Differential expression of *Nrat1* is responsible for Al-tolerance QTL on chromosome 2 in rice

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Received 23 January 2014; Revised 6 April 2014; Accepted 10 April 2014

Abstract

Although rice (*Oryza sativa*) is the most Al-tolerant species among small-grain cereal crops, there is wide genotypic variation in its tolerance to Al toxicity. A number of quantitative trait loci (QTLs) for Al tolerance have been detected, but the responsible genes have not been identified. By using chromosome segment substitution lines, this work found that *Nrat1*, a gene encoding an Al transporter, is responsible for a QTL previously detected on chromosome 2. Substitution of the chromosome segment containing *Nrat1* from Koshihikari (Al-tolerant variety) by that from Kasalath (Al-sensitive variety) decreased *Nrat1* expression and Al uptake and tolerance, but increased binding of Al to the cell wall. *Nrat1* in Kasalath showed tissue localization similar to Koshihikari in the roots. Although Koshihikari and Kasalath differed in four amino acids in *Nrat1* protein, *Nrat1* from Kasalath also showed transport activity for Al. Analysis with site-directed mutagenesis revealed that these differences did not affect the Al-transport activity much. Furthermore, there was no correlation between Al tolerance and the open-reading-frame sequence differences in other rice varieties. On the other hand, there was good correlation between *Nrat1* expression and Al tolerance; however, sequence comparison of the promoter region up to 2.1 kb did not give a clear difference between the Al-tolerant and -sensitive varieties. Taken together, these results indicate that differential expression of *Nrat1* is responsible for the QTL for Al tolerance on chromosome 2, although the mechanism controlling *Nrat1* expression remains to be examined.

Key words: Al QTL, Al tolerance, expression, genotypic difference, *Nrat1*, *Oryza sativa*.

Introduction

Aluminium (Al) toxicity is a major limiting factor of crop production on acid soils, but there is a wide difference in Al tolerance between species and cultivars within a species (Ma et al., 2002; Famoso et al., 2011). Among small-grain cereal crops, rice (*Oryza sativa*) shows the highest tolerance to Al toxicity, but genotypic differences also exist; usually Japonica varieties show higher Al tolerance than Indica varieties (Ma et al., 2002). Furthermore, the relative degree of Al tolerance in the five sub-populations follows the order temperate japonica > tropical japonica > aromatic > indica = aus (Famoso et al., 2011).

A number of quantitative trait loci (QTLs) for Al tolerance have been identified by using different mapping populations and phenotyping methods (Ma and Furukawa, 2002). Wu et al. (2000) identified four QTLs for Al tolerance on chromosomes 1, 3, 9, and 12, using relative root length as a physiological parameter in a random inbred mapping population derived from Azucena (Al-tolerant) and IR1552 (Al-sensitive). Five and 10 QTLs, respectively, for Al tolerance scattering on different chromosomes were detected in populations derived from Chiembau × Omon 269-65 and from CT9993 × IR62266 (Nguyen et al., 2001, 2003). On the other hand, Ma et al. (2002) used relative root elongation as a parameter and identified three QTLs for Al tolerance on chromosomes 1, 2, and 6 in a population of 183
backcross inbred lines derived from a cross of Koshihikari (Al-tolerant) and Kasalath (Al-sensitive). Xue et al. (2007) mapped three QTLs for Al tolerance on chromosomes 1, 9, and 11 in a recombinant inbred line population derived from a cross between the tolerant japonica cultivar Asominori and the sensitive indica cultivar IR24 based on relative root elongation. Recently, a total of 48 distinct Al-tolerance genomic regions were detected by genome-wide association mapping based on relative root growth (Famoso et al., 2011). However, the genes responsible for these QTLs have not been identified.

On the other hand, by using mutant approaches, a number of Al-tolerance genes have been identified in Japonica varieties (Ma et al., 2014). ART1 (Al-tolerance transcription factor 1), a C2H2 zinc-finger type transcription factor, was reported to be involved in Al tolerance (Yamaji et al., 2009). ART1 regulates at least 31 genes by binding to the core cis-acting element [GGN(T/g/a/c)/C(A/G)S(C/G)] in the promoter of these genes (Tatsui et al., 2011). The expression and localization of ART1 is not induced by Al, but the expression of downstream genes is upregulated by Al within hours. Seven ART1-regulated genes (STAR1, STAR2, Nrat1, OsALS1, OsFRDL4, OsMGT1, and OsCDT3) have been functionally characterized. STAR1 and STAR2 encode a ATP-binding domain and a membrane-binding domain, respectively, of a bacterial type ABC transporter (Huang et al., 2009). The STAR1–STAR2 complex localized to vesicles transports UDP-glucose, which may be involved in cell-wall modification, resulting in decreased Al accumulation in the cell wall. OsFRDL4 is responsible for the secretion of citrate in response to Al (Yokosho et al., 2011), while OsCDT3, encoding a small cysteine-rich peptide, shows binding activity with Al, thereby preventing Al entering into the root cells (Xia et al., 2013). On the other hand, OsMGT1 functions as a Mg transporter (Chen et al., 2012), and upregulation of OsMGT1 could alleviate internal Al toxicity by enhancing Mg uptake. Nrat1, a member of Nramp family, takes up trivalent Al at the plasma membrane (Xia et al., 2010), which is required for subsequent sequestration of Al into the vacuoles for final detoxification. Vacular sequestration of Al is mediated by OsALS1, a half-size ABC transporter localized to the tonoplast (Huang et al., 2012). Among these genes examined, OsFRDL4 showed a good correlation between its expression level and Al tolerance (Yokosho et al., 2011), indicating that this gene may be responsible for the genotypic difference in Al tolerance. By contrast, there is no correlation between the genotypic variation of expression levels of STAR1, OsMGT1, OsCDT3, and OsALS1 and Al tolerance (Huang et al., 2009, 2012; Chen et al., 2012; Xia et al., 2013), suggesting that they are involved in fundamental Al detoxification processes common in most rice varieties.

Recently, genome-wide association detected a single, highly significant region on chromosome 2 that was unique to the aus subpopulation (Famoso et al., 2011). This region, which contained the Nrat1 candidate gene (Os02g0131800), is the same as the location of a QTL for Al tolerance on chromosome 2 detected previously in a population from Kasalath and Koshihikari (Ma et al., 2002). This QTL explained 7.3% of the variation of Al tolerance. Physiological characterization showed that although Al-induced secretion of citrate from the roots was higher in Koshihikari than in Kasalath at higher Al concentrations, the difference between the two varieties was not significant (Ma et al., 2002).

The present study investigated whether Nrat1 is responsible for the QTL detected by using chromosomal segment substitution lines. Furthermore, expression level, tissue localization, and Al transport activity were compared between Al-tolerant and –sensitive varieties. This work found that differential Nrat1 expression level is partially responsible for the genotypic difference in Al tolerance in rice.

### Materials and methods

#### Plant materials and growth conditions

Two chromosome segment substitution lines (SL204 and SL205) were provided by the Rice Genome Resource Center (http://www.rgrc.dna.affrc.go.jp). In SL204, the segment from marker C1357 to G132 (0–60.3 cM of chromosome 2) containing Nrat1 was substituted by the Kasalath segment in Koshihikari background, while in SL205, the segment from marker G132 to C747 (60.3–107.7 cM of chromosome 2) was substituted (Supplementary Fig. S1 available at JXB online), which was used as a negative control. Rice seeds (Kasalath, Koshihikari, SL204, SL205) were soaked in tap water overnight at 30 °C in the dark and then transferred to a net floating on 0.5 mM CaCl2 in a 1.5-l plastic container. Seedlings were grown for 4–7 d at 25 °C. Similar size seedlings were selected and used for the following experiments.

#### Evaluation of Al tolerance

Six rice seedlings (5-d-old) per each genotype were exposed to 0.5 mM CaCl2 containing 0, 30, or 50 μM Al (pH 4.5) for 24 h. Root lengths were measured with a ruler before and after treatments. Relative root elongation was calculated as follows: (root elongation with Al) / (root elongation without Al) × 100. Six roots were measured for each treatment.

#### Al determination in cell sap and cell wall

For determining Al accumulation in the root tips, 5-d-old seedlings (Kasalath, Koshihikari, SL204, SL205) were exposed to 50 μM Al (pH 4.5) for 8 h and then root segments (0–1 cm, 20 roots each) were excised after washing three times with 0.5 mM CaCl2. To obtain root cell sap, the root segments were put in ultra-freeze-thermic filter units (Millipore) and centrifuged at 3000 g for 10 min at 4 °C to remove apoplastic solution. The roots were then frozen at −80 °C overnight. The root cell sap solution was obtained by thawing the samples at room temperature and centrifuging at 20 400 g for 10 min. The residual cell wall were washed three times with 70% ethanol and then immersed in a 0.5 M HCl for at least 24 h with occasional vortex. The Al concentration in solution was determined by inductively coupled plasma mass spectrometry (ICP-MS, 7700X, Agilent Technologies).

#### RNA isolation and gene expression analysis

To examine the expression pattern of Nrat1, rice seedlings (7-d-old) were exposed to different Al concentrations (0–50 μM) for 8 h. Root tips (0–1 cm) were excised. All samples with three replicates were subjected to RNA extraction. Total RNA was extracted using an RNeasy Mini Kit (Qiagen). Total RNA (1 μg) was used for
first-strand cDNA synthesis using a SuperScript II kit (Invitrogen), following the manufacturer’s instructions, with an oligo(dT)12-18 primer. Expression was determined with SYBR Premix Ex Taq (Takara) by Mastercycler ep reaclipse (Eppendorf). The primer sequences used for reverse-transcription PCR were as follows: *Nrat1*, forward 5'-GAG GCCGTG TGCAAGGAG-3' and reverse 5'-GGAGGTTA AATCTGCGA CATGCTCT-3'; and *ARTI*, forward 5'-CAGTGTCTCT CGTGGGTCTT-3' and reverse 5'-CTCAGTACGTCCACGATGCT-3'. *HistonE3* (forward 5'-AGTCT GCCGCACTGCAAGCTCTGATG-3' and reverse 5'-TCAC AGATTGACCACTGACG-3') was used as an internal control.

**Immunohistological staining**

Immunostaining with an antibody specific to *Nrat1* was performed, obtained by immunizing rabbits with the synthetic peptide N-MEGTGMREVGRETLLHG-C (positions 1–18 of *Nrat1*) (Xia et al., 2010). After the roots of Koshihikari and Kasalath were exposed or not to 30 μM Al for 12h, the root tips (2mm) were excised. The procedures for immunostaining were the same as described previously (Yamaji and Ma, 2007).

**Yeast experiments**

The cDNA fragment containing the entire open reading frame (ORF) for *Nrat1* derived from Koshihikari or Kasalath was amplified by PCR using the primers 5'-GGTGACA AAAATGAAGGG ACTGTTGAGA GTGA-3' and 5'-CTCAAGCTGAAGCAC-3' (forward and reverse, respectively). The fragment was first cloned into the pGEM-T vector (Promega). After sequence confirmation, *Nrat1* cDNA was excised with *KpnI* and *NolI* for cloning into pYES2 (Invitrogen). Site-directed mutants of *Nrat1* (Koshihikari) were generated on the *Nrat1*-pYES2 construct by PCR using the primers 5'-CGGGTGC-3' and reverse 5'-ACATAGTGCGGGTACTC-3' for amplification; for mutation of *Nrat1* derived from Koshihikari or Kasalath was amplified on the *Nrat1*-pYES2 construct by PCR using the primers 5'-GGTACCAACACGTGCAAGCTCTGATG-3' and 5'-CTCGAG ATTCTATGGTGATGAAGGATC-3' (forward and reverse, respectively). To clone the full-length ORF sequence of *Nrat1*, total RNA was extracted from rice roots using a RNeasy Plant Mini Kit and then converted to cDNA using the protocol supplied by the manufacturers of SuperScript II. The full-length ORF was amplified by PCR using the primers used for amplification of *Nrat1*. The fragments of the amplified promoters and cDNAs were cloned into the vector pGEM-T (Promega) and the sequences were confirmed using a sequence analyser (ABI PRISM 310 Genetic Analyzer, Applied Biosciences).

**Results**

*Nrat1* is responsible for Al QTL on chromosome 2

3 QTLS controlling Al tolerance were previously detected on chromosomes 1, 2, and 6 by using 183 backcross inbred lines derived from Koshihikari (Al tolerant) and Kasalath (Al sensitive) (Ma et al., 2002). A recently identified Al-tolerance gene, *Nrat1*, is located to the QTL region on chromosome 2. To examine whether *Nrat1* is responsible for the QTL, this work obtained two chromosome segment substitution lines, which contained the *Nrat1* allele from Kasalath (SL204) or not (SL205 as a negative control) in Koshihikari background (Supplementary Fig. S1 available at JXB online). First, Al tolerance to different Al concentrations was compared among Koshihikari, Kasalath, SL204, and SL205. In the absence of Al, the four lines showed similar root elongation (Fig. 1A). However, in the presence of 30 and 50 μM Al, relative root elongation was significantly lower in Kasalath.
and SL204 than in Koshihikari and SL205 (Fig. 1B). Relative root elongation of SL204 was higher than that of Kasalath, indicating that other QTLs also contribute to Al tolerance in Koshihikari. This is consistent with this study group’s previous results (Ma et al., 2002).

Nrat1 is an Al transporter localized to the plasma membrane of the root cells and knockout of Nrat1 results in decreased Al uptake (Xia et al., 2010). If Nrat1 is responsible for the QTL on chromosome 2, SL204 should show a phenotype similar to the knockout line. Therefore, Al concentrations in the root cell sap were compared. At the root tip (0–1 cm), Al concentration in root cell sap was significantly lower in Kasalath and SL204 than in Koshihikari and SL205 (Fig. 1C) and Al content in cell walls was higher in Kasalath and SL204 than in Koshihikari and SL205 (Fig. 1D).

Furthermore, this work compared the expression level of Nrat1 in the root tips of Koshihikari, Kasalath, SL204, and SL205. In the absence of Al, the expression level of Nrat1 was almost double in Koshihikari than in Kasalath at root tips (Supplementary Fig. S2 available at JXB online). In the presence of Al, Nrat1 was induced in both Koshihikari and Kasalath, but Kasalath showed expression of Nrat1 lower than Koshihikari (Fig. 2A).

Expression analysis of ART1

A previous study has shown that the expression level of Nrat1 is regulated by ART1 (Xia et al., 2010). To investigate whether differential expression of Nrat1 is caused by variations in ART1 expression between Kasalath and Koshihikari, this work compared the expression of ART1 in two varieties. There was no difference in the expression level of ART1 in the root tips between Kasalath and Koshihikari (Fig. 2B).

Comparison of the promoter and coding region sequences of Nrat1 between Koshihikari and Kasalath

To understand the mechanisms underlying different expression of Nrat1 in two varieties, this work compared the
Genotypic difference in Nrat1 expression in rice | 4301

promoter sequence of Nrat1 up to 2.1 kb upstream from the translation initiation site. Five nucleotide substitutions and a 5-bp insertion were found in the promoter region of Nrat1 of Kasalath (Supplementary Fig. S3 available at JXB online). However, the number of cis-acting elements of ART1 was the same in the promoters between two varieties (Supplementary Fig. S3 available at JXB online).

This work also compared the sequence of the Nrat1 coding region between Koshihikari and Kasalath. There were five nucleotide differences, resulting in four amino acid changes at positions E120K, V326I, T500M, and V515A (Supplementary Fig. S4 available at JXB online). Predication with SOSUI showed that these amino acid changes did not affect the topology of Nrat1, which contains 10 transmembrane domains (Supplementary Fig. S5 available at JXB online).

Localization of Nrat1 protein

Nrat1 has been reported to be localized to all cells of root tips except epidermal cells in a Japonica variety, Nipponbare (Xia et al., 2010). To examine whether the differences in the promoter and coding region of Nrat1 affected localization in tissues, this work performed immunostaining using an antibody specifically against Nrat1. In the root tip (2 mm from the root tip), Nrat1 was localized to all root cells except the epidermal cells in both Koshihikari and Kasalath (Fig. 3). Furthermore, the signal was enhanced in the roots exposed to Al in both varieties. Compared with Kasalath, the signal in the Al-exposed roots of Koshihikari was stronger (Fig. 3).

Transport activity of Nrat1 for Al in yeast

To test whether amino acid substitutions in the Nrat1 protein affect the transport activity for Al, this work expressed different Nrat1 alleles in yeast and compared Al uptake. Expression of Nrat1 alleles from both Kasalath and Koshihikari did not affect yeast growth in the absence of Al (Fig. 4A). However, in the presence of Al, expression of Nrat1 from either Koshihikari or Kasalath increased the sensitivity of yeast to Al toxicity (Fig. 4A), indicating that Nrat1 from Kasalath also has transport activity for Al.

To quantify Al uptake, Al content in yeast expressing different Nrat1 alleles was determined. The Al uptake ability of Nrat1 from Koshihikari was 34% higher than that from Kasalath (Fig. 4B). Since this difference might result from different expression levels of Nrat1 proteins in yeast, protein levels were determined by Western blot with an antibody specific to Nrat1. The results showed that yeast expressing Nrat1 from Koshihikari and Kasalath produced similar levels of Nrat1 (Supplementary Fig. S6 available at JXB online).

Since Nrat1 proteins from Koshihikari and Kasalath differ in four amino acids, this work performed a site-directed mutation analysis to examine which amino acid is important for Al transport activity. It was found that a mutation at the position of 326 from V to I seemed to slightly reduce Al transport activity (Fig. 5), whereas other mutations (E120K, T500M, and V515A) did not affect Al transport activity.

Genotypic differences in Nrat1 expression and promoter and ORF sequences

To further investigate whether Nrat1 is responsible for genotypic difference in Al tolerance in other varieties, the expression levels and Al tolerance were compared in nine varieties differing in Al tolerance. A good correlation (r=0.82) was found between Nrat1 expression level and Al tolerance (relative root elongation), irrespective of the subspecies (Fig. 6).

This work also compared the promoter (2.1 kb) sequence in nine varieties. However, there was no consistent trend between promoter sequence and Nrat1 expression (Supplementary Fig. S3 available at JXB online). Also, no trend was found between amino acid sequence and Al tolerance in nine varieties (Supplementary Fig. S4 available at JXB online).

Discussion

Nrat1 belongs to the Nramp (natural resistance-associated macrophage protein) family but, unlike other members, it
transports trivalent Al (Xia et al., 2010). Knockout of Nrat1 results in decreased Al tolerance of rice (cv. Nipponbare, a Japonica variety) and decreased Al uptake (root cell sap concentration) but increased Al binding to the cell wall (Xia et al., 2010). Therefore, Nrat1 is involved in Al tolerance in rice by transporting Al into the root cells prior to final detoxification in the vacuoles, which is mediated by a tonoplast-localized transporter, OsALS1 (Xia et al., 2010; Huang et al., 2012).

The location of Nrat1 is within the region of a QTL for Al tolerance on chromosome 2 (Ma et al., 2002; Yamaji et al., 2009). Furthermore, another recent study with genome-wide association also showed that variations in Nrat1 are probably responsible for the genotypic variation in Al tolerance in the aus subpopulation (Famoso et al., 2011). The present study confirmed this possibility by using chromosome segment substitution lines. When the Nrat1 region of Koshihikari (Al-tolerant) was substituted by that of Kasalath

Fig. 3. Localization of Nrat1 in roots. Immunostaining with an anti-Nrat1 antibody was performed in 2 mm from the root apex of Kasalath (A, B) and Koshihikari (C, D) in roots exposed for 12 h to 0.5 mM CaCl2 without Al (A, C) or 0.5 mM CaCl2 containing 30 μM Al (B, D). Bars, 100 μm.

Fig. 4. Transport activity of Nrat1 for aluminium in yeast. (A) Effect of Nrat1 expression on Al tolerance; S. cerevisiae BY4741 carrying empty vector pYES2, Nrat1 (Kasalath), or Nrat1 (Koshihikari) were spotted on LPM without uracil medium (from left to right: 10 μl cell suspension with OD 0.2, 0.02, 0.002, and 0.0002) with or without AlCl3 at different dilutions and incubated at 30 °C for 3 d. (B) Transport activity of Nrat1 (Kasalath) and Nrat1 (Koshihikari) for Al3+: yeast cells expressing different Nrat1 alleles were exposed for 4 h to 50 μM AlCl3 (pH 4.2); Al in the yeast was determined by ICP-MS after digestion with 2 M HCl; data are mean±SD of three biological replicates. Different letters above the bars indicate significant differences (P<0.05, Tukey’s test).
Al tolerance exist in Koshihikari. This is in agreement with Kasalath (Magalhaes et al., 2010), therefore, different expression of Nrat1 may be caused by variation of ART1. However, there was no difference in ART1 expression between Koshihikari and Kasalath (Fig. 2B). The numbers of cis-acting elements recognized by ART1 in the Nrat1 promoter region were also similar between two varieties (Supplementary Fig. S3 available at JXB online). These results indicate that ART1 is unlikely to be a factor responsible for the genotypic difference in Nrat1 expression.

Al-sensitive, the expression of Nrat1 was reduced (Fig. 2A) and Al tolerance was also decreased (Fig. 1B). Furthermore, the Al concentration in the root cell sap was decreased (Fig. 1C) but that in the cell wall was increased (Fig. 1D). These phenotypes are similar to a Nrat1 mutant, although these phenotypic changes is different (Xia et al., 2010). Furthermore, there was a good correlation between Nrat1 expression and Al tolerance in other varieties differing in Al tolerance (Fig. 6). Taken together, these results support that Nrat1 is responsible for the QTL detected on chromosome 2. In addition, SL204 was more tolerant to Al compared with Kasalath (Fig. 1B), suggesting that other QTLs controlling Al tolerance exist in Koshihikari. This is in agreement with previous results that have shown that there are two QTLs for Al tolerance on chromosomes 1 and 2 detected in Koshihikari (Ma et al., 2002).

Variations in Nrat1 may result from protein mutation and different gene expression. The localization of Nrat1 protein to the root was similar in Koshihikari and Kasalath (Fig. 3), but there are four amino acid differences in Nrat1 between Koshihikari and Kasalath, at positions E120K, V326I, T500M, and V515A (Supplementary Fig. S4 available at JXB online). These changes do not seem to affect the membrane topology of Nrat1 (Supplementary Fig. S5 available at JXB online). When the Nrat1 allele from Kasalath was expressed in yeast, it also showed transport activity for Al (Fig. 4B), indicating that the loss of function does not result from variation in Nrat1. Quantitative analysis showed that Al uptake mediated by the Nrat1 allele from Koshihikari was slightly higher than that from Kasalath (Fig. 4B). Furthermore, an amino acid change at the position of 326 from V to I seems important to Al transport activity (Fig. 5). Genome-wide association analysis (Famoso et al., 2011) suggested that an amino acid change from valine to alanine (but should be threonine to methionine, amino acid 500 in the paper) is responsible for natural variation in Nrat1 because this amino acid site was predicted to be involved in PKA-type AGC kinase phosphorylation. However, site-directed mutation analysis showed that this mutation did not affect the transport activity for Al in yeast (Fig. 5). Overall, alterations in protein sequence are unlikely to be a major factor responsible for genotypic difference in Al tolerance because these alterations were not found in other Al-sensitive varieties (Supplementary Fig. S4 available at JXB online). However, the possibility could not be excluded that all four mutations are required for a difference in Al transport activity, which remain to be examined in future.

By contrast, the expression level of Nrat1 is responsible for the genotypic differences in Al tolerance. This is supported by a good correlation between Nrat1 expression level and Al tolerance in different rice varieties (Fig. 6). The expression of Nrat1 is regulated by ART1 (Yamaji et al., 2009; Xia et al., 2010), therefore, different expression of Nrat1 may be caused by variation of ART1. However, there was no difference in ART1 expression between Koshihikari and Kasalath (Fig. 2B). The numbers of cis-acting elements recognized by ART1 in the Nrat1 promoter region were also similar between two varieties (Supplementary Fig. S3 available at JXB online). These results indicate that ART1 is unlikely to be a factor responsible for the genotypic difference in Nrat1 expression.

Alterations in the promoter region have been revealed to be the mechanisms for regulating the expression of Al-tolerance genes in several studies (Delhaize et al., 2012; Ma et al., 2014). For example, the high expression level of ALMT1 is associated with tandem repeated elements in the promoter region in most Al-tolerant lines of wheat (Sasaki et al., 2006; Ryan et al., 2010). In sorghum, tourist-like miniature inverted-repeat transposable elements in the promoter region of ShMATE were suggested to be involved in regulating the expression of this gene (Magalhaes et al., 2007). In barley, a 1-kb insertion (CACTA-like transposon) in the 5′-untranscribed region of HvAACT1 enhances its expression in Al-tolerant accessions
(Fujii et al., 2012). Recently, the higher expression level of TaMATE1B in several Brazilian wheat lines was found to be associated with the presence of a Sukkula-like transposable element (11.1-kb) in its promoter (Tovkach et al., 2013). More recently, in Yorkshire fog (Holcus lanatus), higher HIALMT1 expression was achieved by an increase in the number of cis-acting elements for transcription factor HI/ART1 in the promoter region (Chen et al., 2013). Maron et al. (2013) found that Al-tolerant cultivars of maize have three copies of ZmMATE1 in the genome, which are identical and part of a tandem triplication. Therefore, this work compared the promoter sequences of Nrat1 in Al-sensitive and Al-tolerant rice varieties. However, no clear differences were found in the promoter region up to 2.1 kb (Supplementary Fig. S3 available at JXB online). There are two possibilities for this result: either the regulatory factor is present further upstream of the promoter region or the expression of Nrat1 may be regulated differently. Further work is required to understand the mechanism underlying the Nrat1 expression.

In conclusion, these results show that differential expression of Nrat1 is mainly responsible for the Al-tolerance QTL located on chromosome 2 in enhancement. Enhancement of Nrat1 expression in Al-sensitive varieties may increase their Al tolerance.

Supplementary material

Supplementary data are available at JXB online.

Supplementary Fig. S1. Genotypes of SL204 and SL205.

Supplementary Fig. S2. Expression level of Nrat1 in two rice cultivars with and without Al exposure.

Supplementary Fig. S3. Alignment of 2.1-kb promoter region of Nrat1 from Kasalath, Koshihikari, and other varieties.

Supplementary Fig. S4. Alignment of the amino acid sequence of Nrat1 from Kasalath, Koshihikari, and other varieties.

Supplementary Fig. S5. Transmembrane domains of Nrat1 from Kasalath and Koshihikari predicated by SOSUI.

Supplementary Fig. S6. Western blot analysis for Nrat1 expressed in yeast.

Acknowledgements

The research was supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant-in-Aid for Scientific Research on Innovative Areas, no. 22119002 to J.F.M.) and the Ohara Foundation for Agriculture Research. This work was also partially supported by the National Key Basic Research Program of China (no. 2014CB441000 to R.F.S.) and the Natural Science Foundation of China (no. 41025005). The authors thank the Rice Genome Resource Center in Tsukuba for providing chromosome segment substitution lines.

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