G3	0
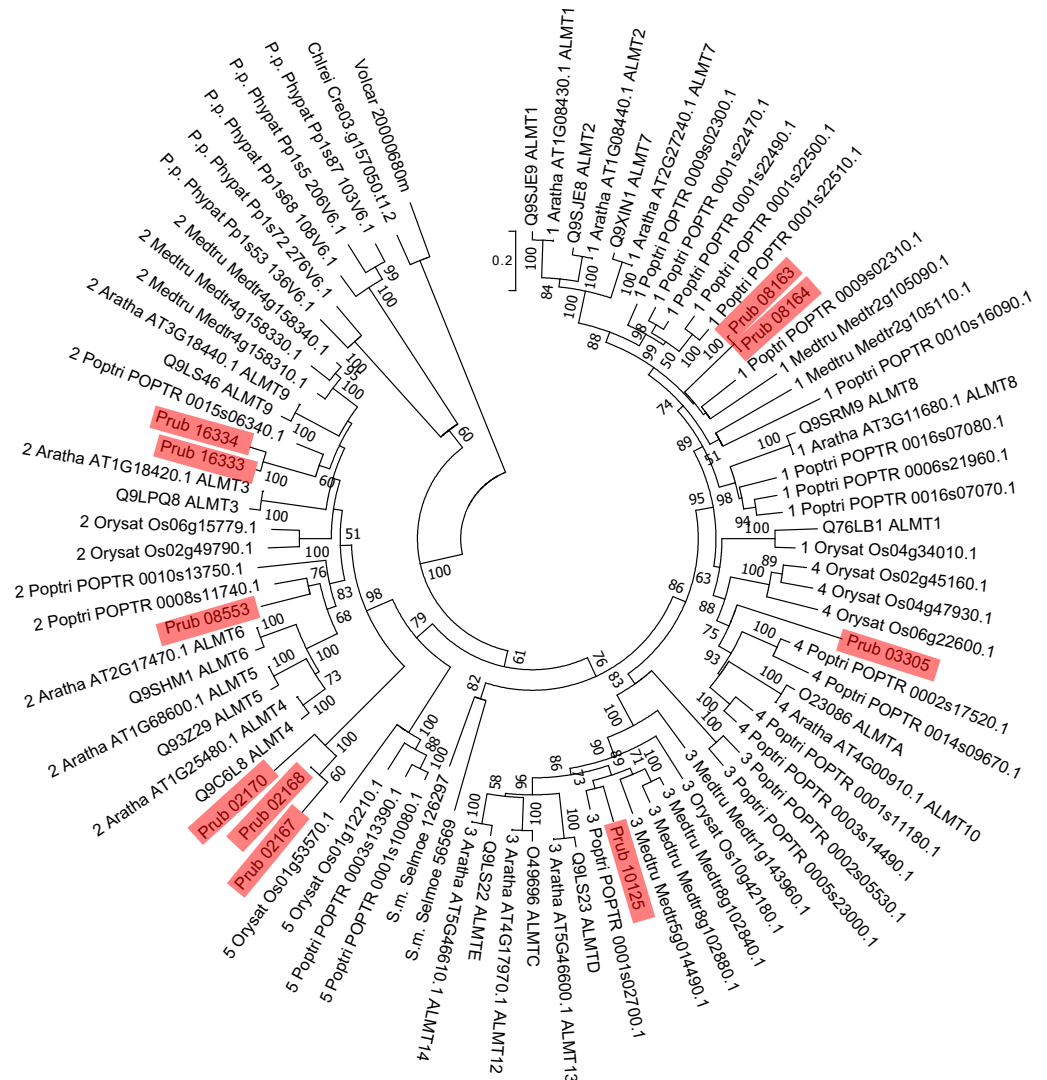
Figure 2 **Phylogenetic tree of ALMTs of *P. rubra* and related proteins.** Sequences of *P. rubra* are shown using red shades. For sequences other than *P. rubra* ALMTs, the labels show the UniProt database ID and ALMT type, and ones from *Dreyer et al. (2012)*. Number at each node is the bootstrap value.

RESULTS AND DISCUSSION

Our RNA-seq analysis of *P. rubra* yielded 57,110,261 paired-end reads, of which 53,994,410 remained after filtering. De novo assembly of the remaining reads resulted in 131,578 contigs (Table 1), in which we found 24,687 non-redundant amino acid sequences; 22,798 of them were expected to originate from *P. rubra* itself as indicated by BLASTP analysis of TAIR10 (the remaining 1,889 sequences were almost no-hit in Swiss-Prot database or included those from microorganisms, etc). Among these 22,798 sequences, 19,701 ones were hit against Swiss-Prot database, and gene ontology (GO) numbers were found in 1,0348 ones. From these sequences, we found 14 ALMTs and 12 MATEs (Table 2).
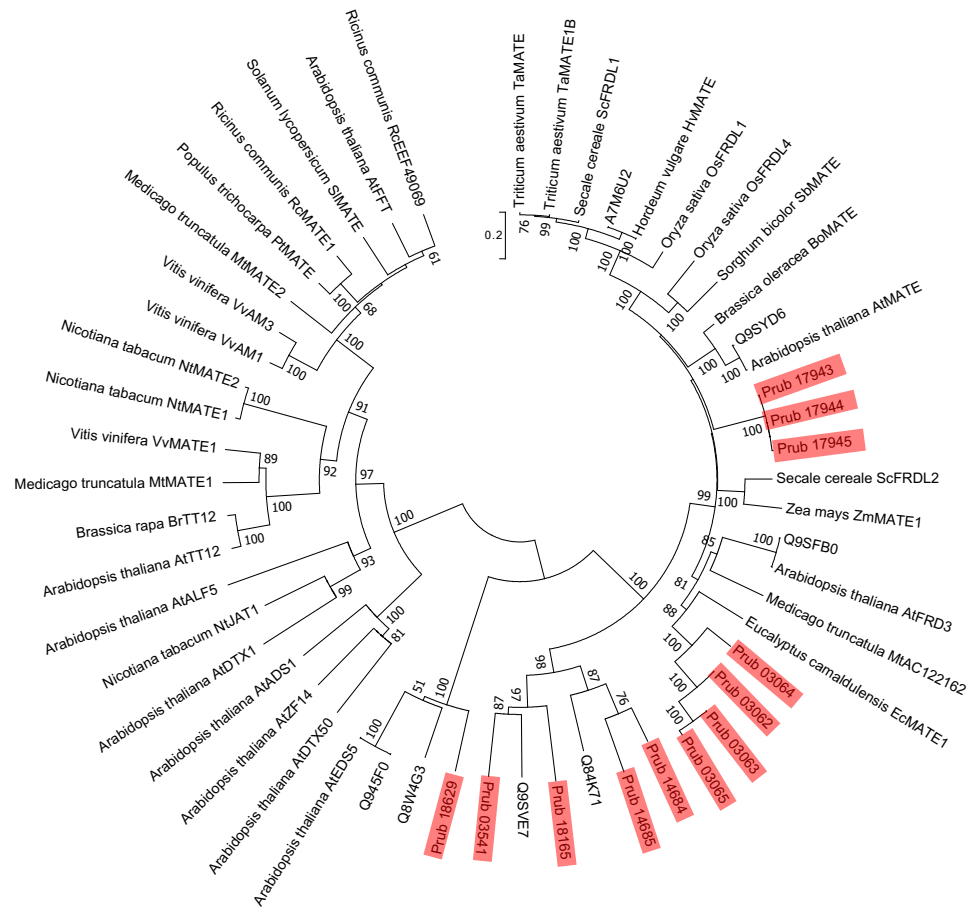
Figure 3 **Phylogenetic tree of MATEs of *P. rubra* and related proteins.** Sequences of *P. rubra* are shown using red shades. For sequences other than *P. rubra* MATEs, the labels are UniProt database IDs, and ones from *Liu et al. (2016)*. Number at each node is the bootstrap value.

Using 14 ALMTs from *P. rubra* as queries in a BLASTP search against the Swiss-Prot database, we found 14 homologs, all of plant origin (mainly from *Arabidopsis*). Using a similar approach, we found 13 homologs of MATEs (six from *Arabidopsis* and seven from non-plant organisms). Molecular phylogenetic analysis did not detect *P. rubra* orthologs of TaALMT1 (UniProt ID: Q76LB1) (Fig. 2). ScALMT1 (accession number: ABA62397) from rye (*Secale cereale*) is the only known clear ortholog of TaALMT1 (*Delhaize, Gruber & Ryan, 2007*). ALMT1 of *Arabidopsis* (UniProt ID: Q9SJE9), encoded by an aluminum tolerance gene (*Hoekenga et al., 2006*), is clearly distinct from TaALMT1 (*Delhaize, Gruber & Ryan, 2007*). Thus, ALMTs related to aluminum tolerance may have multiple origins. Molecular phylogenetic analysis of MATEs revealed no clear orthologs of HvAACT1 (UniProt ID: A7M6U2) in *P. rubra* (Fig. 3).

Expression and functional analyses of ALMTs and MATEs of *P. rubra* would be useful for understanding their roles in soil adaptation of *Psychotria* (e.g., with and without Al treatment). Another aluminum tolerance mechanism of plants (different from releasing

organic acids), aluminum accumulation, has been reported in several species of the Rubiaceae (*Jansen et al., 2003*). Genes related to this function are also good targets for future studies to explain the molecular basis of acid soil adaptation of *P. rubra*.

CONCLUSIONS

We succeeded in identifying transcriptome sequences including ALMTs and MATEs from *P. rubra* in this study. Comparative transcriptome analysis of several *Psychotria* species would help us to clarify the physiological and ecological aspects of diversification of this genus (e.g., adaptation to metalliferous soils; *Merlot et al., 2014*). In particular, *Psychotria manillensis*, which is closely related to *P. rubra*, is reportedly adapted to non-acid soils (*Miyawaki, 1989*). Thus, comparative analysis of *P. rubra* and *P. manillensis* should help to explain how soil adaptation–related genes are involved in adaptive evolution of *Psychotria* species.

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Akira Iguchi conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Kazutsuka Sanmiya and Kenta Watanabe performed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

Sequence data are accessible at the DNA Data Bank of Japan (DDBJ) Sequence Read Archive: DRA008339.

Data Availability

The raw data is available at Figshare: Iguchi, Akira (2019): Transcriptome of *Psychotria rubra* (Rubiaceae). figshare. Dataset. https://doi.org/10.6084/m9.figshare.7848425.v2.

REFERENCES

Davis AP, Bridson D, Jarvis C, Govaerts R. 2001. The typification and characterization of the genus *Psychotria* L. (Rubiaceae). *Botanical Journal of the Linnean Society* **135**:35–42 DOI 10.1111/j.1095-8339.2001.tb02366.x.

Delhaize E, Gruber BD, Ryan PR. 2007. The roles of organic anion permeases in aluminium resistance and mineral nutrition. *FEBS Letters* **581**:2255–2262 DOI 10.1016/j.febslet.2007.03.057.

Delhaize E, Ma JF, Ryan PR. 2012. Transcriptional regulation of aluminium tolerance genes. *Trends in Plant Science* **17**:341–348 DOI 10.1016/j.tplants.2012.02.008.

Delhaize E, Ryan PR, Hebb DM, Yamamoto Y, Sasaki T, Matsumoto H. 2004. Engineering high-level aluminum tolerance in barley with the ALMT1 gene. *Proceedings of the National Academy of Sciences of the United States of America* **101**:15249–15254 DOI 10.1073/pnas.0406258101.

Dreyer I, Gomez-Porras JL, Riaño Pachón DM, Hedrich R, Geiger D. 2012. Molecular evolution of slow and quick anion channels (SLACs and QUACs/ALMTs). *Frontiers in Plant Science* **3**:263 DOI 10.3389/fpls.2012.00263.

Furukawa J, Yamaji N, Wang H, Mitani N, Murata Y, Sato K, Ma JF. 2007. An aluminum-activated citrate transporter in barley. *Plant and Cell Physiology* **48**:1081–1091 DOI 10.1093/pcp/pcm091.

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Chen Z. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* **29**:644–652 DOI 10.1038/nbt.1883.

Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, MacManes MD. 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols* **8**:1494–1512 DOI 10.1038/nprot.2013.084.

Hamilton CW. 1989. A revision of Mesoamerican *Psychotria* subgenus *Psychotria* (Rubiaceae), part I: introduction and species 1-16. *Annals of the Missouri Botanical Garden* **76**:67–111 DOI 10.2307/2399343.

Hiradate S, Ma JF, Matsumoto H. 2007. Strategies of plants to adapt to mineral stresses in problem soils. *Advances in Agronomy* **96**:65–132 DOI 10.1016/S0065-2113(07)96004-6.

Hoekenga OA, Maron LG, Piñeros MA, Cançado GM, Shaff J, Kobayashi Y, Matsumoto H. 2006. *AtALMT1*, which encodes a malate transporter, is identified as one of several genes critical for aluminum tolerance in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America* **103**:9738–9743 DOI 10.1073/pnas.0602868103.

Jansen S, Watanabe T, Dessein S, Smets E, Robbrecht E. 2003. A comparative study of metal levels in leaves of some Al-accumulating Rubiaceae. *Annals of Botany* **91**:657–663 DOI 10.1093/aob/mcg071.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**:772–780 DOI 10.1093/molbev/mst010.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**:1870–1874 DOI 10.1093/molbev/msw054.

Li W, Godzik A. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* **22**:1658–1659 DOI 10.1093/bioinformatics/btl158.

Liu J, Li Y, Wang W, Gai J, Li Y. 2016. Genome-wide analysis of MATE transporters and expression patterns of a subgroup of MATE genes in response to aluminum toxicity in soybean. *BMC Genomics* **17**:223 DOI 10.1186/s12864-016-2559-8.

Liu J, Magalhaes JV, Shaff J, Kochian LV. 2009. Aluminum-activated citrate and malate transporters from the MATE and ALMT families function independently to confer *Arabidopsis* aluminum tolerance. *The Plant Journal* **57**:389–399 DOI 10.1111/j.1365-313X.2008.03696.x.

Ma JF. 2007. Syndrome of aluminum toxicity and diversity of aluminum resistance in higher plants. *International Review of Cytology* **264**:225–252 DOI 10.1016/S0074-7696(07)64005-4.

Ma JF, Chen ZC, Shen RF. 2014. Molecular mechanisms of Al tolerance in gramineous plants. *Plant and Soil* **381**:1–12 DOI 10.1007/s11104-014-2073-1.

Maron LG, Piñeros MA, Guimarães CT, Magalhaes JV, Pleiman JK, Mao C, Kochian LV. 2010. Two functionally distinct members of the MATE (multi-drug and toxic compound extrusion) family of transporters potentially underlie two major aluminum tolerance QTLs in maize. *The Plant Journal* **61**:728–740 DOI 10.1111/j.1365-313X.2009.04103.x.

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. Journal* **17**:10–12 DOI 10.14806/ej.17.1.200.

Merlot S, Hannibal L, Martins S, Martinelli L, Amir H, Lebrun M, Thomine S. 2014. The metal transporter PgIREG1 from the hyperaccumulator *Psychotria gabriellae* is a candidate gene for nickel tolerance and accumulation. *Journal of Experimental Botany* **65**:1551–1564 DOI 10.1093/jxb/eru025.

Miyawaki A. 1989. *Vegetation of Japan 10 Okinawa/Ogasawara*. Tokyo: Shibundo [in Japanese].

Razafimandimbison SG, Taylor CM, Wikström N, Pailler T, Khodabandeh A, Bremer B. 2014. Phylogeny and generic limits in the sister tribes Psychotrieae and Palicoureeae (Rubiaceae): evolution of schizocarps in *Psychotria* and origins of bacterial leaf nodules of the Malagasy species. *American Journal of Botany* **101**:1102–1126 DOI 10.3732/ajb.1400076.

Sasaki T, Yamamoto Y, Ezaki B, Katsuhara M, Ahn SJ, Ryan PR, Matsumoto H. 2004. A wheat gene encoding an aluminum-activated malate transporter. *The Plant Journal* **37**:645–653 DOI 10.1111/j.1365-313X.2003.01991.x.

Shen W, Le S, Li Y, Hu F. 2016. SeqKit: a cross-platform and ultrafast toolkit for FASTA/Q file manipulation. *PLOS ONE* **11**:e0163962 DOI 10.1371/journal.pone.0163962.

Smeds L, Künstner A. 2011. ConDeTri-a content dependent read trimmer for Illumina data. *PLOS ONE* **6**:e26314 DOI 10.1371/journal.pone.0026314.

Swarbreck D, Wilks C, Lamesch P, Berardini TZ, Garcia-Hernandez M, Foerster H, Radenbaugh A. 2007. The *Arabidopsis* Information Resource (TAIR): gene structure and function annotation. *Nucleic Acids Research* **36**:D1009–D1014 DOI 10.1093/nar/gkm965.

Von Uexküll HR, Mutert E. 1995. Global extent, development and economic impact of acid soils. *Plant and Soil* **171**:1–15 DOI 10.1007/BF00009558.