Genome-wide association mapping for grain manganese in rice (*Oryza sativa* L.) using a multi-experiment approach

Panthita Ruang-areerate[1,2*], Anthony J. Travis[1], Shannon R. M. Pinson[3], Lee Tarpley[4], Georgia C. Eizenga[3], Mary Lou Guerinot[5], David E. Salt[6], Alex Douglas[1], Adam H. Price[1] and Gareth J. Norton[1]

[1] Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, AB24 3UU, UK

[2] National Omics Center, National Science and Technology Development Agency (NSTDA), Pathum Thani, 12120, Thailand

[3] USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR 72160, USA

[4] Texas A&M System AgriLife Research Center, Beaumont, TX 77713, USA

[5] Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA

[6] Future Food Beacon of Excellence and the School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK

* Corresponding author. *E-mail address*: panthita.rua@nstda.or.th (P. Ruang-areerate)

ABSTRACT

Manganese (Mn) is an essential trace element for plants and commonly contributes to human health; however, the understanding of the genes controlling natural variation in Mn in crop plants is limited. Here, the integration of two of genome-wide association study approaches were used to increase the identification of valuable quantitative trait loci (QTL) and candidate genes responsible for the concentration of grain Mn across 389 diverse rice cultivars grown in Arkansas and Texas, USA in multiple years. Single-trait analysis was initially performed using three different SNP datasets. As a result, significant loci could be detected using the high-density SNP dataset. Based on the 5.2M SNP dataset, major QTLs were located on chromosomes 3 and 7 for Mn containing six candidate genes. Additionally, the phenotypic data of grain Mn concentration was combined from three flooded-field experiments from the two sites and three years using multi-experiment analysis based on the 5.2M SNP dataset. Two previous QTLs on chromosome 3 were identified across experiments, whereas new Mn QTLs were identified that were not found in individual experiments, on chromosomes 3, 4, 9 and 11. *OsMTP8.1* was identified in both approaches and is a good candidate gene that could be controlling grain Mn concentration. This work demonstrates the utilisation of multi-experiment analysis to identify constitutive QTLs and candidate genes associated with the grain Mn concentration. Hence, the approach should be advantageous to facilitate genomic breeding programs in rice and other crops considering QTLs and genes associated with complex traits in natural populations.

Keywords: manganese, rice grain, QTL, GWAS and multi-experiment analysis

Introduction

Genome-wide association (GWA) mapping is a powerful approach to identify genetic loci associated with complex traits in natural populations. The approach has been successfully applied in plants such as *Arabidopsis thaliana* (Atwell et al. 2010; Baxter et al. 2010), maize (Kump et al. 2011) and rice (Huang et al. 2010, 2012; Zhao et al. 2011; Norton et al. 2014; McCouch et al. 2016; Bettembourg et al. 2017) for identifying important agronomic, disease resistance and ionomic (the elemental composition of biological samples) loci. GWA mapping for ionomic traits in plants has been commonly used to perform QTL analysis related on a single trait in individual experiments (Atwell et al. 2010; Baxter et al. 2010; Li et al. 2010; Norton et al. 2014, 2018; Dimkpa et al. 2016; Yang et al. 2018). However, these studies have not always identified QTLs for a trait from several experiments. There are several reasons why a number of ionomic QTLs have not been consistently detected, including a different range of phenotypic values in experiments due to large environmental effects such as different geographic locations and climate data (temperature and humidity) (Pinson et al. 2015; Huang and Salt 2016), and complex genetic architecture such as distributed allelic variants, each of which have small effects (Korte and Farlow 2013). These reasons may reduce the power of statistical association tests in GWA mapping using single-trait analysis in only one experiment.

Recently, multi-trait approaches have been developed to improve QTL detection by increasing the statistical power with correlated traits and multiple experiments (Korte et al. 2012; Lippert et al. 2014; Zhou and Stephens 2014; Loh et al. 2015). Zhou and Stephens (2014) developed a genome-wide efficient mixed model association (GEMMA) software for testing multiple traits for each single genetic marker with a multivariate linear mixed model (mvLMM), which controls population stratification and accounts for relatedness between individuals. It was developed from the efficient mixed model association (EMMA) algorithm for single-trait analysis (Kang et al. 2008) with which identified QTLs could be compared with multi-trait analysis based on the EMMA algorithm.

Central to effective GWA mapping is the population that is used. The Rice Diversity Panel 1 (RDP1) is a rice panel representing the broad range of rice varieties from >70 countries (Eizenga et al. 2014). This panel was initially genotyped using 44,100 SNPs (Zhao et al. 2011), subsequently it was genotyped with 700,000 SNPs (McCouch et al. 2016) and in the latest iteration 5,231,435 SNPs have been imputed on this panel by comparing the 700,000 SNPs with whole genome sequence data of the 3,000 sequenced rice cultivars (Wang et al. 2018).

Manganese (Mn) is an essential trace element for plants and humans. It is an important co-factor or activator of many enzymes, and is involved in photosynthesis in plants (Marschner 1995; Soetan et al. 2010). Mn deficiency in plants can cause a reduction in growth and yield (Marschner 1995; Hebbern et al. 2005), whereas if the Mn concentration is elevated it can become toxic to plants (Ducic and Polle 2005; Millaleo et al. 2010). Mn homeostasis in the embryo is required for efficient seed germination (Eroglu et al. 2017). Mn deficiency in humans is rare, however it can lead to a range of health impacts including severe birth defects, impaired reproductive functions, skeletal defects, and asthma (Bashir et al. 2013), while overexposure can lead to neurological disorders (Crossgrove and Zheng 2004; O'Neal and Zheng 2015). The recommended daily intake of Mn for an adult is 2.3 mg day^{-1} while the recommended tolerable levels are 11 mg day^{-1} (Institute of Medicine 2001). Rice grain concentrations of Mn are variable, but a recent dietary study of rice consumers in West Bengal, India demonstrated that rice alone can contribute between 0.82 – 4.21 mg day^{-1} for an adult, 35.7% – 183% of the recommend daily amount of Mn (Halder et al. 2020).

The mechanisms of Mn uptake, transport, accumulation and detoxification have been studied in plants (Ducic and Polle 2005; Millaleo et al. 2010; Socha and Guerinot 2014). For rice, a number of Mn transporter genes have been identified such as natural resistance-associated macrophage protein 3 (*OsNRAMP3*), *OsNRAMP5* and *OsNRAMP6* (Ishimaru et al. 2012; Sasaki et al. 2012; Yang et al. 2013, 2014; Peris-Peris et al. 2017), yellow stripe-like protein 2 (*OsYSL2*) and *OsYSL6* (Koike et al. 2004; Ishimaru et al. 2010; Sasaki et al. 2011) and cation diffusion facilitator/metal tolerance protein 8.1 (*OsMTP8.1*), *OsMTP8.2*, *OsMTP9* and *OsMTP11* (Chen et al. 2013, 2016; Ueno et al. 2015; Takemoto et al. 2017; Zhang and Liu 2017; Ma et al. 2018). In addition to these genes, a number of studies have identified QTLs of grain Mn concentration in rice grains based on bi-parental mapping (Stangoulis et al. 2007; Lu et al. 2008; Ishikawa et al. 2010; Norton et al. 2010, 2012a; Du et al. 2013; Zhang et al. 2014). For example, QTLs have been detected on chromosomes 1 (Stangoulis et al. 2007; Lu et al. 2008), 1, 2, 7 and 12 (Ishikawa et al. 2010), 10 and 11 (Norton et al. 2010), 3, 5, 7, 8 and 9 (Norton et al. 2012a), 3, 6, 8 and 9 (Du et al. 2013), and 2, 3, 4, 6, 7, 8, 11 and 12 (Zhang et al. 2014).

Knowledge of natural genetic variation that regulates Mn concentration in grains among rice landraces and cultivars is limited. To address this, we conducted GWA mapping of grain Mn concentration in the RDP1 from four field experiments in Arkansas and Texas, USA. The aims of this study were 1) to compare the impact of increasing marker density on detecting loci in GWA mapping for grain Mn concentration, and 2) to identify QTLs and candidate genes associated with grain Mn concentration across experiments in multiple locations and years using both single-trait and multi-experiment GWA analyses.

Materials and methods

Sample data

A total of 389 rice accessions from the RDP1 (Zhao et al. 2011; McCouch et al. 2016) consisting of 57 *aus* (AUS), 78 *indica* (IND), 100 *temperate japonica* (TEJ), 96 *tropical japonica* (TRJ) and 14 *aromatic* (ARO) as well as 44 admixtures were used in this study (Supplementary Table S1). There were two major varietal groups, *Indica* (AUS and IND) and *Japonica* (TEJ, TRJ and ARO) (McCouch et al. 2016).

The experimental design, planting methods and rice growth conditions were described in Norton et al. (2012b). Briefly, the RDP1 was grown in two locations under either flooded or unflooded cultivation. The locations were Stuttgart, Arkansas (USDA-ARS Dale Bumpers National Rice Research Center) and Beaumont, Texas (Texas A&M AgriLife Research Center), USA. In Arkansas, the rice cultivars were grown in the same location using nearby fields in 2006 and 2007, the field layout in both years was a randomized complete block design (RCBD) with two replications, with identical field management practices wherein fields were flooded when plants were at the five-leaf stage then drained before harvest (datasets referred to as ArFl06 and ArFl07, respectively). Seeds of each cultivar were sown with a seed drill to approximately 2 cm deep in a single row 5 m long with spacing of 25 cm between the plants and 50 cm between the rows. Fields were flush-irrigated twice before a permanent flood was applied to the fields approximately 2–3 weeks after seedling emergence. In Texas, three replications of the RDP1 accessions were grown in 2009 using two different water treatment conditions; flooded and unflooded (datasets referred to as TxFl09 and TxUnfl09, respectively), with all other field management practices the same. The experiment was set up in a RCBD. Five seeds per cultivar were drill-seeded 2 cm deep into 13-cm length lines, hereafter called hillplots. Five hillplots were planted per row with 61 cm between hillplots within each field row, and 25 cm between rows.

Accessions were represented by one hillplot per replication. The 10 cm depth flood were applied when average plant height was approximately 18 cm and maintained until harvest, whereas the unflooded treatment received regular flush irrigations (once or twice a week) to keep the root zone damp but aerated.

For the Arkansas field experiments rice grains for three plants per row for each of the two replications were collected. Seed collection was done by hand and threshed with an Almaco small bundle thresher to obtain the seeds for the grain Mn determination. For the Texas field experiment twenty fully mature seeds per hillplot were dehulled, from which three seeds were randomly selected for analysis of grain Mn.

The concentrations of Mn were determined in the harvested grains using inductively coupled plasma mass spectrometry (ICP-MS) described in Norton et al. (2012b, 2014) and Pinson et al. (2015). In brief, three whole grains of dehusked rice (c. 0.05 g) were digested with 1.0 ml of concentrated nitric acid and heated. The temperature was ramped up from ambient to 110^{o}C over a period of 12 h. An internal standard of indium (final concentration of 20 µg l^{-1}) was added to each sample. Samples were diluted to 10.0 ml and analysed on a PerkinElmer (Waltham, MA, USA) Elan DRCe ICP-MS for Mn. To control for drift the samples were combined and used as a matrix-matched standard and measured every nine samples.

Phenotypic analysis

Phenotypic variances for Mn concentrations were calculated and parsed using two-way ANOVA conducted in R (version 3.3.0) (R Core Team 2016). Across the two field locations (Arkansas and Texas), four field experiments were conducted, designated as ArFl06, ArFl07, TxFl09 and TxUnfl09. Across the Arkansas experiments (ArFl06 and ArFl07), the phenotypic variance was parsed into proportions estimated by genotypes, years, and interaction between genotype and year. For the Texas experiments (TxFl09 and TxUnfl09), the phenotypic variance was parsed into genotypes, water treatments and genotype by water treatment interaction effects.

The average Mn concentration (Supplementary Table S1) of each accession per experiment and treatment was used for the GWA mapping. Prior to GWA mapping, the trait data were visualised to assess normality.

Genotypic data and analysis

The rice accessions in the RDP1 have three publicly available SNP datasets consisting of 36,901 (44K) SNPs (Zhao et al. 2011), 700,000 (700K) SNPs (McCouch et al. 2016) and 5,231,435 (5.2M) SNPs (Wang et al. 2018). The 44K and 700K SNP datasets were generated by genotyping using 44K SNP array and High-Density Rice Array (HDRA), respectively

(Zhao et al. 2011; McCouch et al. 2016), whereas the 5.2M SNP dataset, which contains no missing data, was generated by imputing from the set of the intersection of 700K and 18M SNPs (missing data <5% and minor allele frequency (MAF) >1%) with 4.8M SNPs of the 3,000 Rice Genome Project (Wang et al. 2018).

The SNPs in each dataset were initially filtered using PLINK version 1.9 (Chang et al. 2015), where by SNPs were removed when the percentage of missing genotype data for a single SNP exceeded 20% (the 5.2M SNP dataset had no missing data, due to being imputed) and MAF was less than 5%.

GWA mapping with single-trait analysis

GWA mapping was performed using the three SNP datasets based on LMMs from EMMAX (version beta-7Mar2010) (Kang et al. 2010) using the PIQUE (Parallel Identification of QTLs using EMMAX) pipeline (https://github.com/tony-travis/PIQUE). Phenotype-genotype association was analysed for all accessions (ALL) and four subpopulations (AUS, IND, TEJ and TRJ) in the four field experiments. Due to low accession numbers (<30) from the *aromatic* (ARO) subpopulation and the mixed genetic background of admixtures, these accessions were not analysed as separate subpopulations. Population structure was estimated by performing a principal component analysis (PCA) on the informative SNP data and the eigenvectors for the first four principal components were included in the model as fixed effects for the analysis of the whole (ALL) population (Price et al. 2010) (note: population structure was not included in the analysis of the subpopulations). Relatedness (K matrix) between accessions was estimated by calculating pairwise identity-by-state (IBS) using the SNP data and was included in the models as random effects. For the ALL population GWA analyses relatedness was estimated using the accessions which had phenotype data in each experiment. For the subpopulation analyses relatedness was estimated using accessions from each subpopulation for which phenotype data was collected. The significance threshold for association between SNP and traits was set at *P*-value <0.0001, a value previously used for this population (Famoso et al. 2011; Norton et al. 2014). To further filter these SNPs for false discovery rates (FDR), the *P*-values calculated by the GWA mapping analysis were adjusted using the Benjamini-Hochberg method (Benjamini and Hochberg 1995). To be reported as a SNP significantly associated with the trait the SNP had to both meet the *P*-value <0.0001 and meet the criteria of a 5% FDR. Manhattan plots were used to visualise SNP positions on chromosomes with $-\log_{10}(P)$ and Q-Q plots were used to visualise observed versus expected values probabilities using the qqman package in R version 3.3.0 (Turner 2014).

GWA mapping with multi-experiment analysis

Multi-experiment analysis of GWA mapping for grain Mn concentration for the three flooded-field experiments (ArFl06, ArFl07 and TxFl09) was performed. For this analysis each environment was viewed as one trait. A total of 303 rice accessions (all accessions common among the three experiments) were used for the analysis with the 3,430,260 filtered SNPs (MAF >0.05%) using the mvLMM in the GEMMA version 0.97 (Zhou and Stephens 2014). The mvLMM accounts for both population stratification and relatedness among samples to control confounding factors. The eigenvectors of the first four principal components were calculated using the smartpca program in EIGENSOFT (Patterson et al. 2006) and included in the model as fixed effects. One eigen-decomposition of the centered relatedness matrix (the n by n relatedness matrix; n = the number of samples) for random effects was computed from all filtered SNPs using the relatedness matrix function in GEMMA. The null hypothesis is SNP effects of a single SNP in all experiments are zero, whereas the alternative hypothesis is nonzero effects of at least one SNP tested by a Wald test. *P*-values of all association tests were presented with Manhattan plots and observed *P*-

values against expected *P*-values were presented by Q-Q plots using the qqman package in R (Turner 2014). The guideline of reliability for significant SNPs was 0.0001 (Famoso et al. 2011; Norton et al. 2014). SNPs were also tested to a 5% FDR based on the Benjamini-Hochberg procedure (Benjamini and Hochberg 1995), as previously described.

Clustering significant SNPs and comparing QTLs on rice chromosomes

The grouping function CLUMP was used in PLINK to define candidate regions in the ALL analysis based on the 5.2M SNP. Index SNPs were identified with *P-value* <0.0001 (Norton et al. 2014) and neighbouring SNPs were clumped with *P-value* <0.01 (default value) and squared allele frequency correlation (r^2) >0.5 (applying the criteria from Butardo et al. (2017) based on the 700K SNP dataset) with the index SNPs of each peak within 500 kb, which was the LD-decay average of all accessions in the RDP1 (Zhao et al. 2011). The candidate regions/QTLs were then mapped and compared with previously reported QTLs based on physical genome positions on the 12 rice chromosomes.

Local linkage disequilibrium decay analysis

To determine LD blocks in subpopulations that supported the significant peaks in the ALL analysis, a subset of the 5.2M SNP data surrounding (1 Mbp) a significant peak was extracted using PLINK. Two methods were used; 1) local LD decay was estimated at $r^2 = 0.2$, where r^2 values were calculated using PLINK and estimated by binning the average r^2 values of 10 kb windows (Biscarini et al. 2016; Norton et al. 2018); 2) r^2 values in each SNP pair in each region 500 kb upstream and downstream were calculated and visualised as a local Manhattan plot against a LD heatmap using the LD.heatmap package in R version 3.3.0 (Shin et al. 2006), and then LD blocks were estimated using $r^2 \geq 0.6$ (high LD) (Ripke et al. 2014; Yano et al. 2016).

Candidate gene identification

Within each candidate region, positional genes were identified based on genes identified in the Rice Genome Annotation Project (version 7; http://rice.plantbiology.msu.edu). Retrotransposons and transposon genes were excluded. Genes located within candidate regions were examined and used to identify potential positional functional candidate genes; e.g. genes involved in the uptake, transport and accumulation of elements, associated with Mn. In addition, protein sequences (http://rice.plantbiology.msu.edu) of the list of candidate genes that were not matched with genes previously related to Mn were compared with protein database using BLASTp (https://blast.ncbi.nlm.nih.gov) to investigate gene-sequence homology with other species, in which genes were reported and characterised with functions involving Mn. In addition to gene validation, the gene expression profiles across a range of rice organs and tissues of all identified candidate genes obtained from RiceXPro (http://ricexpro.dna.affrc.go.jp, Sakai et al. 2013) were used to confirm the validity of candidate genes.

Differential gene expression of candidate genes was determined based on the gene expression analysis conducted by Campbell et al. (2020). This data set is transcriptomic data from shoots of young plants from 91 accessions from the RDP1. Initially the data was screened to identify which of the proposed candidate genes were expressed. Low and high grain Mn accessions were identified based on being in the highest 20% and lowest 20% for grain Mn concentration, for the three flooded experiments. Then only low or high grain Mn concentration accessions were selected for further analysis if they were low or high in at least two of the experiments. A total of 14 accessions were identified as high and 18 identified as low grain Mn accessions for which with transcript data was available (Supplementary Table S2). The expression of candidate genes were examined for evidence of differential expression based on this grouping. An ANOVA was used to determine if the gene expression was different between the two groups.

Estimation of phenotypic variance explained by significant SNPs

To determine the effect size of the QTLs, two approaches were taken. Either the smallest *P*-value/index SNPs or the most significant SNP located in candidate genes based on the 5.2M SNP dataset were analysed. The proportion of phenotypic variance explained by each SNP was estimated using linear models, correcting for population structure and contrasting with the population structure effects for all accessions (Zhao et al. 2011). ANOVA was used to contrast the linear models. For subpopulations, the phenotypic variance distribution of a significant SNP was estimated using a simple linear model without correcting for population structure.

Effect sizes by index SNPs in multi-experiment analysis

Effect sizes in each index SNP of QTLs newly identified in multi-experiment analysis were observed in the individual experiments estimated by the mvLMM model.

Results

Variation of grain Mn concentration in the RDP1

In Arkansas, grain Mn concentration for the accessions in 2006 and 2007 ranged from 21.4 to 62.7 mg kg^{-1} and from 20.6 to 68.5 mg kg^{-1}, with means of 34.6 and 40.8 mg kg^{-1}, respectively (Fig. 1a and Table 1). There were significant differences ($P < 2\times10^{-16}$, df = 321) in grain Mn concentration among genotypes, years and a significant interaction between years and genotypes that explained 39%, 15% and 16% of the phenotypic variance, respectively. In Texas, grain Mn concentration for the accessions in 2009 under flooded and unflooded conditions range from 10.6 to 33.5 mg kg^{-1} and from 16.4 to 63.8 mg kg^{-1}, with means of 20.9 and 34.8 mg kg^{-1}, respectively (Fig. 1b and Table 1). The grain Mn concentration under the unflooded condition was significantly higher (1.7 times; $P < 2\times10^{-16}$, df = 367) than the concentration under the flooded condition. The rice grain Mn concentrations were affected by genotypes, water treatments and their interaction, which explained 14%, 61% and 11% of the phenotypic variance, respectively.

To compare the grain Mn accumulation among subpopulations, only those subpopulations with at least 30 accessions (AUS, IND, TEJ and TRJ) were studied. There was a significant difference in grain Mn concentration among the subpopulations (Fig. 2). In the three flooded-field experiments, the *Japonica* (TEJ and TRJ) subgroups had higher average grain Mn concentration than the *Indica* (AUS and IND) subgroups. In contrast, the TRJ subpopulation had the lowest average grain Mn concentration in TxUnfl09.

The accessions screen at these field sites are known to vary in the length of time to heading (Norton et al. 2012b), therefore a correlation analysis was conducted to determine if there was a relationship between heading date and grain manganese concentration. For Arkansas 2007 and flooded experiment in Texas, there was no correlation between flowering time and grain Mn concentration. However, at the Arkansas 2006 experiment there was a significant week positive correlation ($r = 0.235$, $P < 0.001$) between grain manganese and flowering time, while at the Texas unflooded field site there was a significant weak negative correlation ($r = -0.278$, $P < 0.001$) between grain manganese concentration and flowering time.

Density of SNPs among all accessions and subpopulations

To obtain high quality SNPs in each SNP dataset, SNPs were filtered with genotype missing >20% and MAF <0.05 (Supplementary Table S3). After SNP filtering, for example, average SNP density of 11.40, 0.99 and 0.11 kb per SNP were observed for the 44K, 700K and 5.2M SNP datasets, respectively for the ArFl06 dataset.

For subpopulations, it is noteworthy that the final number of filtered SNPs was lower in the TEJ and TRJ subpopulations compared to the AUS and IND subpopulations (Supplementary Table S3). For example, the SNP density in the TEJ subpopulation was 1 SNP per 0.41 kb, whereas the SNP density in the IND subpopulation was 1 SNP per 0.17 kb, when using the 5.2M SNP dataset.

Single-trait GWA mapping for grain Mn concentration

Using the three SNP datasets, GWA mapping for grain Mn concentration was performed for all accessions (Fig. 3a and Supplementary Fig. S1–S3) and for the four subpopulations using the 5.2M SNP dataset only (Supplementary Fig. S4–S7) in the four-field experiments.

Increasing the SNP density increased the number of significant SNPs associated with the trait in analyses of all accessions and in subpopulation analysis (Fig. 3a, Supplementary Fig. S1–S7 and Supplementary Table S4). For example, no significant SNPs for grain Mn in the ALL analysis in ArFl06 were identified using the 44K dataset, while 6 and 16 SNPs were significant using the 700K and 5.2M SNP datasets, respectively (Supplementary Table S4). In addition, there were no significant SNPs associated with Mn accumulation in several subpopulations based on the 44K SNP dataset, whereas a number of significant SNPs were identified based on the 700K and 5.2M SNP datasets. For example, in the TEJ subpopulation in ArFl07, no significant SNPs were detected using the 44K SNP dataset, while 6 and 11 significant SNPs for grain Mn were detected using the 700K and 5.2M SNP datasets, respectively (Supplementary Table S4).

Identification of grain Mn QTLs and candidate genes based on single-trait analysis

Based on the high-density SNP dataset (5.2M SNPs), a number of candidate regions/QTLs in the four experiments (Supplementary Table S5) were mapped on rice chromosomes and compared with previously reported QTLs (Fig. 3b). QTLs were further focused on when SNPs within QTLs passed the 5% FDR (Table 2). Consequently, there were three QTLs on chromosome 3 and two QTLs on chromosome 7 that were significantly associated with grain Mn concentration under flooded and unflooded conditions that met the criteria (Table 2). Based on overlap regions from the CLUMP analysis in experiments, these QTLs on chromosome 3 were at 5.33–6.14, 6.39–7.23 and 7.02–7.87 Mbp. For two of these QTL regions, there are a number of candidate genes including LOC_Os03g11010 (*OsNRAMP2*), LOC_Os03g11734 (*OsFRDL1*) and LOC_Os03g12530 (*OsMTP8.1*). On chromosome 7, the two overlapping QTL regions were at 7.21–8.06 and 7.78–8.57 Mbp. For the first of the two QTL regions, there was a good candidate gene; LOC_Os07g12900 (*OsHMA3*) but this gene is outside the candidate region for the second. The expression profiles of all candidate genes under normal growth conditions were obtained from the RiceXPro database (Supplementary Fig. S8–S11).

All four candidate genes mentioned above were identified as being expressed in shoots (Campbell et al. 2020). Of these four genes two of the candidate genes (*OsMTP8.1* and *OsHMA3*) were found to be differentially expressed between the low grain Mn and high grain Mn accessions (Supplementary Fig. S12a, b). The expression of LOC_Os03g12530 (*OsMTP8.1*) was higher in the accessions with low grain Mn compared to the accessions with high grain Mn, while the expression of LOC_Os07g12900 (*OsHMA3*) was higher in the accessions identified as having high grain Mn compared to the low grain Mn accessions.

Identification of grain Mn QTLs in subpopulations and candidate genes based on single-trait analysis

Due to the complex population structure in the RDP1, the estimation of linkage disequilibrium (LD) decay for single QTL across the whole panel is difficult. Therefore, to estimate the size of QTL regions based on LD, QTL analysis was conducted in the individual subpopulations (AUS, IND, TEJ and TRJ). In QTLs that were detected for the whole population and one of the subpopulations, the subpopulation analysis was used to estimate local LD.

For grain Mn QTLs in subpopulations, one significant QTL on chromosome 7 was identified in only the TEJ subpopulation based on the 5.2M SNP dataset (Fig. 4a, Supplementary Fig. S4–S7 and Supplementary Table S6) that were concordant with significant SNPs on chromosome 7 in the all analysis at the 5% FDR. To determine the accurate genomic position of the QTLs, local LD was analysed with two approaches, LD decay and LD heatmap. The QTL was identified at ~8.26 Mbp in the TEJ subpopulation (Fig. 4a). The average local LD decay between 7 and 9 Mbp on chromosome 7 was high at >1 Mbp (r^2 >0.2) (Fig. 4b). The result was concordant with LD heatmap that showed a large LD block at approximately 1.23 Mbp from 7.64 to 8.87 Mbp (r^2 ≈0.6) (Fig. 4c). One candidate gene, *OsNRAMP5* (~8.87 Mbp), was found to be located within the QTL. *OsHMA3* at 7.40 Mbp which was identified as a candidate gene for the QTL detected here in the ALL analysis is just before this block, while *OsNRAMP1* which is at 8.97 Mbp is just after it (Fig. 4c). In this QTL, the significant SNP mlid0048878287 (8.78 Mbp, P = 8.11E^{-07}), which located close to *OsNRAMP5* and *OsNRAMP1*, explained approximately 8% and 29% of phenotypic variance in ALL and TEJ, respectively. Rice accessions with the TT genotype at this SNP had high Mn accumulation in grains compared to the rice accessions with the CT and CC genotypes (Fig. 4d). The expression profile of *OsNRAMP5* and *OsNRAMP1* under normal growth conditions was obtained from the RiceXPro database (Supplementary Fig. S13–S14). Both *OsNRAMP5* and *OsNRAMP1* was identified as being expressed in shoots of rice plants (Campbell et al. 2020). Of these two genes *OsNRAMP1* was found to be differentially expressed between the low grain Mn and high grain Mn accessions (Supplementary Fig. S12c). The expression of LOC_Os07g15460 (*OsNRAMP1*) was higher in the accessions identified as having grain Mn compared to the low grain Mn accessions.

Multi-experiment GWA mapping for grain Mn concentration and candidate genes

To increase the power of GWA mapping, a single GWA mapping was conducted for grain Mn concentration of 303 accessions for the three flooded-field experiments (ArFl06, ArFl07 and TxFl09) based on the 5.2M SNP dataset (MAF >0.05; 3,430,260 filtered SNPs) using the mvLMM in the GEMMA software. A total of 64 SNPs were significantly associated with grain Mn concentration. Eight QTLs across the 12 rice chromosomes were identified (Fig. 5a and Supplementary Table S7). Two of these QTLs on chromosome 3, 5.97–6.95 and 6.63–7.51 Mbp including *OsFRDL1 and OsMTP8.1* (Fig. 3b, 5b and Supplementary Table S7) were consistent with the QTLs identified based on single-trait analysis. However, a total of 6 QTLs for grain Mn not detected by single-trait analysis were identified using multi-experiment analysis (Fig. 5 and Supplementary Table S7). The six QTLs of interest were at 1.16–1.38 Mbp on chromosome 3, 2.40–3.33 and 3.41–4.27 Mbp on chromosome 4, 0.39–1.00 Mbp on chromosome 9, and 11.39–12.30 and 25.61–25.62 Mbp on chromosome 11. All of these QTLs were novel for grain Mn concentration.

Comparison of the effect sizes of index SNPs for the putative QTLs in each experiment estimated by the mvLMM showed that they were various (Table 3). For example, the QTL on chromosome 3 had similar small positive SNP effects in all experiments, whereas the two QTLs on chromosome 4 had negative SNP effects in ArFl07 compared to other experiments.

Discussion

This study has identified QTLs for grain Mn in rice. Some of those co-localise with previously identified QTLs and known genes involved in Mn accumulation in rice, while some are novel putative QTLs. One of the key objectives of QTL mapping is the identification of stable QTLs (e.g. those are detected in multiple environments). Using a multi-experiment GWA mapping approach, we have been able to identify these stable QTLs.

The environmental factors (different years, locations, and water management treatments) and genetic composition of the accessions effected the concentration of Mn in rice grains. In Arkansas, the average grain Mn concentrations between 2006 and 2007 were significantly different and year explained ~15% of the phenotypic variance. In Texas, the Mn concentration in grains under non-flooded condition significantly increased when compared to the rice cultivation under flooded condition with flooding explaining ~61% of the variation. This is in agreement with Pinson et al. (2015) who reported that water treatment effects had higher impact for element accumulation in rice grains than year effects, and the average grain Mn concentration under unflooded condition was greater than the average grain Mn concentration under flooded condition among 1,763 rice accessions grown in Texas in 2007 and 2008. Senewiratne and Mikkelsen (1961) found Mn concentration in rice leaves were 7.7 fold higher under unflooded condition as compared with flooded condition. One genetic factor that could have an influence on grain element concentrations is flowering time. As this population is comprised of a wide range of different accessions the flowering window (the time from the first accession flowering to the last) is quite large (Norton et al. 2012b). During this time the environmental conditions can change which may affect the availability and therefore the accumulation of manganese. However, in this study only at two sites were relationships between flowering time and grain manganese concentration overserved and in both cases the relationships explained only a small component of the variation.

The genetic differences among subpopulations also affected grain Mn concentrations such as higher grain Mn concentration in the TEJ and TRJ subpopulations grown under flooded conditions compared with the AUS and IND subpopulations (Fig. 2). In another study under flooded conditions, *Japonica* subgroup accessions had higher Mn concentrations in their rice grains than *Indica* subgroup accessions (Yang et al. 2018). Pinson et al. (2015) have shown that although water management treatments had a high impact, genetic backgrounds in the 1,763 rice accessions was a major factor for grain element accumulation in both flooded (average broad sense heritability (H^2) of 16 elements: 0.49, Mn: 0.58) and unflooded (average H^2: 0.57, Mn: 0.70) conditions.

The efficiency of GWA mapping depends on several factors such as the proportion of variation explained by genotype (heritability), the underlying population structure within the panel, sample size, and marker density. McCouch et al. (2016) suggested that increasing SNP density increases the ability to detect genetic loci. In this study, we tested the impact of marker density while using the same rice accessions, the same phenotype data and the same statistical modelling approach to account for population structure and kinship (Kang et al. 2010). We demonstrated the number of markers covering the genome affects the efficacy of GWA mapping (Fig. 3a and Supplementary Fig. S1–S3). Our results revealed that higher marker density increases the number of significant loci associated with the trait. A similar observation has been made in a recent study, where GWA mapping for root cone angle in rice was conducted using 15,000 and 300,000 SNPs (Bettembourg et al. 2017). Wang et al. (2018) also showed that increasing from 700K to 4.8M SNPs in GWA mapping for the grain amylose content in 326 *indica* accessions provided increased confidence in QTLs, as well as revealing new ones. In the present study, increasing marker density improved the identification of genetic loci using GWA mapping. However, at this stage it is unknown what the optimal marker density for GWA mapping in rice is.

From single-trait analyses using 5.2M SNPs, five QTLs on chromosomes 3 and 7 were found to affect grain Mn concentration, and the QTL sizes ranged from 789–852 kb (Table 2). Some of these QTLs co-localise with QTLs previously identified in rice (Fig. 3b). For example, Norton et al. (2012a) detected grain Mn QTLs using the Bala × Azucena mapping population, located similarly with QTLs detected in this study (chromosome 3 at approximately 3.49–6.65 Mbp and chromosome 7 at approximately 7.12–9.14 Mbp). Zhang et al. (2014) detected QTLs for grain Mn under flooded growing conditions in a TeQing-into-Lemont backcross introgression population on chromosome 3 (4–6 Mbp) and chromosome 7 (10–14 Mbp); with the chromosome 7 QTL being identified also in an independent population of Lemont × TeQing recombinant inbred lines. To further narrow down a QTL on chromosome 7, Liu et al. (2017) characterised a major QTL for grain Mn accumulation in recombinant inbred lines from the cross of 93-11 (low grain Mn) with PA64s (high grain Mn) grown in two environments. A major QTL located on the short arm of chromosome 7 was fine mapped between two markers (L8857 and L8906), 49.3 kb region encompassing the known Mn transporter, *OsNRAMP5* (Liu et al. 2017). Recently, Shrestha et al. (2018) conducted GWA mapping for shoot Mn toxicity in 271 RDP1 accessions based on the 700K SNP dataset. Numerous significant SNPs were identified in a large region on the top of chromosome 7. Although they did not report an exact QTL size, both *OsNRAMP5* and *OsNRAMP1* were identified as candidate genes. These results reveal that our identified QTLs based on GWA mapping with the high SNP density were smaller than the comparable genomic regions when using other mapping approaches. As a result, the identified QTLs contained a smaller number of positional candidates, which means the identification of genes underpinning the QTLs should be easier.

Within the grain Mn QTLs on chromosomes 3 and 7, six genes are proposed as contributing to the natural variation observed in grain Mn concentration in the RDP1. The candidate genes were highly expressed in roots, shoots, reproductive organs or embryo and endosperm tissues (Supplementary Fig. S8–S11 and S13–S14). On chromosome 3, three candidate genes were identified as *OsNRAMP2* (LOC_Os03g11010), *OsFRDL1* (LOC_Os03g11734) and *OsMTP8.1* (LOC_Os03g12530). While the function of *OsNRAMP2* is unknown in rice, *OsNRAMP2* has high structural similarity with an Mn transporter from *Eremococcus coleocola* (Mani and Sankaranarayanan 2018). *OsNRAMP2* in rice is also an orthologous gene with *AtNRAMP2* in *Arabidopsis* (Thomine et al. 2000) that is a trans-Golgi network-localised Mn transporter in roots under Mn deficiency (Gao et al. 2018). *OsFRDL1* is a good candidate gene as a knockout of *OsFRDL1* in rice resulted in lower leaf Fe concentration, and higher accumulation of Zn and Mn in leaves of rice (Yokosho et al. 2009). *OsMTP8.1* has been shown to be involved in Mn homeostasis achieved by sequestering excess Mn into vacuoles of rice (Chen et al. 2013, 2016), and to be an orthologous gene with *AtMTP8* involving in the localisation of Mn and Fe in *Arabidopsis* seeds (Chu et al. 2017). On chromosome 7, there were three candidate genes; *OsHMA3* (LOC_Os07g12900), *OsNRAMP5* (LOC_Os07g15370) and *OsNRAMP1* (LOC_Os07g15460). *OsHMA3* is a known tonoplast-localised transporter for Zn and Cd in rice roots, but it is reported that the overexpression of *OsHMA3* affected Mn concentration in roots and shoots (Sasaki et al. 2014). *OsNRAMP5* is a major transporter for Mn as well as for Fe and Cd in rice (Ishimaru et al. 2012; Sasaki et al. 2012; Yang et al. 2014; Liu et al. 2017). Although *OsNRAMP1* is an Fe transporter that is involved in Cd accumulations in rice (Takahashi et al. 2011), a phylogenetic analysis of *NRAMP* sequences in plants showed that *OsNRAMP1* was most similar to *OsNRAMP5* (Vatansever et al. 2016). Sheartha et al (2018) also identified a QTL for Mn toxicity in rice using GWA mapping that encompassed both *OsNRAMP1* and

OsNRAMP5. Therefore, *OsNRAMP1* is possibly involved in Mn transport or cross-talk between Fe and Mn homeostasis (Vatansever et al. 2016).

Due to genetic similarity within the TEJ subpopulation, local LD for the identified QTL on the chromosome 7 was analysed and estimated to define their candidate regions. The average LD decay from 7 to 9 Mbp in the TEJ subpopulation was high at >1 Mbp with the threshold of $r^2 = 0.2$ (Fig. 4b). To determine if this large LD decay was specific to the TEJ subpopulation the LD decay in the other subpopulations was determined (Supplementary Fig. S15). The LD decay across the other subpopulations with only the average LD decay in the IND subpopulation being lower. In addition to LD heatmap, the estimated LD distance in the region (9,017 SNPs in 6.5–9.5 Mbp) in the TEJ subpopulation was 1.23 Mbp from 7.64 to 8.87 Mbp indicating few historical recombination events. It was similar to a large LD block in the AUS (23,041 SNPs) subpopulation, whereas several LD blocks in the IND (13,731 SNPs) and TRJ (6,513 SNPs) subpopulations were observed (Supplementary Fig. S15).

For multi-experiment analysis, conducting GWA mapping for grain Mn concentration with the phenotypic values of the three flooded-field experiments (ArFl06, ArFl07 and TxFl09) using the mvLMM, there were 2 QTLs which had previously been detected in the single site analysis and 6 newly identified QTLs (Fig. 5). Similarly, Korte et al. (2012) reanalysed the flowering time data of Li et al. (2010) in 459 *A. thaliana* accessions grown over two seasons in each of two different locations using MTMM (Multi-trait mixed model) to reveal new QTLs. Three detected loci were involved in the differential flowering response to different environments that were not detected in the individual screens. Indeed, multi-trait analysis is an efficient tool for detecting loci/QTLs associated with multiple traits, because of the increased power obtained from additional data from correlated traits or a single trait in multiple experiments (Korte et al. 2012; Zhou and Stephens 2014). Thus, this approach should be used to identify stable QTLs, and is potentially beneficial in terms of GWAS of complex traits. The validation of the new QTLs could be further studied for identification of candidate genes underlying these QTLs that may contribute to the ultimate grain Mn concentration in rice.

While gene expression data was not collected for the plants grown in this experiment, recently transcriptomic analysis for 91 of the RDP1 accessions was conducted (Campbell et al. 2020). This database consists of gene expression data from shoots, and can be used to determine if genes are differentially expressed between accessions. For candidate genes discussed, all were found to be expressed in shoots with *OsMTP8.1*, *OsHMA3* and *OsNRAMP1* differentially expressed between the low and high grain Mn accessions (Supplementary Fig. S12). Differential gene expression means that these genes are very good candidates for the trait as this expression difference could be driving the QTLs. However, future analysis of gene expression between low and high Mn accumulating accessions during grain filling will give a further insight into the role these genes play in the Mn accumulation in the grain.

Conclusion

This study uses data from multiple field experiments (locations, years and irrigation treatments) to conduct GWA mapping for a grain elemental trait, Mn concentration, in rice. We have demonstrated that multi-experiment analysis has a number of potential benefits, including the identification of QTLs not detected in individual analyses. Future study would be required to validate these genes, and identify the alleles that are responsible for variation in Mn accumulation in rice grains.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was part supported by the US National Science Foundation, Plant Genome Research Program (grant #IOS 0701119 to DES, MLG and SRMP) and The US National Institutes of Health (grant 2P4ES007373 to MLG and DES). PR is a PhD student funded by the Thai Government Scholarship.

References

Atwell S, Huang YS, Vilhjálmsson BJ, Willems G, Horton M, Li Y et al. (2010) Genome-wide association study of 107 phenotypes in Arabidopsis thaliana inbred lines. Nature 465:627–631

Bashir K, Takahashi R, Nakanishi H, Nishizawa NK (2013) The road to micronutrient biofortification of rice: progress and prospects. Front Plant Sci 4:15

Baxter I, Brazelton JN, Yu D, Huang YS, Lahner B, Yakubova E et al. (2010) A coastal cline in sodium accumulation in Arabidopsis thaliana is driven by natural variation of the sodium transporter AtHKT1;1. PLoS Genet 6:e1001193

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B 57:289–300

Bettembourg M, Dardou A, Audebert A, Thomas E, Frouin J, Guiderdoni E et al. (2017) Genome-wide association mapping for root cone angle in rice. Rice 10:45

Biscarini F, Cozzi P, Casella L, Riccardi P, Vattari A, Orasen G et al. (2016) Genome-wide association study for traits related to plant and grain morphology, and root architecture in temperate rice accessions. PLoS One 11:e0155425

Butardo VM, Anacleto R, Parween S, Samson I, de Guzman K, Alhambra CM et al. (2017) Systems genetics identifies a novel regulatory domain of amylose synthesis. Plant Physiol 173:887–906

Campbell MT, Du Q, Liu K, Sharma S, Zhang C, Walia H (2020) Characterization of the transcriptional divergence between the subspecies of cultivated rice (Oryza sativa). BMC Genomics 21:394

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 4:7

Chen Z, Fujii Y, Yamaji N, Masuda S, Takemoto Y, Kamiya T et al. (2013) Mn tolerance in rice is mediated by MTP8.1, a member of the cation diffusion facilitator family. J Exp Bot 64:4375–4387

Chen X, Li J, Wang L, Ma G, Zhang W (2016) A mutagenic study identifying critical residues for the structure and function of rice manganese transporter OsMTP8.1. Sci Rep 6:32073

Chu HH, Car S, Socha AL, Hindt MN, Punshon T, Guerinot ML (2017) The Arabidopsis MTP8 transporter determines the localization of manganese and iron in seeds. Sci Rep 7:11024

Crossgrove J, Zheng W (2004) Manganese toxicity upon overexposure. NMR Biomed 17:544–553

Dimkpa SON, Lahari Z, Shrestha R, Douglas A, Gheysen G, Price AH (2016) A genome-wide association study of a global rice panel reveals resistance in Oryza sativa to root-knot nematodes. J Exp Bot 67:1191–1200

Du J, Zeng D, Wang B, Qian Q, Zheng S, Ling HQ (2013) Environmental effects on mineral accumulation in rice grains and identification of ecological specific QTLs. Environ Geochem Health 35:161–170

Ducic T, Polle A (2005) Transport and detoxification of manganese and copper in plants. Braz J Plant Physiol 17:103–112

Eizenga GC, Ali ML, Bryant RJ, Yeater KM, McClung AM, McCouch SR (2014) Registration of the rice diversity panel 1 for genomewide association studies. J Plant Regist 8:109–116

Eroglu S, Giehl RFH, Meier B, Takahashi M, Terada Y, Ignatyev K et al. (2017) Metal tolerance protein 8 mediates manganese homeostasis and iron reallocation during seed development and germination. Plant Physiol 174:1633–1647

Famoso AN, Zhao K, Clark RT, Tung C-W, Wright MH, Bustamante C et al. (2011) Genetic architecture of aluminum tolerance in rice (Oryza sativa) determined through genome-wide association analysis and QTL mapping. PLoS Genet 7:e1002221

Gao H, Xie W, Yang C, Xu J, Li J, Wang H et al. (2018) NRAMP2, a trans-golgi network-localized manganese transporter, is required for Arabidopsis root growth under manganese deficiency. N Phytologist 217:179–193

Halder D, Saha JK, Biswas A (2020) Accumulation of essential and non-essential trace elements in rice grain: possible health impacts on rice consumers in West Bengal, India. Sci Total Env 706:135944

Hebbern CA, Pedas P, Schjoerring JK, Knudsen L, Husted S (2005) Genotypic differences in manganese efficiency: field experiments with winter barley (Hordeum vulgare L.). Plant Soil 272:233–244

Huang XY, Salt DE (2016) Plant ionomics: from elemental profiling to environmental adaptation. Mol Plant 9:787–797

Huang X, Wei X, Sang T, Zhao Q, Feng Q, Zhao Y et al. (2010) Genome-wide association studies of 14 agronomic traits in rice landraces. Nat Genet 42:961–967

Huang X, Zhao Y, Wei X, Li C, Wang A, Zhao Q et al. (2012) Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. Nat Genet 44:32–39

Institute of Medicine (2001) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academies Press: Washington DC

Ishikawa S, Abe T, Kuramata M, Yamaguchi M, Ando T, Yamamoto T et al. (2010) A major quantitative trait locus for increasing cadmium-specific concentration in rice grain is located on the short arm of chromosome 7. J Exp Bot 61:923–934

Ishimaru Y, Masuda H, Bashir K, Inoue H, Tsukamoto T, Takahashi M et al. (2010) Rice metal-nicotianamine transporter, OsYSL2, is required for the long-distance transport of iron and manganese. Plant J 62:379–390.

Ishimaru Y, Takahashi R, Bashir K, Shimo H, Senoura T, Sugimoto K et al. (2012) Characterizing the role of rice NRAMP5 in manganese, iron and cadmium transport. Sci Rep 2:286

Kang HM, Sul JH, Service SK, Zaitlen NA, Kong S-Y, Freimer NB et al. (2010) Variance component model to account for sample structure in genome-wide association studies. Nat Genet 42:348–354

Kang HM, Zaitlen NA, Wade CM, Kirby A, Heckerman D, Daly MJ et al. (2008) Efficient control of population structure in model organism association mapping. Genetics 178:1709–1723

Koike S, Inoue H, Mizuno D, Takahashi M, Nakanishi H, Mori S et al. (2004) OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. Plant J 39:415–424

Korte A, Farlow A (2013) The advantages and limitations of trait analysis with GWAS: a review. Plant Methods 9:29

Korte A, Vilhjálmsson BJ, Segura V, Platt A, Long Q, Nordborg M (2012) A mixed-model approach for genome-wide association studies of correlated traits in structured populations. Nat Genet 44:1066–1071

Kump KL, Bradbury PJ, Wisser RJ, Buckler ES, Belcher AR, Oropeza-Rosas MA et al. (2011) Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nat Genet 43:163–168

Li Y, Huang Y, Bergelson J, Nordborg M, Borevitz JO (2010) Association mapping of local climate-sensitive quantitative trait loci in Arabidopsis thaliana. Proc Natl Acad Sci 107:21199–21204

Lippert C, Casale FP, Rakitsch B, Stegle O (2014) LIMIX: genetic analysis of multiple traits. bioRxiv

Liu C, Chen G, Li Y, Peng Y, Zhang A, Hong K et al. (2017) Characterization of a major QTL for manganese accumulation in rice grain. Sci Rep 7:17704

Loh P-R, Tucker G, Bulik-Sullivan BK, Vilhjálmsson BJ, Finucane HK, Salem RM et al. (2015) Efficient bayesian mixed-model analysis increases association power in large cohorts. Nat Genet 47:284–290

Lu K, Li L, Zheng X, Zhang Z, Mou T, Hu Z (2008) Quantitative trait loci controlling Cu, Ca, Zn, Mn and Fe content in rice grains. J Genet 87:305–310

Ma G, Li J, Li J, Li Y, Gu D, Chen C et al. (2018) OsMTP11, a trans-golgi network localized transporter, is involved in manganese tolerance in rice. Plant Sci 274:59–69

Mani A, Sankaranarayanan K (2018) In silico analysis of natural resistance-associated macrophage protein (NRAMP) family of transporters in rice. Protein J 37:237–247

Marschner H (1995) Mineral nutrition of higher plants. Academic Press: London

McCouch SR, Wright MH, Tung C-W, Maron LG, McNally KL, Fitzgerald M et al. (2016) Open access resources for genome-wide association mapping in rice. Nat Commun

7:10532

Millaleo R, Reyes- Diaz M, Ivanov AG, Mora ML, Alberdi M (2010) Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. J Soil Sci Plant Nutr 10:476–494

Norton GJ, Deacon CM, Xiong L, Huang S, Meharg AA, Price AH (2010) Genetic mapping of the rice ionome in leaves and grain: identification of QTLs for 17 elements including arsenic, cadmium, iron and selenium. Plant Soil 329:139–153

Norton GJ, Douglas A, Lahner B, Yakubova E, Guerinot ML, Pinson SRM et al. (2014) Genome wide association mapping of grain arsenic, copper, molybdenum and zinc in rice (Oryza sativa L.) grown at four international field sites. PLoS One 9:e89685

Norton GJ, Duan GL, Lei M, Zhu YG, Meharg AA, Price AH (2012a) Identification of quantitative trait loci for rice grain element composition on an arsenic impacted soil: influence of flowering time on genetic loci. Ann Appl Biol 161:46–56

Norton GJ, Pinson SRM, Alexander J, McKay S, Hansen H, Duan GL et al. (2012b) Variation in grain arsenic assessed in a diverse panel of rice (Oryza sativa) grown in multiple sites. N Phytologist 193:650–664

Norton GJ, Travis AJ, Douglas A, Fairley S, Alves EDP, Ruang-areerate P et al. (2018) Genome wide association mapping of grain and straw biomass traits in the rice Bengal and Assam Aus Panel (BAAP) grown under alternate wetting and drying and permanently flooded irrigation. Front Plant Sci 9:1223

O'Neal SL, Zheng W (2015) Manganese toxicity upon overexposure: a decade in review. Curr Environ Heal Rep 2:315–328

Patterson N, Price AL, Reich D (2006) Population structure and eigenanalysis. PLoS Genet 2:e190

Peris-Peris C, Serra-Cardona A, Sánchez-Sanuy F, Campo S, Ariño J (2017) Two NRAMP6 isoforms function as iron and manganese transporters and contribute to disease resistance in rice. Mol Plant-Microbe Interact 30:385–398

Pinson SRM, Tarpley L, Yan W, Yeater K, Lahner B, Yakubova E et al. (2015) Worldwide genetic diversity for mineral element concentrations in rice grain. Crop Sci 55:294–311

Price AL, Zaitlen NA, Reich D, Patterson N (2010) New approaches to population stratification in genome-wide association studies. Nat Rev Genet 11:459–463

R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Ripke S, Neale BM, Corvin A, Walters JTR, Farh KH, Holmans PA et al. (2014) Biological insights from 108 schizophrenia-associated genetic loci. Nature 511:421–427

Sakai H, Lee SS, Tanaka T, Numa H, Kim J, Kawahara Y et al. (2013) Rice annotation project database (RAP-DB): an integrative and interactive database for rice genomics. Plant Cell Physiol 54:e6

Sasaki A, Yamaji N, Ma JF (2014) Overexpression of OsHMA3 enhances Cd tolerance and expression of Zn transporter genes in rice. J Exp Bot 65:6013–6021

Sasaki A, Yamaji N, Xia J, Ma JF (2011) OsYSL6 is involved in the detoxification of excess manganese in rice. Plant Physiol 157:1832–1840

Sasaki A, Yamaji N, Yokosho K, Ma JF (2012) Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. Plant Cell 24:2155–2167

Senewiratne ST, Mikkelsen DS (1961) Physiological factors limiting growth and yield of Oryza sativa under unflooded conditions. Plant Soil 14:127–146

Shin JH, Blay S, McNeney B, Graham J (2006) LDheatmap: an R function for graphical display of pairwise linkage disequilibria between single nucleotide polymorphisms. J Stat Softw 16:c03

Shrestha A, Dziwornu AK, Ueda Y, Wu LB, Mathew B, Frei M (2018) Genome-wide association study to identify candidate loci and genes for Mn toxicity tolerance in rice. PLoS One 13:e0192116

Socha AL, Guerinot ML (2014) Mn-euvering manganese: the role of transporter gene family members in manganese uptake and mobilization in plants. Front Plant Sci 5:106

Soetan K, Olaiya CO, Oyewole OE (2010) The importance of mineral elements for humans, domestic animals and plants: a review. African J Food Sci 4:200–222

Stangoulis JCR, Huynh BL, Welch RM, Choi EY, Graham RD (2007) Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. Euphytica 154:289–294

Takahashi R, Ishimaru Y, Nakanishi H, Nishizawa NK (2011) Role of the iron transporter OsNRAMP1 in cadmium uptake and accumulation in rice. Plant Signal Behav 6:1813–1816

Takemoto Y, Tsunemitsu Y, Fujii-Kashino M, Mitani-Ueno N, Yamaji N, Ma JF et al. (2017) The tonoplast-localized transporter MTP8.2 contributes to manganese detoxification in the shoots and roots of Oryza sativa L. Plant Cell Physiol 58:1573–1582

Thomine S, Wang R, Ward JM, Crawford NM, Schroede JI (2000) Cadmium and iron transport by members of a plant metal transporter family in Arabidopsis with homology to Nramp genes. Proc Natl Acad Sci 97:4991–4996

Turner SD (2014) qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. bioRxiv

Ueno D, Sasaki A, Yamaji N, Miyaji T, Fujii Y, Takemoto Y et al. (2015) A polarly localized transporter for efficient manganese uptake in rice. Nat Plants 1:15170

Vatansever R, Filiz E, Ozyigit II (2016) In silico analysis of Mn transporters (NRAMP1) in various plant species. Mol Biol Rep 43:151–163

Wang DR, Agosto-Pérez FJ, Chebotarov D, Shi Y, Marchini J, Fitzgerald M et al. (2018) An imputation platform to enhance integration of rice genetic resources. Nat Commun 9:3519

Yang M, Lu K, Zhao F-J, Xie W, Ramakrishna P, Wang G et al. (2018) Genome-wide association studies reveal the genetic basis of ionomic variation in rice. Plant Cell 30:2720–2740

Yang M, Zhang W, Dong H, Zhang Y, Lv K, Wang D et al. (2013) OsNRAMP3 is a vascular bundles-specific manganese transporter that is responsible for manganese distribution in rice. PLoS One 8:e83990

Yang M, Zhang Y, Zhang L, Hu J, Zhang X, Lu K et al. (2014) OsNRAMP5 contributes to manganese translocation and distribution in rice shoots. J Exp Bot 65:4849–4861

Yano K, Yamamoto E, Aya K, Takeuchi H, Lo P, Hu L et al. (2016) Genome-wide association study using whole-genome sequencing rapidly identifies new genes influencing agronomic traits in rice. Nat Genet 48:927–934

Yokosho K, Yamaji N, Ueno D, Mitani N, Ma JF (2009) OsFRDL1 is a citrate transporter required for efficient translocation of iron in rice. Plant Physiol 149:297–305

Zhang M, Liu B (2017) Identification of a rice metal tolerance protein OsMTP11 as a manganese transporter. PLoS One 12:e0174987

Zhang M, Pinson SRM, Tarpley L, Huang XY, Lahner B, Yakubova E et al. (2014) Mapping and validation of quantitative trait loci associated with concentrations of 16 elements in unmilled rice grain. Theor Appl Genet 127:137–165

Zhao K, Tung C-W, Eizenga GC, Wright MH, Ali ML, Price AH et al. (2011) Genome-wide association mapping reveals a rich genetic architecture of complex traits in Oryza sativa. Nat Commun 2:467

Zhou X, Stephens M (2014) Efficient multivariate linear mixed model algorithms for genome-wide association studies. Nat Methods 11:407–409

Figures

Fig. 1 Grain Mn distributions in all rice accessions. (a) Distribution of grain Mn concentration in Arkansas under flooded condition in 2006 and 2007. (b) Distribution of grain Mn concentration in Texas under flooded and unflooded conditions in 2009.

Fig. 2 Distribution of grain Mn concentration in rice in four subpopulations in four field experiments. The horizontal black bar is the median of grain Mn concentration.

Fig. 3 Genome-wide association mapping results for grain Mn concentration in rice using single-trait analysis in all accessions grown in Arkansas under flooded condition in 2006. (a) Manhattan (left) and Q-Q (right) plots are presented for the 44K, 700K and 5.2M SNP datasets. The blue horizontal line represents the $-\log_{10}(P)$ threshold at 4. The red dot indicates SNP loci passed 5% FDR. (b) Location of QTLs associated with grain Mn concentration in rice for four field experiments based on the 5.2M SNP dataset using single-trait analysis and previously reported QTLs. The four field experiments are ArFl06 – light blue, ArFl07 – blue, TxFl09 – orange and TxUnfl09 – brown. Previous reported QTLs are displayed in purple with the letter indicating the study they were detected in; a = Stangoulis et al. (2007), b = Lu et al. (2008), c = Norton et al. (2010), d = Ishikawa et al. (2010), e = Norton et al. (2012a), f = Du et al. (2013) and g = Zhang et al. (2014). Known Mn-transporter locations are indicated by horizontal black lines, whereas the locations of candidate genes are indicated by horizontal red lines.

Fig. 4 Genome-wide association mapping results for grain Mn concentration in the *temperate japonica* subpopulation based on the 5.2M SNP dataset, as well as local linkage disequilibrium analysis and SNP allele effects. (a) Manhattan (left) and Q-Q (right) plots are presented in four-field experiments as ArFl06: Arkansas flooded 2006, ArFl07: Arkansas flooded 2007, TxFl09: Texas flooded 2009 and TxUnfl09: Texas unflooded 2009. The blue horizontal line represents the $-\log_{10}(P)$ threshold at 4. The red dot indicates SNP loci passed 5% FDR. (b) LD decay and (c) local Manhattan plot (top) as well as LD heatmap (bottom) for grain Mn concentration in ArFl07 on chromosome 7 at 7–9 Mbp and 6.5–9.5 Mbp, respectively. (d) The effect of SNP alleles on grain Mn concentration for the QTL on chromosome 7 with the SNP mlid0048878287 (8,781,883 bp) in all accessions (left) and the *temperate japonica* subpopulation (right) in ArFl07.

Fig. 5 Genome-wide association mapping results for grain Mn concentration in rice based on the 5.2M SNP dataset using multi-experiment analysis. (a) Manhattan (left) and Q-Q (right) plots are presented. The blue horizontal line represents the $-\log_{10}(P)$ threshold at 4. The red dot indicates SNP loci passed 5% FDR. The blue arrows point to new QTLs based on multi-experiment analysis. (b) Location of QTLs associated with grain Mn concentration in rice based on the 5.2M SNP dataset using multi-experiment analysis and previously reported QTLs. QTLs in ArFl06-ArFl07-TxFl09 is presented in red. Previous reported QTLs are displayed in purple with the letter indicating the study they were detected in; a = Stangoulis et al. (2007), b = Lu et al. (2008), c = Norton et al. (2010), d = Ishikawa et al. (2010), e = Norton et al. (2012a), f = Du et al. (2013) and g = Zhang et al. (2014). Known Mn-transporter locations are indicated by horizontal black lines, whereas the locations of candidate genes are indicated by horizontal red lines.

Tables

Table 1 Grain Mn concentration (mg kg^{-1}) in the RDP1 accessions at each field experiment.

Table 2 Significant QTLs on chromosomes 3 and 7, and candidate genes for grain Mn concentration in all rice accessions under flooded and unflooded conditions based on $P < 0.0001$ and passing 5% FDR using the 5.2M SNP dataset. All QTLs are concordant with previously reported QTLs of which references are given in Fig. 3.

Table 3 New putative QTLs and candidate genes for grain Mn concentration based on the 5.2M SNP dataset using multi-experiment analysis.

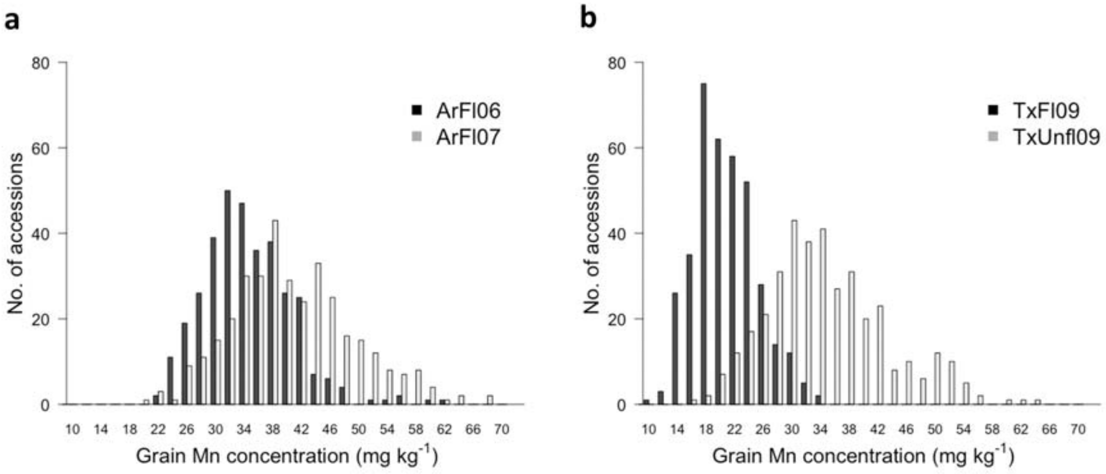

Fig. 1 Grain Mn distributions in all rice accessions. (a) Distribution of grain Mn concentration in Arkansas under flooded condition in 2006 and 2007. (b) Distribution of grain Mn concentration in Texas under flooded and unflooded conditions in 2009.

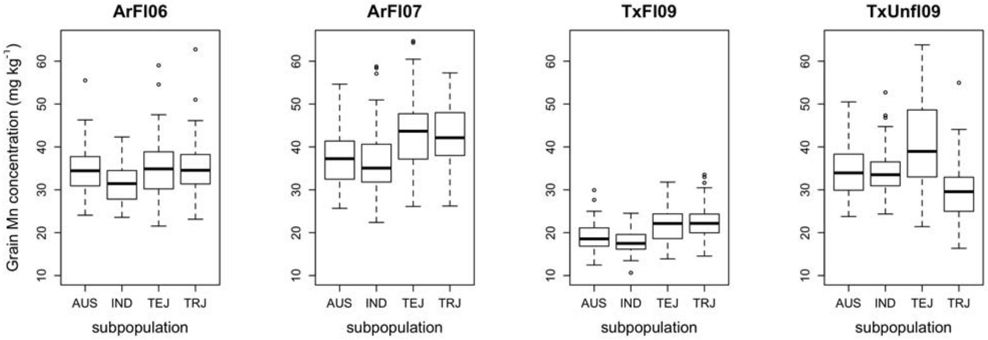

Fig. 2 Distribution of grain Mn concentration in rice in four subpopulations in four field experiments. The horizontal black bar is the median of grain Mn concentration.

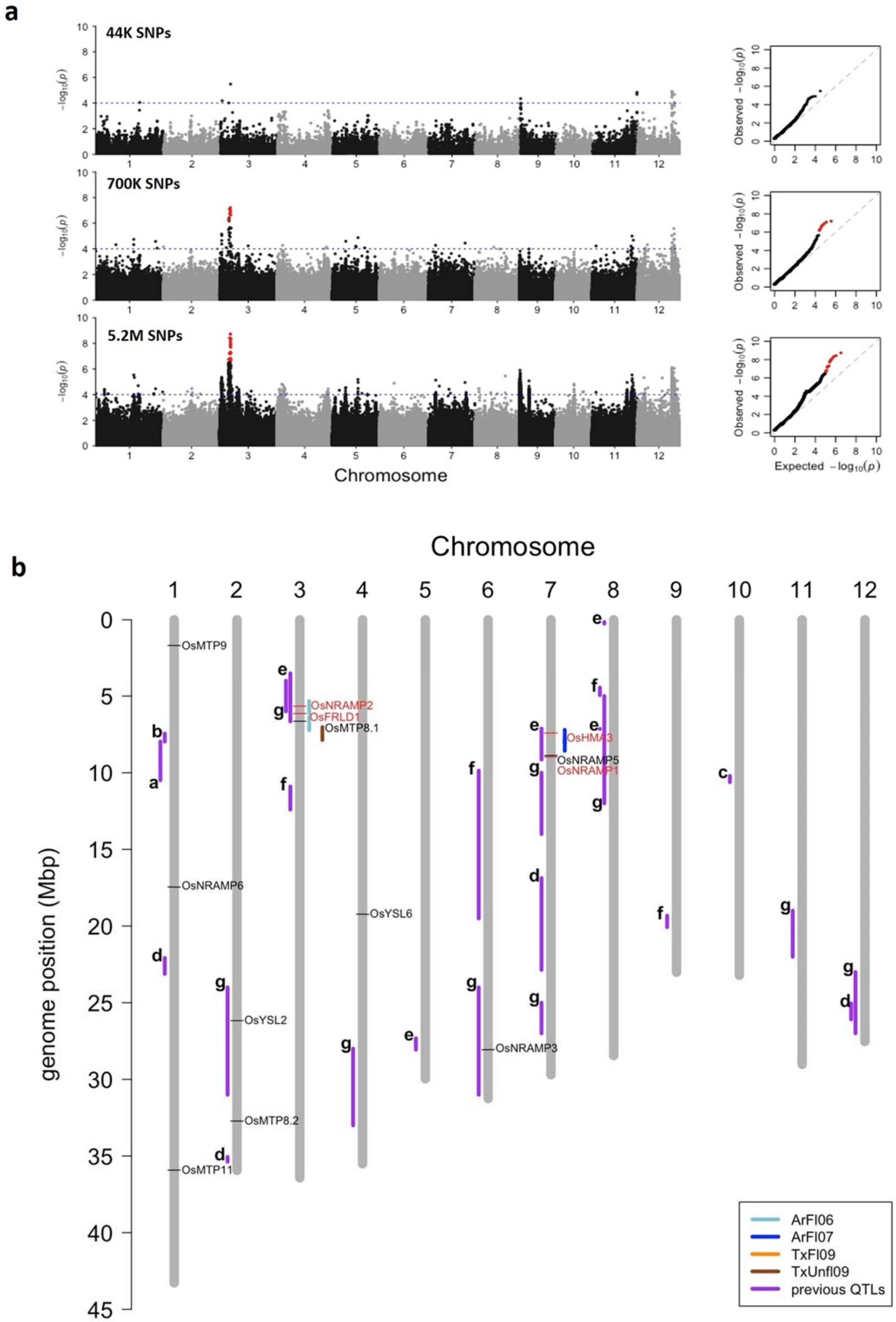

Fig. 3 Genome-wide association mapping results for grain Mn concentration in rice using single-trait analysis in all accessions grown in Arkansas under flooded condition in 2006. (a) Manhattan (left) and Q-Q (right) plots are presented for the 44K, 700K and 5.2M SNP datasets. The blue horizontal line represents the $-\log_{10}(P)$ threshold at 4. The red dot indicates

SNP loci passed 5% FDR. (b) Location of QTLs associated with grain Mn concentration in rice for four field experiments based on the 5.2M SNP dataset using single-trait analysis and previously reported QTLs. The four field experiments are ArFl06 – light blue, ArFl07 – blue, TxFl09 – orange and TxUnfl09 – brown. Previous reported QTLs are displayed in purple with the letter indicating the study they were detected in; a = Stangoulis et al. (2007), b = Lu et al. (2008), c = Norton et al. (2010), d = Ishikawa et al. (2010), e = Norton et al. (2012a), f = Du et al. (2013) and g = Zhang et al. (2014). Known Mn-transporter locations are indicated by horizontal black lines, whereas the locations of candidate genes are indicated by horizontal red lines.

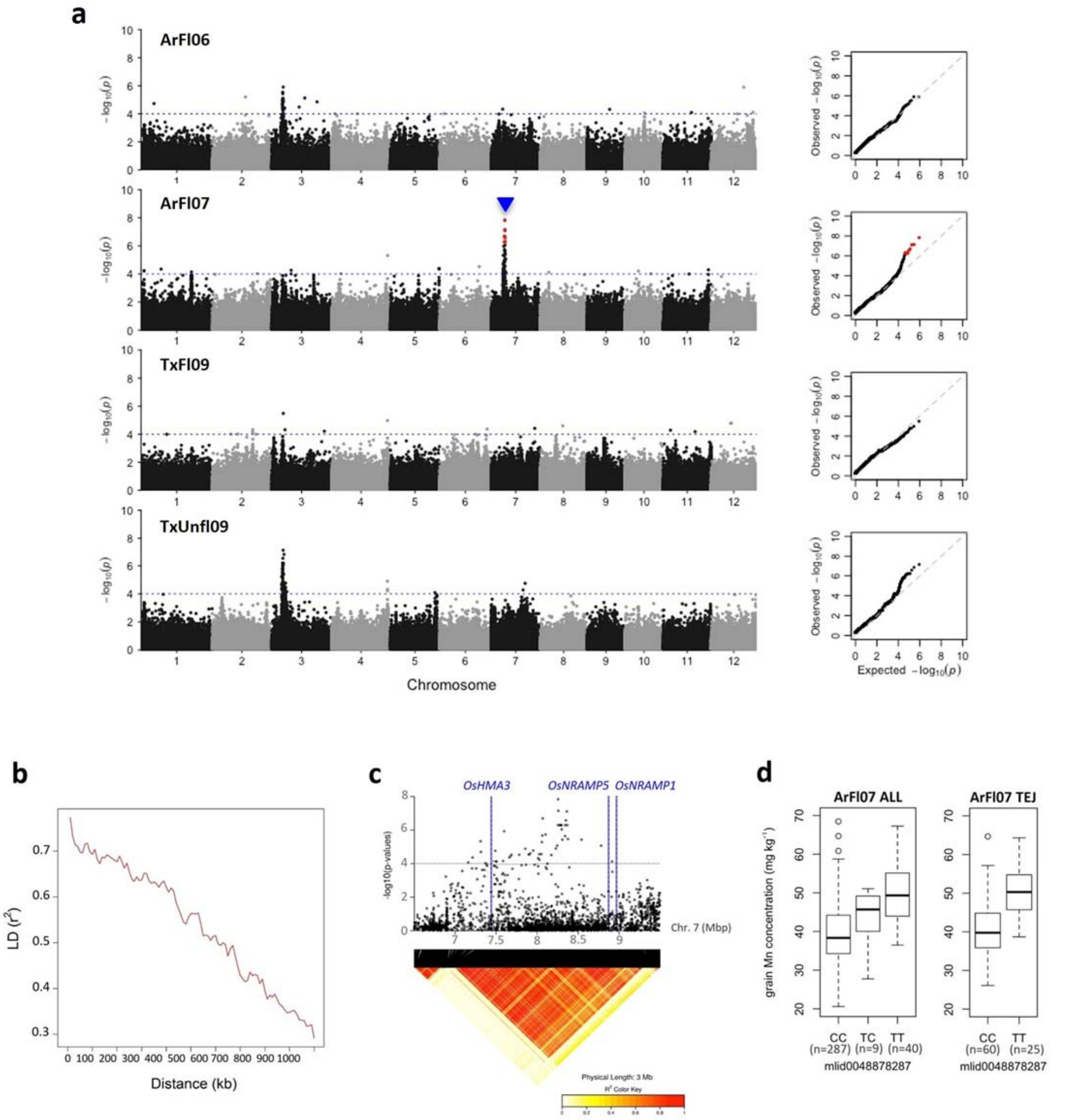

Fig. 4 Genome-wide association mapping results for grain Mn concentration in the *temperate japonica* subpopulation based on the 5.2M SNP dataset, as well as local linkage disequilibrium analysis and SNP allele effects. (a) Manhattan (left) and Q-Q (right) plots are presented in four-field experiments as ArFl06: Arkansas flooded 2006, ArFl07: Arkansas flooded 2007, TxFl09: Texas flooded 2009 and TxUnfl09: Texas unflooded 2009. The blue horizontal line represents the $-\log_{10}(P)$ threshold at 4. The red dot indicates SNP loci passed 5% FDR. (b) LD decay and (c) local Manhattan plot (top) as well as LD heatmap (bottom) for grain Mn concentration in ArFl07 on chromosome 7 at 7–9 Mbp and 6.5–9.5 Mbp, respectively. (d) The effect of SNP alleles on grain Mn concentration for the QTL on chromosome 7 with the SNP mlid0048878287 (8,781,883 bp) in all accessions (left) and the *temperate japonica* subpopulation (right) in ArFl07.

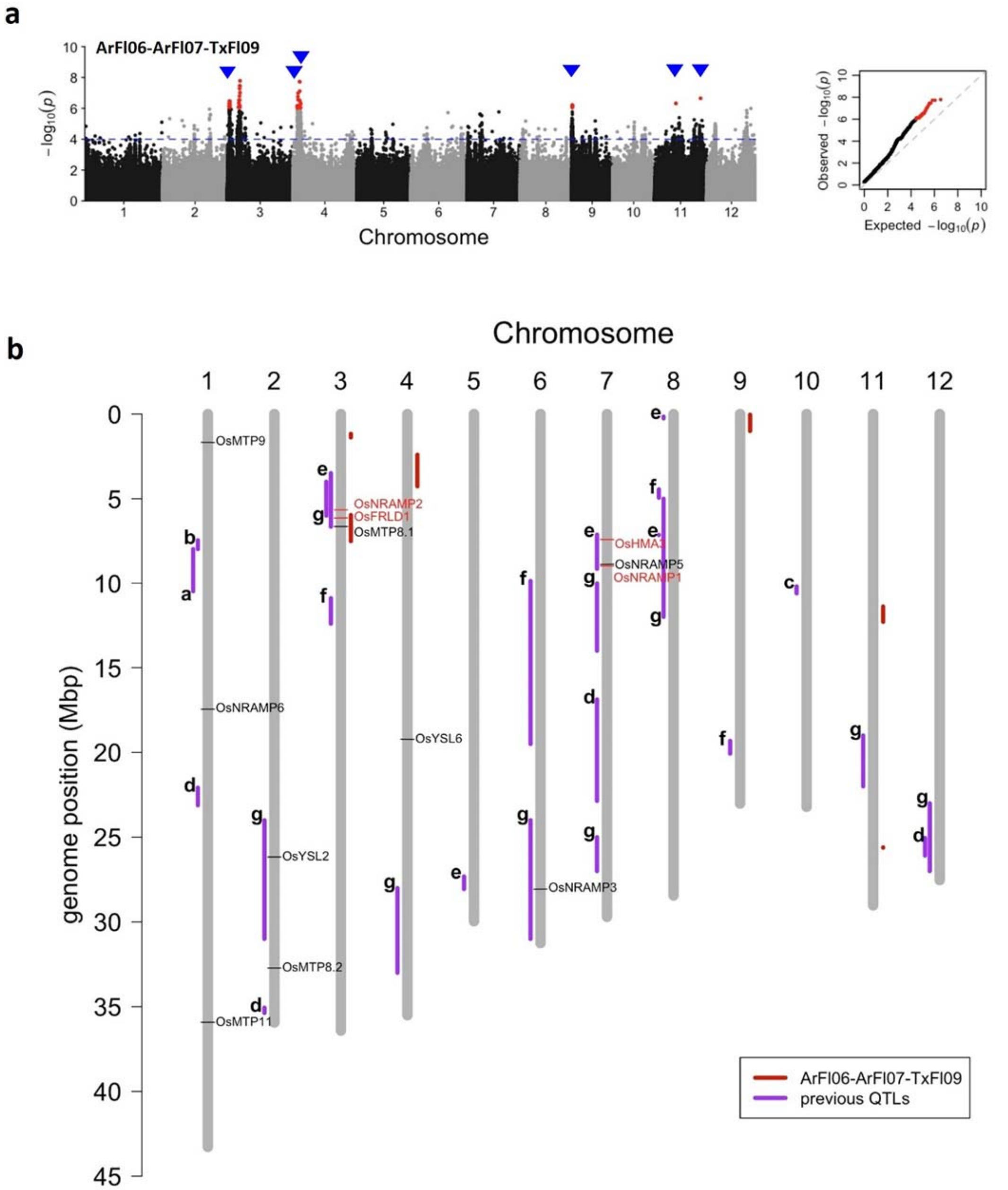

Fig. 5 Genome-wide association mapping results for grain Mn concentration in rice based on the 5.2M SNP dataset using multi-experiment analysis. (a) Manhattan (left) and Q-Q (right) plots are presented. The blue horizontal line represents the $-\log_{10}(P)$ threshold at 4. The red dot indicates SNP loci passed 5% FDR. The blue arrows point to new QTLs based on multi-experiment analysis. (b) Location of QTLs associated with grain Mn concentration in rice based on the 5.2M SNP dataset using multi-experiment analysis and previously reported QTLs. QTLs in ArFl06-ArFl07-TxFl09 is presented in red. Previous reported QTLs are displayed in purple with the letter indicating the study they were detected in; a = Stangoulis et al. (2007), b = Lu et al. (2008), c = Norton et al. (2010), d = Ishikawa et al. (2010), e = Norton et al. (2012a), f = Du et al. (2013) and g = Zhang et al. (2014). Known Mn-transporter

locations are indicated by horizontal black lines, whereas the locations of candidate genes are indicated by horizontal red lines.

Table 1 Grain Mn concentration (mg kg^{-1}) in the RDP1 accessions at each field experiment.

Site	Year	Condition	No. of accessions	Range	Mean	SD	CV (%)
Arkansas	2006	flooded	342	21.4–62.7	34.6	6.2	17.8
	2007	flooded	349	20.6–68.5	40.8	8.6	21.1
Texas	2009	flooded	373	10.6–33.5	20.9	4.3	20.7
	2009	unflooded	370	16.4–63.8	34.8	8.4	24.2

SD, standard deviation; CV, coefficient of variation

Table 2 Significant QTLs on chromosomes 3 and 7, and candidate genes for grain Mn concentration in all rice accessions under flooded and unflooded conditions based on *P* <0.0001 and passing 5% FDR using the 5.2M SNP dataset. All QTLs are concordant with previously reported QTLs of which references are given in Fig. 3.

Chr.	**Loci**	**Experiment**	**Index SNP id**	**Position**	**Minor/Major allele**	**MAF**	***P*-value**	**Estimated candidate region**	**Clumped size (kb)**	**MSU id**	**Gene name**
3	1	ArFl06	mlid0018058877	5,804,440	T/C	0.11	1.77E-07	5,326,994–6,138,356	811.36	LOC_Os03g11010	*OsNRAMP2*
										LOC_Os03g11734	*OsFRDL1*
	2	ArFl06	mlid0018228403	6,819,735	T/C	0.13	1.87E-09	6,387,194–7,233,912	846.72	LOC_Os03g12530	*OsMTP8.1*
	3	TxUnfl09	mlid0018352659	7,507,792	T/A	0.12	1.22E-08	7,019,729–7,866,465	846.74	-	-
7	1	ArFl07	mlid0048560313	7,594,101	A/G	0.14	1.80E-08	7,206,339–8,057,921	851.58	LOC_Os07g12900	*OsHMA3*
	2	ArFl07	mlid0048735332	8,256,487	A/C	0.15	1.44E-08	7,780,036–8,568,541	788.51	-	-

Table 3 New putative QTLs and candidate genes for grain Mn concentration based on the 5.2M SNP dataset using multi-experiment analysis.

Chr.	Index SNP id	Position	Minor/Major allele	MAF	*P*-value	Allele effect			Estimated candidate region	Clumped size (kb)
						ArFl06	ArFl07	TxFl09		
3	mlid0017254736	1,253,009	T/C	0.24	3.43E-07	0.0066	0.0180	0.0075	1,164,504–1,377,789	213.29
4	mlid0025444660	2,891,380	T/C	0.17	1.03E-07	0.0043	-0.0262	0.0085	2,403,945–3,330,179	926.23
4	mlid0025744000	3,767,588	C/G	0.14	1.89E-08	0.0065	-0.0014	0.0105	3,411,664–4,267,559	855.90
9	mlid0060879641	504,844	A/G	0.40	6.17E-07	-0.0113	-0.0397	-0.0135	38,664–1,004,262	965.60
11	mlid0074604290	11,886,926	G/A	0.39	4.80E-07	0.0062	-0.0320	-0.0095	11,391,970–12,295,345	903.38
11	mlid0078299980	25,621,708	A/C	0.05	2.24E-07	0.0094	0.0340	-0.0026	25,619,048–25,621,708	2.76

Supplementary Information

Genome-wide association mapping for grain manganese in rice (*Oryza sativa* L.) using a multi-experiment approach

Panthita Ruang-areerate, Anthony J. Travis, Shannon R. M. Pinson, Lee Tarpley, Georgia C. Eizenga, Mary Lou Guerinot, David E. Salt, Alex Douglas, Adam H. Price and Gareth J. Norton

Table S1. Grain Mn concentration in four field experiments. The average concentration of more two or more replicates is shown. The abbreviation of subpopulations is ADM: admixed, ADM-IND: admixed *indica*, ADM-JAP: admixed *japonica*, AUS: *aus*, IND: *indica*, TEJ: *temperate japonica* and TRJ: *tropical japonica* (Source. McCouch et al. 2016, Supplementary Data 2). The unit of the grain Mn concentration is mg kg^{-1}. NA is not available data. Asterisks represent phenotypic data that were used in GWA analysis based on the 5.2M SNP dataset.

NSFTV ID	HDRA ID	Accession name	Subpopulation	ArFl06	ArFl07	TxFl09	TxUnfl09
1	86f75d2b.0	Agostano	TEJ	36.84*	40.75*	26.45*	43.10*
2	cd22c3de.0	Aichi Asahi	TEJ	NA	44.40*	18.51*	38.00*
3	5ef1be74.0	Ai-Chiao-Hong	IND	25.88*	25.46*	15.83*	26.59*
4	81d03b86.0	NSF-TV 4	AUS	33.37*	31.36*	18.47*	29.90*
5	5533f406.0	NSF-TV 5	ARO	47.49*	46.19*	30.46*	49.51*
6	0d125c0e.0	ARC 7229	AUS	39.01*	46.68*	21.24*	36.08*
7	e37be9e5.0	Arias	TRJ	46.14*	51.98*	26.40*	31.38*
8	30c3073f.0	Asse Y Pung	TRJ	34.79*	34.06*	16.04*	20.87*
9	2a9d8d47.0	Baber	TEJ	29.90*	30.19*	18.14*	26.48*
10	2e1c9c87.0	Baghlani Nangarhar	TEJ	NA	32.71*	22.87*	42.78*
11	1d0066e2.0	Baguamon 14	IND	NA	NA	15.35*	24.36*
12	NA	Basmati	ARO	29.34	38.24	20.83	35.02
13	660f0236.0	NSF-TV 13	AUS	40.83*	54.64*	21.47*	34.29*
14	NA	Basmati 217	ARO	33.36	33.18	24.48	46.63
15	e13b4d7a.0	Beonjo	TEJ	NA	NA	23.47*	35.48*
16	f0328a18.0	Bico Branco	ARO	40.78*	52.25*	26.41*	34.87*
17	446f6c62.0	Binulawan	IND	NA	NA	21.96*	42.12*
18	cbd6b346.0	BJ 1	AUS	24.28*	33.47*	14.10*	27.75*
19	d22c8e33.0	Black Gora	AUS	28.23*	28.24*	17.84*	36.19*
20	6af7c9fc.0	Blue Rose	ADM-JAP	45.25*	62.51*	29.88*	36.35*
21	fa4c4111.0	Byakkoku Y 5006 Seln	IND	35.19*	45.92*	23.57*	34.98*
22	52c6e2ba.0	Caawa/Fortuna 6-103-15	TRJ	30.97*	NA	21.77*	33.08*
23	7741d7c1.0	Canella De Ferro	TRJ	34.77*	48.30*	21.78*	22.72*
24	e074229f.0	Carolina Gold 12033	TRJ	36.68*	40.97*	22.52*	25.57*
25	1ce7093b.0	Carolina Gold 12034	TRJ	31.15*	39.08*	19.34*	24.18*
26	32a15c4d.0	Carolina Gold Sel	TRJ	37.32*	45.09*	24.35*	32.93*
27	1956dd3f.0	NSF-TV 27	TRJ	28.27*	40.53*	21.21*	26.76*
28	b6701089.0	Champa Tong 54	AUS	36.16*	46.39*	23.52*	40.73*
29	30646147.0	Chau	IND	NA	31.81*	18.10*	33.46*
30	dd6e755e.0	Chiem Chanh	IND	31.44	43.13*	17.46*	NA
31	d2da857d.0	Chinese	TEJ	NA	45.72*	23.14*	30.26*
32	068b860d.0	Chodongji	TEJ	38.74*	52.89*	29.39*	35.24*
33	2b4e06fe.0	Chuan 4	AUS	38.17*	36.44*	21.12*	31.18*
34	c03e2c51.0	NSF-TV 34	IND	NA	NA	NA	28.33*
35	c59435a8.0	CO18	IND	NA	NA	19.37	34.71*
36	2593e5a7.0	CS-M3	TEJ	41.56*	64.68*	25.77*	46.08*
37	69527f35.0	Cuba 65	TRJ	34.92*	51.08*	31.63*	38.59*
39	014ab0f1.0	NSF-TV 39	AUS	34.88*	35.76*	17.32*	36.14*
40	76f56677.0	Dam	ADM-JAP	29.93*	37.29*	21.04*	21.83*
43	e5951598.0	Dee Geo Woo Gen	IND	32.98*	36.40*	17.27*	32.66*
44	7d4c6c4e.0	Dhala Shaitta	AUS	46.30*	38.10*	23.33*	49.21*
45	8d6aded4.0	Dom-Sufid	ARO	40.14*	42.16*	26.19*	33.56*
46	68c2ecf8.0	Dourado Agulha	TRJ	29.69*	33.98*	17.44*	23.16*
48	63f63dde.0	NSF-TV 48	ADM-JAP	28.58*	35.80*	25.99*	20.93*
49	21d3f1b3.0	DV85	AUS	33.39*	33.94*	27.64*	39.09*
50	NA	DZ78	AUS	24.19	25.06	17.04	28.38
51	57d7feea.0	Early Wataribune	TEJ	29.78*	43.48*	21.83*	34.55*
52	9e0c626c.0	Eh Ia Chiu	TEJ	NA	NA	28.46*	50.93*

Table S1. Continued

NSFTV ID	HDRA ID	Accession name	Subpopulation	ArFl06	ArFl07	TxFl09	TxUnfl09
53	61b7bf53.0	Firooz	ARO	47.23*	45.65	26.34*	35.43*
54	06334433.0	Fortuna	TRJ	45.41*	46.15*	23.71*	20.59*
55	ef8fd965.0	Gerdeh	ADM-JAP	32.82*	34.31*	25.09*	40.01*
56	652530bb.0	Geumobyeo	TEJ	NA	42.50*	22.31*	45.82*
57	71d5fcf4.0	NSF-TV 57	IND	27.83*	33.08*	17.73*	31.56*
58	9c099b78.0	Ghati Kamma Nangarhar	AUS	38.34*	45.70*	23.45*	38.32*
59	a5ec1b10.0	Gogo Lempuk	TRJ	27.02*	34.47*	20.85*	27.26*
60	7f7fb805.0	Gotak Gatik	ADM-JAP	34.78*	34.51*	19.48*	29.31*
61	NA	Guan-Yin-Tsan	IND	25.18	22.07*	14.25	26.08
62	9ec86ff1.0	Gyehwa 3	TEJ	NA	NA	21.18*	32.11*
63	407f31bd.0	Haginomae Mochi	TEJ	NA	NA	28.01*	38.34*
64	a53530c6.0	Heukgyeong	TEJ	NA	NA	30.34*	36.70*
65	1ab838a2.0	Honduras	TRJ	33.72*	39.71*	25.09*	26.56*
66	7691a376.0	Hsia Chioh Keh Tu	IND	NA	NA	17.70*	27.16*
67	39dd7feb.0	Hu Lo Tao	TEJ	45.80*	55.50*	24.27*	41.39*
68	NA	I-Geo-Tze	IND	NA	40.29	16.43	27.20
69	c14c4e03.0	IAC 25	TRJ	32.74*	42.79*	17.32*	38.20*
70	efbb93de.0	Iguape Cateto	TRJ	27.89*	36.21*	21.04*	28.52*
71	61cc3858.0	IR 36	IND	39.15*	38.86*	22.93*	33.58*
72	12d82364.0	IR 8	IND	26.27*	32.56*	18.72*	33.24*
73	97dcf87d.0	IRAT 177	TRJ	29.83*	43.99*	20.78*	23.95*
74	55d3afae.0	IRGA 409	IND	27.26*	32.41*	18.71*	33.83*
75	b0816082.0	Jambu	TRJ	31.21*	51.25*	20.87*	32.38*
76	cb5e38e6.0	Jaya	IND	29.94*	42.25*	17.63*	26.46*
77	45ec7861.0	JC149	IND	42.13*	45.48*	20.03*	36.73*
78	66d97672.0	Jhona 349	AUS	37.18*	49.68*	24.69*	38.92*
79	c0936cf1.0	Jouiku 393G	TEJ	33.25*	54.77*	21.58*	54.48*
80	02d095ba.0	K 65	ADM	32.75*	34.26*	23.34*	29.07*
81	006dfe9b.0	Kalamkati	AUS	37.62*	43.11*	21.98*	42.30*
83	becfe767.0	Kamenoo	TEJ	38.03*	49.90*	26.35*	37.99*
84	7bee5b9f.0	Kaniranga	TRJ	37.58*	39.19*	26.88*	30.76*
85	328163e1.0	Kasalath	AUS	30.92*	37.47*	17.04*	37.64*
86	NA	Kaw Luyoeng	TEJ	NA	NA	22.85	26.94
87	64fa0112.0	Keriting Tingii	ADM-JAP	32.42*	39.10*	19.97*	21.51*
88	NA	Khao Gaew	AUS	37.53	NA	28.18	33.84
89	897c5eef.0	NSF-TV 89	TRJ	41.79*	37.81*	24.46*	28.29*
90	743f41c1.0	Kiang-Chou-Chiu	IND	29.96*	36.10*	16.51*	30.31*
91	ff836253.0	Kibi	TEJ	NA	NA	21.85*	27.60*
92	5df8f871.0	Kinastano	TRJ	34.36*	42.07*	21.45*	31.40*
93	9f1f4614.0	Kitrana 508	ARO	40.57*	68.48*	31.96*	45.86*
94	20a4c97d.0	Koshihikari	TEJ	29.04*	37.16*	18.13*	35.84*
95	0c85e164.0	NSF-TV 95	ADM	26.91*	34.17*	21.88*	34.84*
96	32a6808e.0	KU115	TRJ	23.14*	42.77*	18.88*	24.56*
97	NA	Kun-Min-Tsieh-Hunan	IND	NA	48.17	21.00	34.79
98	6ab77e3e.0	L-202	TRJ	31.59*	37.10*	20.77*	24.41*
99	7eb7c6a8.0	LAC 23	TRJ	31.65*	37.77*	23.64*	22.09*
100	1f10be3d.0	Lacrosse	ADM-JAP	38.79*	67.28*	28.76*	50.97*
101	9f782e9e.0	Lemont	TRJ	NA	39.97*	28.95*	30.00*
102	6e26f4cc.0	Leung Pratew	IND	NA	35.28*	16.29*	32.63*
103	8c76404c.0	Luk Takhar	TEJ	37.17*	35.72*	18.34*	33.88*
104	8629f76c.0	Mansaku	TEJ	31.74*	46.72*	18.39*	51.37*
105	16463092.0	Mehr	AUS	32.43*	30.95*	19.43*	32.67*
106	8a06320f.0	Ming Hui	IND	32.90*	33.82*	18.00*	30.94*
107	7b7a0d82.0	NSF-TV 107	TRJ	25.85*	30.99*	17.50*	23.24*
108	e3b049a9.0	Moroberekan	TRJ	36.50*	52.94*	22.52*	29.28*
110	11bf5114.0	Mudgo	IND	NA	45.59*	10.64*	43.32*
111	NA	N_22	TRJ	43.02	NA	25.26	32.17
112	531e23fa.0	N12	ARO	39.40*	60.85*	29.46*	38.49*
113	7a723d9e.0	Norin 20	TEJ	23.72*	28.37*	15.72*	28.68*
114	eac19fb8.0	Nova	ADM-JAP	37.70*	57.74*	27.41*	38.16*
115	0bca95e0.0	NPE 835	TEJ	NA	47.06*	19.09*	30.97*

Table S1. Continued

NSFTV ID	HDRA ID	Accession name	Subpopulation	ArFl06	ArFl07	TxFl09	TxUnfl09
116	6cedf6aa.0	NSF-TV 116	TRJ	32.26*	48.20*	27.70*	29.59*
117	5b144b9c.0	O-Luen-Cheung	IND	30.72*	39.38*	17.91*	42.61*
118	71bd9426.0	Oro	TEJ	35.99*	45.71*	25.09*	53.19*
119	3aa51818.0	Oryzica Llanos 5	IND	38.15*	50.95*	NA	NA
120	8e6220e5.0	OS6	TRJ	35.25*	41.32*	19.79*	30.76*
121	280279b3.0	Ostiglia	ADM-JAP	34.25*	57.60*	25.39*	42.79*
122	b6dc1bcc.0	Padi Kasalle	TRJ	37.38*	26.22*	14.55*	24.33*
123	714ac141.0	Pagaiyahan	IND	32.36*	30.46*	17.50*	28.42*
124	6c91b63d.0	Pankhari 203	ARO	NA	NA	18.45*	25.10*
125	63f298ba.0	Pao-Tou-Hung	IND	29.96*	33.44*	13.97*	33.56*
126	0f6a67da.0	Pappaku	IND	NA	40.21*	21.54*	33.14*
127	b403e79f.0	NSFTV127	TRJ	NA	40.71*	20.27*	25.91*
128	76a1efc9.0	Pato De Gallinazo	ADM-JAP	32.54*	43.56*	24.50*	39.83*
129	8fafd383.0	Peh-Kuh	IND	27.83*	29.92*	15.30*	34.73*
130	a796716d.0	Peh-Kuh-Tsao-Tu	IND	27.18*	31.08*	17.71*	32.82*
131	d09d62e7.0	Phudugey	AUS	35.72*	41.71*	20.81*	46.04*
132	02cc7c6d.0	Rathuwee	IND	41.19*	50.64*	24.41*	47.34*
133	1a95985b.0	Rikuto Kemochi	TEJ	NA	47.66*	20.96*	48.63*
134	4ab486ec.0	Romeo	TEJ	30.22*	39.30*	18.62*	38.89*
135	NA	RT 1031-69	TRJ	38.87	54.43	24.14	32.03
136	d72ee9ba.0	RTS12	IND	NA	NA	24.40*	NA
137	8653bbdb.0	RTS14	IND	32.78*	28.38*	16.89*	29.89*
138	1a946dc6.0	RTS4	IND	NA	26.36*	17.41	30.67*
139	bd0d322b.0	S4542A3-49B-2B12	TRJ	35.54*	43.85*	33.52*	35.89*
140	85551f9c.0	Saturn	TRJ	28.50*	NA	22.47*	21.04*
141	a8319fc6.0	Seratoes Hari	IND	38.99*	58.75*	NA	NA
142	806c51cc.0	Shai-Kuh	IND	38.64	46.36*	19.40*	41.40*
143	3ea144c5.0	Shinriki	TEJ	31.31*	35.80*	21.95*	29.85*
144	3269952e.0	Shoemed	TEJ	NA	NA	22.24*	39.78*
145	17c4070a.0	Short Grain	IND	41.83*	37.09*	17.31*	32.71*
146	NA	Shuang-Chiang	IND	NA	NA	13.46	24.36
147	48b4f132.0	Sinampaga Selection	TRJ	31.31*	40.27*	19.97*	21.47*
148	ed1beb9e.0	Sintane Diofor	IND	NA	NA	14.73*	26.64*
149	d56e98bc.0	Sinaguing	TRJ	38.24*	42.06*	23.04*	18.97*
150	128dd425.0	Sultani	TRJ	33.77*	48.69*	24.35*	44.08*
151	dee26171.0	Suweon	TEJ	32.40*	31.59*	19.26*	28.30*
152	e4533c8b.0	T 1	AUS	34.56*	32.47*	19.84*	25.93*
153	40f343f4.0	T26	AUS	41.53*	34.44*	16.86*	42.41*
154	dfdfb828.0	Ta Hung Ku	TEJ	29.99*	36.16*	20.14*	31.37*
155	9127f236.0	Ta Mao Tsao	TEJ	30.20*	47.55*	18.52*	38.96*
156	93387d96.0	Taichung Native 1	IND	27.25*	32.99*	13.84*	36.08*
157	2db62b89.0	Tainan Iku 487	TEJ	NA	51.58*	23.75*	38.98*
158	6516a500.0	Taipei 309	TEJ	NA	49.78*	24.00*	51.82*
159	4ef132dc.0	Tam Cau 9A	IND	NA	NA	21.96*	31.75*
160	f659d521.0	NSF-TV 160	ARO	42.91*	52.38*	33.12*	41.74*
161	4f643bf5.0	TeQing	IND	25.83*	34.68*	19.77*	33.11*
162	2b28e441.0	TKM6	IND	NA	43.63*	18.02*	37.28*
163	192e24ab.0	Taducan	IND	31.32	37.59*	17.34*	35.57*
164	72497775.0	Tondok	TRJ	43.56*	55.47*	30.48*	39.87*
165	9b2223bb.0	Trembese	TRJ	34.55*	37.49*	22.57*	36.74*
166	61152244.0	Tsipala 421	ADM-IND	26.76*	25.35*	13.22*	30.86*
167	8e941960.0	B6616A4-22-Bk-5-4	TRJ	39.25*	42.21*	22.77*	28.75*
168	NA	Vary Vato 462	ADM	NA	22.99	13.91	21.00
169	ba028784.0	WC 6	TEJ	NA	26.11*	20.07*	28.79*
170	715bc18e.0	Wells	TRJ	NA	37.59*	22.62*	31.46*
171	b238d197.0	ZHE 733	IND	33.25*	40.62*	21.09*	39.90*
172	9ac72c9e.0	Zhenshan 2	IND	39.01*	37.31*	20.21*	44.04*
173	0f847952.0	Nipponbare	TEJ	NA	36.78*	17.87*	29.81*
174	08de34ee.0	Azucena	TRJ	37.66*	56.30*	27.71*	30.49*
175	1e91cb13.0	NSF-TV 175	TRJ	35.29*	50.56*	22.93*	25.94*
176	8cfa8fb2.0	583	TRJ	51.02*	38.22*	24.00*	29.20*

Table S1. Continued

NSFTV ID	HDRA ID	Accession name	Subpopulation	ArFl06	ArFl07	TxFl09	TxUnfl09
177	15e6c437.0	68-2	TEJ	42.58*	38.70*	26.13*	61.60*
178	480f7505.0	ARC 6578	AUS	37.57*	43.28*	29.92*	31.64*
179	5505a767.0	Bellardone	TEJ	33.88*	35.63*	19.76*	38.56*
180	f948d7b5.0	Benllok	TEJ	36.44*	39.47*	31.80*	51.77*
181	8cd35626.0	Bergreis	TEJ	54.56*	43.97*	23.94*	38.34*
182	024bbd20.0	Blue Rose Supreme	ADM-JAP	46.27*	46.90*	24.67*	37.96*
183	c08a5ea2.0	Boa Vista	TRJ	43.08*	40.86*	25.15*	25.88*
184	7119ebee.0	Bombon	TEJ	33.68*	45.24*	22.26*	38.07*
185	c97be7ae.0	British Honduras Creole	TRJ	35.31*	43.15*	22.10*	27.64*
186	4559ff3f.0	Bul Zo	TEJ	44.69*	56.08*	25.82*	59.75*
187	60e142c3.0	C57-5043	TRJ	42.15*	50.05*	17.81*	41.33*
188	bf18cbae.0	Coppocina	TRJ	62.74*	53.61*	20.62*	36.33*
189	661eaeaa.0	Criollo La Fria	IND	30.26*	33.53*	16.51*	36.51*
190	bf7afed8.0	Delrex	TRJ	NA	31.52*	23.66*	27.93*
191	97ce6839.0	Dom Zard	ARO	48.75*	41.19*	30.89*	39.16*
192	fdd98970.0	Erythroceros Hokkaido	TEJ	NA	35.82*	19.11*	22.13*
193	5632be21.0	Fossa Av	TRJ	40.28*	51.67*	19.69*	29.09*
195	3c10a4b8.0	IRAT 13	TRJ	31.02*	37.24*	18.07*	23.29*
196	cff871e8.0	JM70	IND	NA	NA	16.20*	34.58*
197	NA	Kaukkyi Ani	ADM	56.50	42.50	21.65	29.15
198	818143d8.0	Leah	TRJ	37.99*	41.90*	20.96*	42.36*
199	958ed737.0	NSF-TV 199	TRJ	33.61*	32.87*	19.39*	24.99*
200	c8a73fe7.0	P 737	AUS	33.25*	37.55*	18.28*	27.87*
201	263f2baf.0	Pate Blanc Mn 1	TRJ	40.84*	51.05*	21.13*	30.25*
202	ccdc6d39.0	Pratao	TRJ	43.72*	35.91*	18.46*	30.69*
203	f46f03bf.0	Radin Ebos 33	IND	26.67*	30.68*	15.27*	35.05*
204	778cea6e.0	Razza 77	TEJ	39.24*	43.80*	23.19*	49.12*
205	348853a5.0	Rinaldo Bersani	TEJ	31.36*	37.68*	18.83*	37.98*
206	37249a74.0	Rojofotsy 738	ADM-IND	27.58*	20.59*	13.84*	21.38*
207	d628ec3a.0	Sigadis	IND	NA	58.39*	20.46*	52.72*
208	e876c9ec.0	SLO 17	IND	42.32*	57.09*	20.71*	40.41*
209	5384be83.0	Tchibanga	IND	32.79*	38.53*	16.53*	34.70*
211	5497b233.0	Tokyo Shino Mochi	ADM-JAP	36.84*	52.50*	30.72*	49.23*
212	330798b4.0	NSF-TV 212	TRJ	32.10*	38.37*	25.90*	32.41*
213	b2320902.0	WC 3397	TRJ	34.55*	45.47*	26.87*	20.39*
214	34df4fca.0	WC 4419	TRJ	41.36*	46.49*	22.10*	36.58*
215	fed1dbae.0	WC 4443	TRJ	32.59*	38.07*	17.57*	29.11*
216	149fb038.0	Yabani Montakhab 7	TEJ	59.02*	45.39*	24.78*	37.64*
217	915c0033.0	YRL-1	ADM-JAP	33.30*	41.31*	20.57*	39.50*
218	85da3a70.0	PI 298967-1	ADM-JAP	36.92*	49.24*	28.90*	NA
219	199f4455.0	Nucleoryza	TEJ	28.53*	34.12*	16.42*	21.41*
220	c5bf98cc.0	Azerbaidjanica	TEJ	34.88*	47.16*	18.79*	35.10*
221	311aaf30.0	Sadri Belyi	ARO	38.07*	37.42*	23.46*	48.28*
222	7cfe4a0c.0	Paraiba Chines Nova	IND	28.19*	25.40*	17.06*	41.11*
223	f73fa9e9.0	Priano Guaira	TRJ	29.29*	37.94*	25.80*	28.39*
224	f2e9bed5.0	Karabaschak	TEJ	33.03*	46.47*	24.39*	38.30*
225	a31b4a06.0	Biser 1	TEJ	36.00*	41.33*	24.55*	45.01*
226	536afc14.0	IRAT 44	TRJ	42.80*	44.91*	21.75*	35.56*
227	a7983b03.0	Riz Local	ADM-IND	41.44	43.75*	18.08*	33.11*
228	195567bf.0	CA 902/B/2/1	AUS	31.93*	30.48*	13.90*	27.20*
229	NA	Niquen	TRJ	24.70	30.61	NA	NA
230	0ede42bd.0	NSF-TV 230	TEJ	38.86*	NA	NA	NA
231	49649a00.0	Hunan Early Dwarf No. 3	IND	23.58*	22.39*	14.07*	42.10*
232	c528bce0.0	Shangyu 394	TEJ	33.02*	36.21*	17.43*	39.43*
233	ca615802.0	Sung Liao 2	TEJ	29.06*	37.16*	22.90*	50.70*
234	32ca1759.0	Aijiaonante	IND	35.72*	30.00*	14.71*	35.37*
235	13ae4f27.0	Sze Guen Zim	IND	30.77*	34.14*	17.89*	29.44*
236	eb17cda6.0	WC 521	ADM-JAP	35.95*	45.13*	23.66*	42.64*
237	dc2cf39b.0	Estrela	ADM-JAP	36.09*	35.09*	14.66*	32.06*
238	9b36ce98.0	WAB 56-104	TRJ	26.34*	43.34*	15.89*	33.95*
239	523d5ec4.0	WAB 502-13-4-1	TRJ	30.93*	47.80*	17.92*	32.33*

Table S1. Continued

NSFTV ID	HDRA ID	Accession name	Subpopulation	ArFl06	ArFl07	TxFl09	TxUnfl09
240	6f263bd1.0	WAB 501-11-5-1	TRJ	35.90*	42.72*	23.05*	33.44*
241	2a7724c1.0	ECIA76-S89-1	IND	32.44*	43.80*	20.68*	44.75*
242	b21f7528.0	27	TRJ	33.14*	44.91*	24.20*	32.66*
243	44aeff79.0	Tropical Rice	TEJ	21.54*	37.59*	14.59*	33.09*
244	5efe8622.0	Arabi	ADM-JAP	31.93*	30.86*	17.44*	32.16*
245	8a41a10a.0	Sab Ini	TEJ	34.33*	43.64*	15.80*	30.03*
246	e0815776.0	Saraya	AUS	34.83*	34.69*	17.54*	37.45*
247	de7ac7bf.0	Desvauxii	TEJ	35.35*	40.05*	19.46*	40.69*
248	9b37187d.0	Caucasica	TEJ	41.68*	41.82*	25.44*	46.07*
249	87621330.0	Pirinae 69	TEJ	38.62*	64.31*	23.60*	52.51*
250	88e1f162.0	Bulgare	TEJ	32.71*	43.16*	15.54*	38.33*
251	f25ad349.0	H256-76-1-1-1	TRJ	35.87*	39.32*	22.63*	42.23*
252	56f5103e.0	Djimoron	IND	25.83*	33.66*	17.25*	34.69*
253	1911c363.0	Guineandao	ADM-JAP	32.56*	56.53*	22.69*	34.43*
254	NA	Hon Chim	IND	29.08	30.62	19.28*	44.65
255	3fb62843.0	Pai Hok Glutinous	IND	NA	27.29*	14.45*	31.15*
256	0be20a08.0	Romanica	TEJ	26.72*	42.63*	17.22*	29.29*
257	cb9c18df.0	Agusita	TEJ	25.17*	27.48*	15.46*	30.56*
258	60c362f1.0	Tia Bura	TRJ	29.54*	31.93*	17.12*	16.35*
259	fe70ec33.0	Sadri Tor Misri	ADM-IND	NA	37.00*	16.78*	24.39*
260	bfa16661.0	NSF-TV 260	ARO	38.42*	30.93*	18.33*	26.48*
261	ceb798a3.0	Shim Balte	AUS	38.81*	35.60*	18.57*	25.65*
262	f30e146b.0	Halwa Gose Red	AUS	39.89*	53.94*	18.19*	33.76*
263	c7d2513e.0	Maratelli	TEJ	42.55*	54.64*	23.33*	53.48*
264	f2f12364.0	Baldo	ADM-JAP	27.18*	39.30*	19.59*	33.86*
265	544f48c0.0	Vialone	ADM-JAP	36.49*	48.90*	22.56*	41.09*
266	f7da2506.0	Hiderisirazu	ADM-JAP	43.27*	44.01*	18.38*	51.77*
267	aceb3352.0	Hatsunishiki	TEJ	30.97*	42.83*	23.57*	37.61*
268	b49ad0db.0	Vavilovi	TEJ	26.37*	35.56*	17.05*	27.94*
269	092f15e7.0	Sundensis	IND	29.05*	43.98*	19.30*	28.62*
270	6815ec6d.0	Osogovka	TEJ	28.41*	34.49*	14.87*	27.89*
271	b67fd5f6.0	M. Blatec	TEJ	37.72*	50.30*	24.01*	50.84*
272	c7301040.0	923	ADM	38.19*	39.16*	18.96*	30.80*
273	493f2b7e.0	Varyla	TRJ	38.88*	57.26*	21.01*	39.91*
274	7dba928e.0	Padi Pagalong	TRJ	41.99*	46.45*	22.24*	30.30*
275	07dac217.0	Sri Malaysia Dua	TEJ	27.24*	35.98*	20.71*	36.34*
276	fc26ce23.0	Kaukau	AUS	38.90*	40.01*	24.08*	28.75*
277	1516d75f.0	Gambiaka Sebela	TEJ	40.96*	50.38*	26.79*	55.87*
278	17627a92.0	C1-6-5-3	ADM	39.96*	27.70*	22.16*	NA
279	642238d9.0	Kon Suito	ADM	21.39*	31.99*	14.64*	26.93*
280	58d5bdc3.0	Saku	TRJ	44.44*	48.33*	28.76*	54.95*
281	85959164.0	Patna	ADM-JAP	41.53*	49.50*	24.21*	38.16*
282	5aa7968d.0	Triomphe Du Maroc	TEJ	37.00*	52.24*	22.66*	47.45*
283	6b37cb3d.0	Chibica	TEJ	41.18*	49.43*	20.59*	49.05*
284	a32e0586.0	IR 44595	IND	34.56*	33.60*	24.53*	38.67*
285	9baa653c.0	Tox 782-20-1	TRJ	31.01*	48.61*	18.36*	27.29*
286	044639de.0	IITA 135	TRJ	34.50*	44.94*	21.49*	33.60*
287	0e787735.0	Zerawchanica Karatalski	TEJ	24.47*	37.14*	17.86*	30.43*
288	bc6b0af5.0	Italica Carolina	TEJ	23.31*	38.33*	14.04*	27.20*
289	7fc911f7.0	Lusitano	TEJ	25.99*	47.22*	21.69*	31.27*
290	d864377c.0	Amposta	TEJ	38.39*	40.62*	23.37*	52.43*
291	b29d3bf4.0	Toploea 70/76	TEJ	32.87*	37.64*	19.62*	41.42*
292	6d9c7d8b.0	Stegaru 65	TEJ	42.85*	57.15*	23.99*	51.95*
293	4c188d7c.0	TOg 7178	ADM-IND	42.35	40.48*	20.36*	29.84*
294	9a60fd42.0	SL 22-613	IND	35.77*	34.09*	20.16*	46.82*
295	e8c621e8.0	Bombilla	TEJ	42.68*	43.70*	28.33*	33.00*
296	2b81cf74.0	Dosel	TEJ	34.86*	43.96*	25.52*	53.92*
297	5ae0bd70.0	Bahia	TEJ	40.22*	57.38*	26.08*	43.95*
298	NA	LD 24	IND	30.19	33.62	17.05	40.10
299	07f246bb.0	SML 242	IND	35.16*	35.06*	16.64*	31.47*
300	16d7e397.0	Sml Kapuri	TEJ	38.56*	44.02*	21.45*	42.60*

Table S1. Continued

NSFTV ID	HDRA ID	Accession name	Subpopulation	ArFl06	ArFl07	TxFl09	TxUnfl09
301	86556239.0	Melanotrix	TEJ	31.42*	NA	13.89*	29.50*
302	234e0df9.0	WIR 3039	TEJ	28.77*	NA	18.35*	22.49*
303	73c4fdfb.0	Kihogo	TEJ	41.19*	47.63*	28.87*	47.68*
304	773e969e.0	519	IND	30.57*	35.32*	14.48*	28.37*
305	8288d278.0	Doble Carolina Rinaldo Barsani	ADM-JAP	31.39*	44.10*	17.94*	40.65*
306	2c583eed.0	WIR 3764	TEJ	29.62*	38.39*	NA	NA
307	c0d6eca3.0	Uzbekskij 2	TEJ	37.41*	59.28*	23.27*	49.82*
308	593def74.0	Llanero 501	TRJ	40.19*	45.73*	20.47*	37.55*
309	a0a3d697.0	Manzano	TRJ	27.23*	41.52*	19.08*	20.50*
310	fbae20bd.0	R 101	TRJ	28.79*	42.70*	19.09*	32.06*
311	137628a5.0	56-122-23	TEJ	34.81*	33.46*	21.96*	51.13*
312	NA	Aswina 330	AUS	36.99	NA	14.79	23.42
313	872ec0b4.0	BR24	IND	25.52*	26.26*	16.12*	32.80*
314	ea28671d.0	CTG 1516	AUS	36.78*	34.04*	21.74*	40.25*
315	74c9fbbc.0	Dawebyan	IND	26.13*	34.84*	13.47*	30.94*
316	b828b757.0	DD 62	AUS	34.32*	37.45*	16.39*	45.74*
317	b956dfb5.0	DJ 123	AUS	29.39*	27.26*	18.60*	34.12*
318	3eb8fab5.0	DJ 24	AUS	34.86*	46.03*	18.53*	33.52*
319	c7aeb39f.0	DK 12	AUS	31.32*	37.38*	20.46*	35.41*
320	cf44b628.0	DM 43	AUS	30.85*	35.29*	20.25*	37.30*
321	1f6f13c3.0	DM 56	AUS	30.90*	34.50*	18.54*	25.56*
322	72cef953.0	DM 59	AUS	36.45*	38.35*	19.79*	45.74*
323	1510e4aa.0	DNJ 140	AUS	55.52*	40.03*	23.67*	31.26*
324	0defa551.0	DV 123	AUS	30.12*	32.44*	17.59*	24.83*
325	d0392fe8.0	EMATA A 16-34	IND	24.40*	27.54*	15.75*	32.02*
326	aeed4684.0	Ghorbhai	AUS	40.26*	41.37*	18.65*	50.50*
327	fb2b3d82.0	Goria	AUS	28.40*	28.71*	14.28*	23.77*
328	1e4f3933.0	Jamir	AUS	27.42*	37.25*	16.82*	34.80*
329	3e58f34c.0	Kachilon	AUS	28.01*	33.96*	12.46*	33.25*
330	084e8b29.0	Khao Pahk Maw	AUS	31.82*	37.70*	16.61*	32.12*
331	d438bfed.0	Khao Tot Long 227	AUS	32.53*	37.87*	15.68*	37.15*
332	7e956f22.0	KPF-16	ADM-IND	24.90*	32.46*	12.80*	33.50*
333	22e60af9.0	Leuang Hawn	TEJ	29.96*	35.82*	17.70*	41.25*
334	09869491.0	Lomello	TEJ	41.06*	39.40*	23.03*	56.24*
335	1e26852c.0	Okshitmayin	ADM-JAP	29.86*	23.18*	14.33*	18.52*
336	fa180a1e.0	Paung Malaung	AUS	35.54*	35.32*	15.80*	41.53*
337	a7bec464.0	Sabharaj	IND	33.10*	42.71*	16.26*	30.48*
338	fed0b9a3.0	Sitpwa	TEJ	47.51*	54.73*	24.69*	63.81*
339	b3a301ea.0	Yodanya	IND	32.97*	38.31*	18.34*	28.47*
340	4b0a7350.0	Berenj	ADM	26.69*	32.10*	19.05*	29.69*
341	9f786a86.0	Shirkati	AUS	38.82*	44.69*	25.00*	37.29*
342	ac7a352c.0	Cenit	TRJ	33.12*	36.48*	19.13*	31.89*
343	b663b3f8.0	Victoria F.A.	TEJ	37.37*	47.74*	22.99*	44.54*
344	a0f35768.0	Habiganj Boro 6	ADM	39.94*	38.81*	NA	33.40*
345	4e35b58a.0	DZ 193	AUS	29.30*	27.75*	16.80*	32.83*
346	80fc89ae.0	Karkati 87	AUS	42.72*	44.32*	18.21*	28.30*
347	853e318c.0	Creole	TRJ	34.82*	44.95*	20.57*	30.78*
348	a446becd.0	China 1039	IND	33.85*	38.24*	18.97*	35.02*
349	a5a6e2dd.0	Chang Ch'Sang Hsu Tao	IND	27.55*	36.62*	16.47*	34.43*
350	8adbe877.0	Ligerito	TRJ	36.46*	56.19*	23.34*	30.95*
351	c6d4b592.0	NSF-TV 351	TEJ	39.20*	NA	NA	NA
352	3f0623af.0	Guatemala 1021	TRJ	36.16*	NA	NA	NA
353	af77442b.0	ARC 10376	AUS	33.36*	31.12*	16.91*	34.58*
354	41be3af3.0	BALA	IND	31.15*	32.30*	20.51*	34.18*
355	122a975b.0	ASD 1	TEJ	38.65*	49.82*	31.14*	40.27*
356	f22d2dc6.0	JC 117	IND	33.44*	31.77*	15.69*	29.19*
357	cc0ef8cf.0	9524	AUS	37.75*	NA	15.83*	30.03*
358	0764178c.0	ARC 10086	TRJ	35.34*	56.11*	22.39*	29.53*
359	c86f37d0.0	Surjamkuhi	AUS	31.70*	27.85*	20.27*	40.03*
360	6f068769.0	PTB 30	AUS	33.80*	30.93*	19.06*	30.11*

Table S1. Continued

NSFTV ID	HDRA ID	Accession name	Subpopulation	ArFl06	ArFl07	TxFl09	TxUnfl09
361	NA	F.R. 13A	TEJ	38.33	59.57	29.78	41.36
362	cbba6af5.0	NSF-TV 362	TRJ	33.68*	NA	NA	NA
363	e32c9d62.0	Edomen Scented	TEJ	35.18*	44.06*	19.23*	NA
364	14e43b02.0	Rikuto Norin 21	ADM-JAP	35.96*	46.27*	21.29*	51.49*
365	f2e723fc.0	Shirogane	TEJ	41.91*	60.46*	22.04*	42.33*
366	73b20824.0	Kiuki No. 46	TEJ	31.65*	44.42*	22.64*	50.80*
367	e59cbfbe.0	Sanbyang-Daeme	ADM-JAP	33.55*	43.40*	18.05*	34.37*
368	db737b9b.0	Deokjeokjodo	TEJ	30.63*	35.91*	17.89*	35.74*
369	dd2bfbfb.0	Sathi	AUS	35.81*	39.93*	20.40*	25.10*
370	bd7aaa87.0	Coarse	AUS	33.81*	38.76*	19.67*	31.31*
371	84ad8457.0	Santhi-Sufaid	AUS	28.90*	42.03*	18.42*	31.51*
372	e8f708a5.0	Sufaid	AUS	26.32*	25.68*	16.82*	25.69*
373	c20dfc59.0	Lambayeque 1	ARO	39.65*	36.36*	25.87*	37.55*
374	b37e6755.0	NSF-TV 374	TEJ	35.16*	NA	NA	NA
375	9aa2c28a.0	Upland	TRJ	32.15*	NA	NA	NA
376	de696d95.0	Breviaristata	ADM-JAP	26.22*	37.51*	20.16*	27.77*
377	2779bba9.0	PR 304	TRJ	39.81*	50.00*	24.01*	32.19*
378	eca85a73.0	Kalubala Vee	AUS	24.08*	30.39*	12.82*	23.79*
379	626cd18d.0	Wanica	TRJ	31.59*	40.57*	21.57*	22.94*
380	238f25f1.0	Tainan-Iku No. 512	TEJ	39.37*	47.49*	30.04*	47.05*
381	77f6728e.0	325	TRJ	39.83*	37.12*	32.97*	29.00*
383	3e1268da.0	NSF-TV 383	TEJ	31.69*	NA	NA	NA
384	9764b3c8.0	318	TRJ	32.12*	38.21*	22.50*	22.03*
385	3b25c24f.0	Nira	IND	27.92*	29.53*	14.26*	33.37*
386	4f4f777a.0	Palmyra	TRJ	29.92*	40.04*	20.50*	36.92*
387	a08839ce.0	M-202	ADM-JAP	39.43*	54.55*	26.25*	39.42*
389	7126c359.0	CI 11011	TRJ	NA	NA	19.54*	25.31*
390	5c592759.0	CI 11026	ADM	30.04*	31.56*	18.70*	27.35*
391	180a155f.0	Della	TRJ	33.00*	49.18*	29.42*	29.26*
392	c4a397e5.0	Edith	TRJ	33.71*	35.76*	25.45*	24.58*
394	1f856ac1.0	Lady Wright Seln	TRJ	40.59*	44.00*	23.31*	31.91*
395	ffde60f9.0	OS 6 (WC 10296)	TRJ	33.17*	33.67*	23.49*	20.57*
396	1eb5d579.0	Cocodrie	TRJ	32.11*	NA	25.64*	40.88*
397	45d3c920.0	Cybonnet	TRJ	31.37*	NA	27.16*	42.38*
398	0c8eb926.0	93-11	IND	32.91*	NA	NA	NA
399	12a175c1.0	Spring	TRJ	29.13*	NA	NA	NA
400	61876b53.0	Yang Dao 6	IND	34.43*	NA	NA	NA

Table S2. Cultivars identified as having low or high grain Mn concentration (top or bottom 20%) across at least two of the flooded field experiments. Only cultivars with transcriptomic data from Campbell et al (2020) are shown.

NSFTV ID	Accession name	Grain Mn concentration
5	NSF-TV 5	high
16	Bico Branco	high
36	CS-M3	high
37	Cuba 65	high
57	NSF-TV 57	low
72	IR 8	low
74	IRGA 409	low
83	Kamenoo	high
107	NSF-TV 107	low
122	Padi Kasalle	low
130	Peh-Kuh-Tsao-Tu	low
132	Rathuwee	high
156	Taichung Native 1	low
177	68-2	high
187	C57-5043	high
188	Coppocina	high
201	Pate Blanc Mn 1	high
228	CA 902/B/2/1	low
231	Hunan Early Dwarf No. 3	low
234	Aijiaonante	low
243	Tropical Rice	low
255	Pai Hok Glutinous	low
262	Halwa Gose Red	high
270	Osogovka	low
295	Bombilla	high
303	Kihogo	high
315	Dawebyan	low
329	Kachilon	low
335	Okshitmayin	low
349	Chang Ch’Sang Hsu Tao	low
356	JC 117	low
381	325	high

Table S3. The information of SNP filtering in the Arkansas flooded 2006 experiment. The criteria of SNP filtering are genotype missing >20% and minor allele frequency <0.05. The abbreviation of analysis sets is ALL: all accessions, AUS: *aus*, IND: *indica*, TEJ: *temperate japonica* and TRJ: *tropical japonica*.

SNP dataset	Number	ALL	AUS	IND	TEJ	TRJ
44K	accessions	330	55	56	76	84
	missing >20%	1,344	3,288	3,215	1,136	1,119
	MAF <0.05	2,822	17,629	16,724	28,518	25,215
	remained SNPs	32,735	15,984	16,962	7,247	10,567
	a SNP per kb	11.40	23.35	22.00	51.50	35.32
700K	Accessions	322	53	52	80	88
	missing >20%	9,137	56,735	46,710	23,618	28,960
	MAF <0.05	312,759	407,489	430,441	545,580	517,299
	remained SNPs	378,104	235,776	222,849	130,802	153,741
	a SNP per kb	0.99	1.58	1.67	2.85	2.43
5.2M	Accessions	324	54	52	80	88
	missing >20%	0	0	0	0	0
	MAF <0.05	1,815,120	3,074,054	3,098,722	4,317,423	3,962,973
	remained SNPs	3,416,313	2,157,379	2,132,711	914,010	1,268,460
	a SNP per kb	0.11	0.17	0.18	0.41	0.29

Table S4. The number of significant SNPs detected in the GWA mapping for grain Mn concentration in four field experiments based on a *P*-value of <0.0001 and 5% FDR.

	No. of significant SNPs														
	44K SNP dataset					**700K SNP dataset**					**5.2M SNP dataset**				
Experiment	ALL	AUS	IND	TEJ	TRJ	ALL	AUS	IND	TEJ	TRJ	ALL	AUS	IND	TEJ	TRJ
ArFl06	0	0	0	0	0	6	0	0	0	0	16	0	0	0	1
ArFl07	0	0	0	0	0	0	0	0	6	0	2	0	0	11	0
TxFl09	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
TxUnfl09	0	0	0	0	0	5	0	1	4	0	1	0	0	0	0

Table S5. Information of QTLs with index SNPs at *P*-value <0.0001 for grain Mn concentration in the analysis of all accessions based on the 5.2M SNP dataset using single-trait analysis. Asterisks represent SNPs passing 5% FDR.

Experiment	Index SNP	Chr.	Position	Minor/Major allele	MAF	*P*-value	Effect size	No. of significant SNPs	Position range	Region size	No. of genes
ArFl07	mlid0000019940	1	123,009	A/G	0.14	4.64E-05	0.0438	2	123,009-165,099	42.09	5
ArFl06	mlid0000033090	1	181,426	A/G	0.08	7.96E-05	0.0073	6	174,826-600,441	425.62	61
ArFl07	mlid0000448047	1	2,146,717	T/C	0.43	3.01E-05	-0.0562	158	2,131,720-2,215,765	84.05	5
TxFl09	mlid0000723178	1	3,459,839	C/T	0.17	2.61E-05	0.0153	296	3,436,376-3,730,200	293.82	45
TxFl09	mlid0000878141	1	4,292,449	T/C	0.47	2.68E-05	0.0080	171	4,217,828-4,311,080	93.25	11
ArFl06	mlid0001146690	1	5,664,904	T/C	0.38	3.88E-05	0.0049	57	5,383,347-5,697,586	314.24	35
TxUnfl09	mlid0001328468	1	6,539,781	A/G	0.08	2.33E-05	0.0020	6	6,536,809-6,585,491	48.68	6
ArFl06	mlid0001628295	1	8,045,294	C/T	0.44	8.67E-05	-0.0044	2	8,045,294-8,045,549	0.26	0
TxFl09	mlid0002185886	1	10,656,792	C/T	0.44	8.16E-05	-0.0115	923	10,226,516-11,057,661	831.15	77
TxUnfl09	mlid0002776198	1	13,048,025	T/C	0.22	2.50E-05	-0.0014	3	12,878,686-13,048,025	169.34	11
ArFl07	mlid0003005243	1	13,920,386	T/C	0.38	7.62E-05	-0.0622	277	13,560,157-14,157,770	597.61	63
ArFl07	mlid0005164163	1	22,292,167	A/G	0.18	7.83E-05	0.0591	76	21,945,024-22,435,786	490.76	46
ArFl06	mlid0005661019	1	24,391,177	A/C	0.25	2.90E-06	-0.0062	5	24,391,177-24,729,936	338.76	34
ArFl06	mlid0006248765	1	27,153,440	T/C	0.25	1.99E-05	-0.0070	59	26,834,385-27,560,934	726.55	78
ArFl07	mlid0006337462	1	27,568,714	A/G	0.33	2.64E-05	-0.0619	10	27,568,714-27,814,025	245.31	32
ArFl06	mlid0006457031	1	28,199,195	C/A	0.17	9.83E-05	-0.0066	17	27,775,114-28,607,327	832.21	116
ArFl07	mlid0006934369	1	30,572,995	G/T	0.39	5.57E-06	-0.0528	70	30,208,786-30,591,047	382.26	54
ArFl07	mlid0006970577	1	30,732,639	T/C	0.09	2.06E-05	-0.0579	5	30,651,782-30,732,639	80.86	12
TxUnfl09	mlid0007008512	1	30,919,865	T/C	0.16	3.22E-05	0.0018	6	30,862,865-30,931,025	68.16	10
TxUnfl09	mlid0007413155	1	33,201,894	T/A	0.18	5.73E-05	0.0031	13	32,756,728-33,201,992	445.26	56
TxUnfl09	mlid0007652134	1	34,397,681	T/C	0.21	3.38E-05	0.0014	69	34,362,967-34,630,675	267.71	41
ArFl06	mlid0008175383	1	37,169,154	C/T	0.35	8.29E-05	-0.0116	14	36,931,912-37,605,080	673.17	96
ArFl06	mlid0009325893	2	60,863	G/T	0.12	8.63E-05	-0.0062	4	17,321-335,545	318.22	48
TxFl09	mlid0010026617	2	3,880,164	T/C	0.10	6.93E-05	0.0088	3	3,879,499-3,882,581	3.08	0
ArFl07	mlid0010796973	2	7,451,014	T/C	0.20	7.50E-05	-0.0784	34	7,444,276-7,882,035	437.76	39
ArFl06	mlid0012726450	2	15,300,734	A/G	0.05	5.76E-05	-0.0101	2	15,296,780-15,300,734	3.95	0
ArFl06	mlid0013375366	2	18,105,789	T/C	0.08	3.35E-05	-0.0076	11	18,006,819-18,348,310	341.49	31
ArFl06	mlid0013438666	2	18,382,286	T/C	0.06	6.97E-05	0.0080	3	18,368,364-18,401,417	33.05	4
ArFl06	mlid0014599910	2	23,520,095	G/A	0.27	8.74E-05	-0.0061	24	23,464,601-23,520,095	55.49	8
ArFl07	mlid0015051855	2	25,239,189	T/A	0.48	1.81E-05	0.0420	267	25,145,898-25,271,445	125.55	19
TxFl09	mlid0017089793	3	248,540	T/C	0.21	2.23E-06	-0.0094	3	2,137-248,540	246.40	31
TxUnfl09	mlid0017139000	3	534,810	G/A	0.29	6.83E-06	0.0021	25	182,400-1,013,046	830.65	125
ArFl07	mlid0017141509	3	548,141	T/G	0.08	6.83E-05	-0.0488	9	450,203-896,900	446.70	67

Table S5. Continued

Experiment	Index SNP	Chr.	Position	Minor/Major allele	MAF	*P*-value	Effect size	No. of significant SNPs	Position range	Region size	No. of genes
TxFl09	mlid0017217822	3	1,034,975	T/G	0.31	1.02E-06	0.0089	88	983,312-1,042,969	59.66	11
ArFl06	mlid0017254736	3	1,253,009	T/C	0.24	5.75E-06	-0.0058	162	1,164,504-1,339,597	175.09	7
TxFl09	mlid0017279658	3	1,377,789	C/G	0.18	4.53E-07	-0.0109	131	1,157,226-1,648,969	491.74	65
TxUnfl09	mlid0017369457	3	1,935,699	G/A	0.39	3.01E-06	-0.0017	566	1,436,180-2,420,174	983.99	141
ArFl06	mlid0017391766	3	2,053,421	A/G	0.25	1.64E-05	0.0075	10	1,940,674-2,053,421	112.75	17
ArFl07	mlid0017403189	3	2,139,454	C/G	0.14	9.42E-06	-0.0483	3	1,733,087-2,139,454	406.37	63
TxFl09	mlid0017403189	3	2,139,454	C/G	0.14	8.81E-07	-0.0095	2	1,955,068-2,139,454	184.39	29
TxUnfl09	mlid0017403189	3	2,139,454	C/G	0.14	3.39E-06	-0.0015	2	1,955,068-2,139,454	184.39	29
ArFl07	mlid0017490028	3	2,609,343	A/G	0.24	1.59E-05	-0.0515	25	2,286,634-3,075,111	788.48	111
TxUnfl09	mlid0017524623	3	2,810,078	A/G	0.11	1.54E-06	-0.0017	6	2,564,544-2,962,701	398.16	55
ArFl06	mlid0017538827	3	2,896,387	G/A	0.08	4.57E-05	0.0080	10	2,570,519-3,180,256	609.74	85
ArFl07	mlid0017825959	3	4,494,487	A/C	0.42	7.03E-05	0.1032	30	4,344,754-4,751,396	406.64	60
ArFl06	mlid0018058877*	3	5,804,440	T/C	0.11	1.77E-07	-0.0085	34	5,326,994-6,138,356	811.36	116
ArFl07	mlid0018109250	3	6,112,919	T/C	0.18	1.43E-06	-0.0551	24	5,652,891-6,576,407	923.52	127
TxUnfl09	mlid0018109250	3	6,112,919	T/C	0.18	1.98E-07	-0.0018	24	5,652,891-6,576,407	923.52	127
ArFl06	mlid0018228403*	3	6,819,735	T/C	0.13	1.87E-09	-0.0097	28	6,387,194-7,233,912	846.72	108
TxUnfl09	mlid0018228403	3	6,819,735	T/C	0.12	7.39E-08	-0.0020	27	6,387,194-7,233,912	846.72	108
TxFl09	mlid0018232940	3	6,850,443	T/C	0.12	1.30E-05	-0.0095	16	6,387,194-7,233,912	846.72	108
ArFl07	mlid0018272219	3	7,068,710	C/A	0.41	7.90E-06	0.1309	1707	6,568,754-7,565,173	996.42	121
TxUnfl09	mlid0018352659*	3	7,507,792	T/A	0.12	1.22E-08	-0.0022	6	7,019,729-7,866,465	846.74	118
TxFl09	mlid0018435354	3	7,960,970	A/G	0.18	6.67E-05	0.0078	46	7,950,154-8,220,761	270.61	29
ArFl06	mlid0018435354	3	7,960,970	A/G	0.19	3.41E-05	0.0058	24	7,960,970-8,220,761	259.79	28
ArFl07	mlid0018480795	3	8,188,534	C/T	0.16	1.75E-06	-0.0579	20	7,765,595-8,509,003	743.41	96
TxUnfl09	mlid0018497934	3	8,272,283	G/A	0.48	1.10E-05	0.0017	584	7,964,037-8,714,866	750.83	91
TxUnfl09	mlid0018696761	3	9,280,288	T/C	0.21	1.80E-05	-0.0015	13	8,844,326-9,660,452	816.13	103
TxFl09	mlid0018818593	3	9,944,846	G/A	0.38	9.51E-05	0.0214	62	9,857,809-10,160,493	302.68	41
ArFl06	mlid0018989251	3	10,877,033	A/G	0.14	2.60E-05	-0.0073	29	10,596,192-11,297,028	700.84	99
TxUnfl09	mlid0019037439	3	11,158,867	A/G	0.19	1.12E-05	-0.0015	38	10,729,062-11,645,807	916.75	119
ArFl07	mlid0019119471	3	11,540,252	A/T	0.13	1.49E-05	-0.0508	2	11,540,252-11,540,388	0.14	0
ArFl06	mlid0019213444	3	12,066,585	A/G	0.34	7.05E-05	-0.0052	35	11,987,123-12,070,220	83.10	7
ArFl06	mlid0019247158	3	12,219,211	A/G	0.38	6.50E-05	-0.0044	44	12,190,862-12,307,385	116.52	12
TxUnfl09	mlid0019267880	3	12,338,820	A/G	0.22	8.47E-05	0.0018	22	11,880,797-12,488,679	607.88	74
TxUnfl09	mlid0019364540	3	12,844,485	T/G	0.18	5.19E-05	-0.0013	10	12,697,481-13,033,575	336.09	38
TxUnfl09	mlid0019440785	3	13,213,054	A/G	0.31	5.76E-05	0.0016	191	13,197,939-13,703,865	505.93	35

Table S5. Continued

Experiment	Index SNP	Chr.	Position	Minor/Major allele	MAF	*P*-value	Effect size	No. of significant SNPs	Position range	Region size	No. of genes
TxUnfl09	mlid0019804810	3	14,724,047	A/C	0.15	3.70E-05	-0.0015	27	14,611,170-15,131,932	520.76	63
ArFl07	mlid0019960770	3	15,357,505	A/G	0.06	5.93E-05	0.0758	21	15,143,056-15,380,006	236.95	20
ArFl07	mlid0021659521	3	22,106,879	A/G	0.17	8.63E-05	0.0514	9	21,644,339-22,578,211	933.87	100
TxUnfl09	mlid0024568427	3	36,301,884	T/G	0.08	6.00E-05	0.0018	10	36,174,146-36,393,037	218.89	33
ArFl06	mlid0025149650	4	1,968,617	G/T	0.14	3.79E-05	-0.0070	501	1,479,804-2,452,868	973.06	62
ArFl07	mlid0025707475	4	3,656,689	A/G	0.24	6.34E-05	-0.0623	2	3,656,689-3,656,691	0.00	0
TxFl09	mlid0025717115	4	3,683,138	T/G	0.25	1.44E-05	-0.0077	260	3,566,693-4,150,560	583.87	31
ArFl06	mlid0025744000	4	3,767,588	C/G	0.15	1.75E-05	-0.0070	475	3,546,454-4,267,559	721.11	42
ArFl07	mlid0026000519	4	4,518,590	G/C	0.45	5.55E-05	0.0441	3	4,518,590-4,561,388	42.80	3
ArFl06	mlid0026026457	4	4,589,724	A/G	0.10	2.65E-05	-0.0077	116	4,143,492-5,076,058	932.57	73
ArFl06	mlid0026549601	4	6,411,467	T/A	0.16	8.97E-05	-0.0057	13	6,274,498-6,910,392	635.89	43
TxUnfl09	mlid0028161429	4	12,700,111	G/A	0.41	7.37E-05	0.0023	45	12,345,772-13,075,898	730.13	61
ArFl07	mlid0028172730	4	12,750,568	A/G	0.07	9.52E-05	0.0635	9	12,713,034-13,247,363	534.33	34
ArFl07	mlid0031106832	4	25,869,921	A/C	0.08	5.74E-05	-0.0545	2	25,816,271-25,869,921	53.65	8
TxUnfl09	mlid0031438547	4	27,723,582	T/C	0.16	1.81E-05	-0.0014	4	27,530,771-27,723,582	192.81	25
TxUnfl09	mlid0032139446	4	31,379,472	A/C	0.25	1.73E-05	0.0012	3	31,379,472-31,396,020	16.55	2
TxUnfl09	mlid0032169157	4	31,517,245	G/A	0.21	7.86E-06	0.0013	24	31,436,463-31,518,249	81.79	10
ArFl06	mlid0032239044	4	31,795,359	A/G	0.06	8.39E-05	0.0094	2	31,674,813-31,795,359	120.55	17
ArFl06	mlid0032355393	4	32,352,335	C/T	0.23	5.55E-05	-0.0052	202	32,191,244-32,416,112	224.87	37
ArFl06	mlid0032431814	4	32,762,655	T/A	0.39	3.59E-05	0.0054	301	32,692,323-32,962,075	269.75	37
TxFl09	mlid0032464069	4	32,946,010	G/C	0.05	5.78E-05	0.0121	237	32,701,018-32,961,093	260.08	35
TxFl09	mlid0032576659	4	33,565,328	A/G	0.14	6.71E-05	-0.0095	8	33,562,872-33,651,420	88.55	12
TxFl09	mlid0032599030	4	33,695,231	C/A	0.07	1.40E-05	-0.0131	7	33,685,641-34,190,893	505.25	85
TxUnfl09	mlid0032618309	4	33,821,719	A/G	0.13	6.97E-08	0.0022	58	33,690,561-34,000,755	310.19	48
TxUnfl09	mlid0033054616	5	788,750	T/A	0.06	2.88E-05	-0.0026	110	474,196-1,126,253	652.06	100
TxUnfl09	mlid0033429210	5	2,559,004	A/C	0.05	1.99E-05	-0.0028	4	2,518,640-2,976,477	457.84	64
ArFl06	mlid0034828288	5	8,514,958	T/C	0.17	2.81E-05	0.0064	729	8,252,741-8,861,638	608.90	58
ArFl07	mlid0035784333	5	11,982,703	G/A	0.16	6.69E-05	0.0792	20	11,627,410-12,391,785	764.38	26
TxFl09	mlid0036699269	5	15,505,854	A/C	0.14	4.01E-05	0.0094	2	15,505,854-15,505,866	0.01	0
ArFl06	mlid0036877832	5	16,216,139	T/G	0.19	6.56E-06	0.0078	9	15,788,068-16,321,462	533.39	43
ArFl07	mlid0037053083	5	17,005,018	G/A	0.06	6.47E-05	0.0633	4	16,896,601-17,005,018	108.42	14
ArFl06	mlid0038239792	5	22,311,347	A/G	0.18	9.30E-05	-0.0079	545	22,085,885-22,678,493	592.61	80
ArFl07	mlid0039149707	5	27,022,326	G/A	0.42	7.28E-05	0.0528	99	26,995,511-27,100,109	104.60	10
TxUnfl09	mlid0039187547	5	27,226,215	T/C	0.11	4.64E-06	0.0016	3	26,888,797-27,244,382	355.59	60
TxUnfl09	mlid0039365198	5	28,218,584	C/T	0.18	1.80E-06	-0.0015	29	27,736,768-28,282,420	545.65	85

Table S5. Continued

Experiment	Index SNP	Chr.	Position	Minor/Major allele	MAF	*P*-value	Effect size	No. of significant SNPs	Position range	Region size	No. of genes
ArFl07	mlid0040021997	6	1,262,141	T/C	0.19	5.16E-05	-0.0544	14	1,223,768-1,269,796	46.03	1
ArFl07	mlid0041121469	6	6,621,721	G/A	0.24	7.18E-05	-0.0650	269	6,482,121-6,681,234	199.11	22
ArFl07	mlid0041502434	6	8,335,458	T/C	0.06	4.21E-05	-0.0673	5	8,334,407-8,828,318	493.91	34
ArFl06	mlid0041742621	6	9,306,770	T/C	0.23	1.32E-05	-0.0067	136	9,262,477-9,649,409	386.93	28
ArFl07	mlid0045073481	6	22,175,896	G/A	0.49	8.18E-05	0.0509	33	22,169,258-22,244,613	75.36	7
TxUnfl09	mlid0045399949	6	23,470,209	G/T	0.48	2.62E-05	0.0015	516	23,242,450-23,610,281	367.83	46
TxFl09	mlid0045461862	6	23,781,926	A/G	0.33	2.27E-05	0.0074	2	23,781,926-23,798,692	16.77	1
TxFl09	mlid0046349982	6	27,986,699	T/C	0.06	6.10E-05	0.0118	21	27,955,019-28,388,068	433.05	50
TxFl09	mlid0047302856	7	1,786,904	G/A	0.11	5.72E-05	-0.0097	46	1,712,575-2,122,805	410.23	60
ArFl07	mlid0047344943	7	1,976,642	T/G	0.08	2.37E-05	-0.0653	21	1,551,405-2,248,771	697.37	111
TxUnfl09	mlid0047349435	7	1,996,390	T/C	0.14	6.20E-07	-0.0018	33	1,712,575-2,122,805	410.23	60
ArFl06	mlid0047878419	7	4,649,020	T/C	0.09	7.36E-06	0.0083	265	4,525,393-4,769,910	244.52	29
TxUnfl09	mlid0048280334	7	6,467,214	T/C	0.07	2.15E-05	0.0026	241	6,396,326-6,655,214	258.89	15
ArFl06	mlid0048394990	7	6,943,451	C/T	0.38	2.18E-05	0.0105	7	6,904,525-6,945,546	41.02	3
TxUnfl09	mlid0048395730	7	6,947,990	A/G	0.18	1.87E-05	-0.0014	14	6,943,808-7,432,428	488.62	50
ArFl07	mlid0048417532	7	7,039,315	T/G	0.09	3.04E-05	0.0609	10	6,896,309-7,188,320	292.01	27
ArFl07	mlid0048560313*	7	7,594,101	A/G	0.14	1.80E-08	-0.0642	62	7,206,339-8,057,921	851.58	95
ArFl07	mlid0048735332*	7	8,256,487	A/C	0.15	1.44E-08	-0.0646	43	7,780,036-8,568,541	788.51	85
ArFl07	mlid0048878287	7	8,781,883	T/C	0.13	8.11E-07	-0.0584	19	8,286,791-8,966,501	679.71	50
TxUnfl09	mlid0048911414	7	8,918,567	A/G	0.17	1.35E-06	-0.0016	26	8,426,282-9,415,605	989.32	70
TxUnfl09	mlid0049262184	7	10,000,611	T/C	0.11	7.50E-05	0.0020	120	9,580,684-10,479,183	898.50	51
TxFl09	mlid0049468238	7	10,792,084	C/A	0.08	7.79E-05	0.0129	48	10,468,770-11,137,290	668.52	38
TxUnfl09	mlid0049975685	7	12,511,952	C/A	0.48	9.56E-05	0.0020	45	12,138,139-12,996,207	858.07	49
ArFl06	mlid0050130596	7	13,115,593	A/C	0.41	3.81E-05	-0.0053	12	12,925,876-13,596,994	671.12	54
ArFl07	mlid0050176584	7	13,293,081	T/A	0.47	2.60E-05	-0.0716	827	12,814,477-13,776,495	962.02	78
TxUnfl09	mlid0050192943	7	13,360,325	A/G	0.12	7.44E-05	0.0021	314	13,150,959-13,858,120	707.16	55
ArFl07	mlid0050369562	7	14,074,821	T/A	0.44	8.48E-05	0.0072	252	13,591,238-14,574,700	983.46	40
ArFl06	mlid0050369562	7	14,074,821	T/A	0.46	7.89E-05	0.0545	8	13,596,994-14,465,797	868.80	32
TxFl09	mlid0050861939	7	15,950,972	A/T	0.08	2.96E-05	-0.0100	10	15,879,438-15,983,624	104.19	9
TxFl09	mlid0050917382	7	16,158,488	C/T	0.35	9.01E-06	-0.0136	524	15,997,895-16,612,767	614.87	48
ArFl07	mlid0051111163	7	16,863,655	T/G	0.09	4.78E-05	-0.0556	17	16,388,691-16,909,831	521.14	39
ArFl07	mlid0051123185	7	16,913,539	T/C	0.05	3.39E-05	0.0615	4	16,913,539-17,312,997	399.46	40
ArFl07	mlid0051160215	7	17,076,700	C/T	0.13	6.99E-05	-0.0443	6	17,045,677-17,085,743	40.07	1
TxFl09	mlid0051330148	7	17,774,245	T/C	0.10	4.46E-06	-0.0108	25	17,590,345-18,241,304	650.96	45
TxUnfl09	mlid0051462630	7	18,399,434	T/G	0.26	3.85E-05	-0.0011	104	18,220,434-18,415,839	195.41	20

Table S5. Continued

Experiment	Index SNP	Chr.	Position	Minor/Major allele	MAF	*P*-value	Effect size	No. of significant SNPs	Position range	Region size	No. of genes
TxFl09	mlid0051475438	7	18,458,422	C/T	0.43	7.44E-05	-0.0071	36	18,432,355-18,461,325	28.97	1
TxUnfl09	mlid0051676689	7	19,362,191	T/C	0.09	5.94E-07	-0.0022	12	18,877,554-19,785,070	907.52	91
TxFl09	mlid0051872874	7	20,201,002	G/A	0.27	4.64E-05	-0.0117	102	20,189,963-20,508,341	318.38	29
TxUnfl09	mlid0051919873	7	20,449,244	C/A	0.13	2.75E-06	-0.0018	163	19,983,846-20,943,211	959.37	103
TxUnfl09	mlid0052008601	7	20,822,294	T/C	0.16	4.19E-05	-0.0014	186	20,777,914-21,312,625	534.71	67
ArFl07	mlid0093681574	7	20,837,538	A/G	0.36	4.71E-05	0.0521	2	20,837,538-21,118,140	280.60	31
TxUnfl09	mlid0052223663	7	21,697,917	C/T	0.10	3.87E-07	-0.0021	529	21,216,322-22,080,444	864.12	108
ArFl06	mlid0052705543	7	24,085,380	A/T	0.49	6.80E-05	-0.0048	31	24,085,380-24,494,687	409.31	57
TxFl09	mlid0053724675	7	29,253,689	T/C	0.40	1.05E-05	-0.0072	4	29,252,102-29,256,983	4.88	0
ArFl07	mlid0054540507	8	3,448,579	C/T	0.40	8.96E-05	-0.0407	11	3,448,579-3,466,494	17.92	3
ArFl06	mlid0057441135	8	14,251,515	A/G	0.07	8.26E-05	0.0092	6	14,251,515-14,658,228	406.71	39
TxUnfl09	mlid0057724097	8	15,404,266	A/T	0.30	1.10E-05	0.0017	99	15,069,541-15,569,082	499.54	40
TxUnfl09	mlid0058629740	8	18,633,227	A/G	0.45	4.93E-05	0.0009	6	18,632,924-18,633,798	0.87	0
ArFl06	mlid0058845647	8	19,441,251	T/C	0.08	3.49E-06	0.0073	3	19,396,502-19,441,251	44.75	3
TxUnfl09	mlid0059904850	8	24,476,847	C/G	0.07	3.73E-05	-0.0017	2	24,476,847-24,537,135	60.29	8
TxFl09	mlid0060088398	8	25,306,049	G/A	0.41	7.64E-05	0.0103	8	25,304,471-25,309,350	4.88	0
TxUnfl09	mlid0060116389	8	25,451,883	A/G	0.35	7.32E-06	0.0014	21	25,431,981-25,452,150	20.17	6
TxUnfl09	mlid0060118314	8	25,457,748	A/G	0.17	7.99E-05	0.0013	7	25,452,903-25,469,768	16.87	1
ArFl06	mlid0060823934	9	265,940	G/A	0.41	1.25E-06	0.0096	1737	38,664-763,797	725.13	36
ArFl06	mlid0062326730	9	6,101,631	A/G	0.07	8.46E-06	-0.0090	76	6,096,839-6,230,575	133.74	12
TxFl09	mlid0063419233	9	10,242,506	T/C	0.30	1.41E-05	-0.0084	90	9,937,552-10,381,365	443.81	38
TxUnfl09	mlid0063897346	9	12,059,032	A/G	0.31	9.98E-06	-0.0011	10	12,054,891-12,077,225	22.33	2
ArFl07	mlid0063932979	9	12,201,784	A/G	0.12	8.26E-05	-0.0456	5	12,078,356-12,201,784	123.43	14
TxFl09	mlid0064157659	9	13,128,154	A/G	0.26	8.51E-05	-0.0084	58	12,660,585-13,574,815	914.23	86
ArFl07	mlid0064176730	9	13,220,840	T/C	0.29	7.21E-05	-0.0653	100	12,922,629-13,656,075	733.45	64
ArFl07	mlid0066552620	10	1,433,318	G/A	0.50	8.96E-05	-0.0485	125	1,021,398-1,521,235	499.84	48
ArFl07	mlid0068753817	10	9,135,984	A/G	0.17	7.24E-05	-0.0659	18	9,034,312-9,581,739	547.43	46
ArFl07	mlid0069308421	10	11,270,727	T/C	0.15	4.87E-06	0.0651	297	10,770,822-11,638,851	868.03	119
ArFl06	mlid0069442493	10	11,819,220	A/G	0.15	1.54E-05	0.0061	131	11,784,075-11,860,967	76.89	9
ArFl06	mlid0070818833	10	17,859,436	G/A	0.39	5.77E-05	0.0114	273	17,560,135-18,024,179	464.04	48
ArFl07	mlid0071294637	10	20,222,070	T/C	0.05	6.17E-05	-0.0787	3	19,926,365-20,237,046	310.68	42
TxFl09	mlid0071609317	10	21,796,956	A/G	0.21	8.93E-05	-0.0185	221	21,615,363-22,079,560	464.20	61
ArFl06	mlid0072361199	11	2,698,985	A/G	0.06	6.59E-05	-0.0074	2	2,698,985-3,040,074	341.09	42
ArFl07	mlid0072447960	11	3,174,953	T/C	0.20	9.85E-05	-0.0438	5	3,064,685-3,174,953	110.27	14
ArFl07	mlid0072977556	11	5,610,752	G/A	0.43	5.39E-05	-0.0543	239	5,454,343-5,998,805	544.46	50

Table S5. Continued

Experiment	Index SNP	Chr.	Position	Minor/Major allele	MAF	*P*-value	Effect size	No. of significant SNPs	Position range	Region size	No. of genes
TxUnfl09	mlid0073114890	11	6,165,503	T/A	0.39	6.25E-05	-0.0013	1188	6,032,253-6,511,266	479.01	55
TxFl09	mlid0073321131	11	6,882,891	G/C	0.17	2.99E-05	0.0112	17	6,515,329-7,198,896	683.57	58
ArFl07	mlid0073516248	11	7,720,491	G/A	0.25	8.93E-05	-0.0648	390	7,542,683-8,000,900	458.22	45
TxUnfl09	mlid0073588741	11	8,042,077	G/A	0.06	2.39E-05	-0.0022	25	8,041,460-8,412,015	370.56	36
ArFl07	mlid0073894380	11	9,187,040	C/T	0.23	5.76E-05	-0.0884	371	8,921,323-9,423,133	501.81	49
ArFl07	mlid0076536806	11	18,849,758	C/T	0.41	7.63E-05	-0.0704	135	18,424,490-18,959,363	534.87	45
TxFl09	mlid0076988314	11	20,676,859	A/T	0.10	1.02E-05	-0.0116	620	20,359,638-20,702,309	342.67	41
TxUnfl09	mlid0077484051	11	22,425,949	G/A	0.36	5.36E-05	-0.0011	148	22,318,664-22,428,638	109.97	8
ArFl06	mlid0077498371	11	22,473,981	C/T	0.35	1.12E-05	0.0057	104	22,287,380-22,921,862	634.48	74
TxFl09	mlid0077561546	11	22,736,597	A/G	0.18	1.03E-06	0.0104	243	22,239,670-22,958,836	719.17	81
ArFl06	mlid0078153893	11	25,033,501	A/G	0.05	6.34E-05	-0.0096	76	24,975,860-25,135,667	159.81	8
ArFl06	mlid0078353755	11	25,818,971	C/T	0.05	5.59E-06	-0.0095	9	25,606,178-25,849,741	243.56	26
ArFl06	mlid0078388730	11	25,947,679	T/A	0.22	7.09E-05	-0.0053	25	25,897,420-26,030,224	132.80	5
ArFl06	mlid0078489434	11	26,357,777	T/C	0.23	1.20E-05	-0.0057	13	26,098,105-26,531,387	433.28	44
ArFl06	mlid0078771642	11	27,391,131	A/G	0.06	7.94E-05	-0.0093	54	27,027,670-27,830,326	802.66	81
ArFl07	mlid0078777862	11	27,412,331	T/C	0.06	9.71E-06	0.0590	4	27,382,797-27,412,942	30.15	4
TxFl09	mlid0079128359	11	28,765,760	T/C	0.26	6.36E-05	-0.0070	150	28,361,729-28,796,600	434.87	49
ArFl06	mlid0079597426	12	2,160,756	G/A	0.35	8.83E-05	0.0146	28	1,723,629-2,559,097	835.47	114
TxUnfl09	mlid0079901644	12	3,613,472	T/C	0.24	6.93E-05	0.0013	12	3,612,538-3,638,198	25.66	4
ArFl06	mlid0079970698	12	3,923,305	A/C	0.08	8.39E-05	-0.0073	11	3,844,627-4,190,069	345.44	48
ArFl06	mlid0080262794	12	5,098,184	A/G	0.19	3.64E-05	-0.0061	27	4,634,609-5,330,735	696.13	63
TxUnfl09	mlid0080556281	12	6,229,277	T/G	0.50	9.87E-05	0.0012	1193	5,774,262-6,659,161	884.90	60
ArFl06	mlid0080684017	12	6,740,964	A/C	0.12	2.94E-05	-0.0071	6	6,740,964-7,230,369	489.41	37
ArFl07	mlid0081693964	12	10,264,614	A/G	0.14	1.35E-05	-0.0533	2	10,264,614-10,271,635	7.02	0
ArFl07	mlid0082841259	12	14,152,000	T/C	0.08	6.22E-05	0.0715	6	14,105,006-14,264,032	159.03	8
ArFl06	mlid0083359334	12	15,889,073	C/G	0.08	5.00E-06	0.0084	3	15,829,690-15,889,073	59.38	5
ArFl06	mlid0084408685	12	19,851,795	T/C	0.12	4.36E-05	-0.0068	4	19,670,268-19,851,840	181.57	20
TxUnfl09	mlid0084491921	12	20,174,978	T/C	0.28	5.86E-05	0.0015	9	20,104,393-20,376,884	272.49	12
ArFl06	mlid0084893680	12	22,058,105	C/G	0.08	7.74E-07	0.0099	771	21,997,826-22,453,255	455.43	38
ArFl06	mlid0085270959	12	23,577,179	T/C	0.45	8.93E-07	-0.0067	138	23,495,787-23,662,701	166.91	23
ArFl06	mlid0085583646	12	25,155,287	A/G	0.15	9.82E-05	-0.0058	7	25,121,845-25,207,138	85.29	3
ArFl06	mlid0085642863	12	25,419,976	T/C	0.12	8.39E-06	-0.0073	22	25,258,977-25,816,872	557.90	73
TxUnfl09	mlid0085928877	12	26,805,183	T/A	0.47	8.48E-05	-0.0016	11	26,805,183-27,260,581	455.40	53

Table S6. List of significant SNPs (*P*-value <0.0001 and passing 5% FDR) for grain Mn concentration in the association peak on chromosome 7 in the *temperate japonica* subpopulation in the Arkansas flooded 2007 experiment based on the 5.2M SNP dataset using single trait analysis.

SNP id	**Chr.**	**Position**	**Minor/Major allele**	**MAF**	***P*-value**	**Effect size**
mlid0048683628	7	8,068,469	T/C	0.40	2.04E-07	-0.0773
mlid0048735174	7	8,255,712	A/G	0.32	5.10E-07	-0.0829
mlid0048735332	7	8,256,487	A/C	0.35	1.47E-08	-0.0870
mlid0048736042	7	8,259,474	G/A	0.34	7.07E-08	-0.0849
mlid0048739213	7	8,274,209	A/G	0.32	5.10E-07	-0.0829
mlid0048741969	7	8,286,791	T/C	0.32	5.10E-07	-0.0829
mlid0048744587	7	8,297,556	T/G	0.32	5.10E-07	-0.0829
mlid0048755700	7	8,342,845	G/C	0.32	5.10E-07	-0.0829
mlid0048757951	7	8,351,860	A/G	0.33	2.66E-07	-0.0832
mlid0048760588	7	8,362,721	T/C	0.35	7.79E-08	-0.0843
mlid0048762728	7	8,368,012	G/C	0.32	5.10E-07	-0.0829

Table S7. Information of QTLs with index SNPs at *P*-value <0.0001 for grain Mn concentration in 303 rice accessions based on the 5.2M SNP dataset using multi-experiment analysis. Asterisks represent SNPs passing 5% FDR. Bold font indicates QTLs that were not detected using single-trait analysis based on *P*-value <0.0001, but they were identified using multi-experiment analysis.

Index SNP	Chr.	Position	Minor/Major allele	MAF	*P*-value	ArFl06 effect size	ArFl07 effect size	TxFl09 effect size	No. of significant SNPs	Position range	Region size	No. of genes
mlid0000119132	1	615,232	T/C	0.29	1.45E-05	-0.0015	-0.0554	-0.0008	76	454,506-622,002	167.50	25
mlid0001151802	1	5,690,218	T/G	0.32	6.24E-05	-0.0055	-0.0294	-0.0070	9	5,660,309-5,697,586	37.28	7
mlid0005661019	1	24,391,177	A/C	0.24	7.21E-05	0.0061	0.0293	0.0050	3	24,391,177-24,406,734	15.56	4
mlid0006421683	1	28,000,525	C/A	0.45	5.56E-05	-0.0006	0.0489	0.0021	546	27,749,433-28,282,038	532.61	66
mlid0006934369	1	30,572,995	G/T	0.38	4.30E-05	0.0052	0.0508	0.0016	59	30,497,194-30,591,047	93.85	12
mlid0010026617	2	3,880,164	T/C	0.11	4.70E-06	-0.0035	0.0080	-0.0093	3	3,879,499-3,882,581	3.08	0
mlid0011856950	**2**	**11,863,144**	**A/G**	**0.37**	**1.41E-05**	**0.0051**	**0.0011**	**-0.0013**	**17**	**11,618,987-12,090,182**	**471.20**	**39**
mlid0013232407	**2**	**17,548,954**	**T/C**	**0.11**	**1.32E-05**	**0.0076**	**0.0513**	**0.0030**	**190**	**17,371,671-17,807,609**	**435.94**	**35**
mlid0015205876	**2**	**25,996,854**	**G/T**	**0.30**	**1.14E-06**	**0.0036**	**-0.0394**	**0.0036**	**7**	**25,832,178-26,009,875**	**177.70**	**18**
mlid0015314364	**2**	**26,510,097**	**T/C**	**0.38**	**2.60E-06**	**-0.0022**	**0.1015**	**0.0070**	**1199**	**26,010,310-26,826,269**	**815.96**	**109**
mlid0016839251	**2**	**34,744,900**	**T/C**	**0.38**	**9.60E-05**	**-0.0011**	**-0.0360**	**-0.0014**	**3**	**34,681,316-34,744,900**	**63.58**	**5**
mlid0017089793	3	241,484	A/C	0.18	5.16E-05	-0.0107	-0.0472	-0.0184	2	198,229-248,540	50.31	35
mlid0017087587	**3**	**248,540**	**T/C**	**0.19**	**1.33E-05**	**0.0048**	**0.0256**	**0.0090**	**213**	**217,406-468,920**	**251.51**	**4**
mlid0017254736*	**3**	**1,253,009**	**T/C**	**0.24**	**3.43E-07**	**0.0066**	**0.0180**	**0.0075**	**168**	**1,164,504-1,377,789**	**213.29**	**22**
mlid0017312969	3	1,576,033	T/G	0.18	7.89E-06	0.0040	0.0457	0.0080	10	1,561,296-1,906,322	345.03	52
mlid0017485103	3	2,573,901	A/G	0.23	1.19E-05	0.0052	0.0440	0.0102	23	2,081,528-3,013,586	932.06	130
mlid0018081677	3	5,941,461	A/G	0.13	6.53E-06	0.0075	0.0445	0.0067	31	5,482,084-6,387,194	905.11	128
mlid0018163783*	3	6,451,980	T/C	0.13	2.96E-07	0.0083	0.0512	0.0088	18	5,966,229-6,946,412	980.18	116
mlid0018263668*	3	7,019,729	T/C	0.13	1.63E-08	0.0089	0.0552	0.0112	19	6,633,863-7,507,792	873.93	110
mlid0018475760	3	8,158,810	T/C	0.12	2.09E-06	0.0070	0.0635	0.0102	19	7,671,825-8,394,062	722.24	97
mlid0018818253	3	9,943,206	G/A	0.38	4.32E-06	-0.0011	-0.1257	-0.0179	266	9,793,467-10,166,279	372.81	50
mlid0020245537	**3**	**16,537,232**	**A/G**	**0.37**	**4.72E-05**	**0.0088**	**0.0005**	**-0.0034**	**5**	**16,537,232-16,978,380**	**441.15**	**39**
mlid0021261713	**3**	**20,475,126**	**T/C**	**0.09**	**7.85E-05**	**0.0067**	**0.0268**	**-0.0002**	**19**	**20,109,360-20,866,631**	**757.27**	**54**
mlid0023316606	**3**	**29,673,238**	**T/C**	**0.13**	**1.75E-05**	**0.0067**	**-0.0159**	**0.0017**	**5**	**29,651,332-29,703,696**	**52.36**	**3**
mlid0025444660*	**4**	**2,891,380**	**T/C**	**0.17**	**1.03E-07**	**0.0043**	**-0.0262**	**0.0085**	**325**	**2,403,945-3,330,179**	**926.23**	**44**
mlid0025744000*	**4**	**3,767,588**	**C/G**	**0.14**	**1.89E-08**	**0.0065**	**-0.0014**	**0.0105**	**479**	**3,411,664-4,267,559**	**855.90**	**48**

Table S7. Continued

Index SNP	Chr.	Position	Minor/Major allele	MAF	*P*-value	ArFl06 effect size	ArFl07 effect size	TxFl09 effect size	No. of significant SNPs	Position range	Region size	No. of genes
mlid0025923404	4	4,270,357	A/G	0.17	5.39E-07	0.0048	0.0022	0.0102	332	3,772,040-4,723,318	951.28	66
mlid0026136232	**4**	**4,962,085**	**T/C**	**0.10**	**8.72E-05**	**0.0059**	**0.0070**	**0.0076**	**3**	**4,561,606-4,962,085**	**400.48**	**30**
mlid0026570948	4	6,488,383	T/C	0.13	6.07E-05	0.0059	0.0046	0.0093	9	6,317,929-6,939,295	621.37	43
mlid0032196084	4	31,629,134	T/C	0.27	5.41E-05	-0.0041	-0.0250	-0.0129	35	31,596,359-31,893,463	297.10	44
mlid0032464069	4	32,946,010	G/C	0.06	3.31E-05	-0.0079	-0.0372	-0.0133	241	32,701,044-32,961,659	260.62	35
mlid0032618309	4	33,821,719	A/G	0.10	5.45E-05	-0.0069	-0.0472	-0.0109	17	33,794,465-34,000,755	206.29	32
mlid0034764646	5	8,295,089	G/A	0.21	2.64E-05	-0.0078	-0.0295	-0.0033	243	8,252,741-8,767,546	514.81	51
mlid0036877832	5	16,216,139	T/G	0.18	4.87E-05	-0.0079	-0.0350	-0.0066	9	16,153,431-16,321,462	168.03	18
mlid0037757883	**5**	**20,056,165**	**T/G**	**0.12**	**1.08E-05**	**0.0027**	**-0.0449**	**0.0016**	**27**	**20,053,176-20,500,839**	**447.66**	**44**
mlid0041294108	**6**	**7,399,042**	**G/A**	**0.29**	**5.53E-05**	**0.0034**	**0.0318**	**0.0119**	**33**	**7,210,399-7,400,220**	**189.82**	**23**
mlid0041674617	**6**	**9,005,159**	**T/G**	**0.06**	**1.80E-05**	**0.0013**	**-0.0365**	**0.0147**	**31**	**8,981,585-9,371,897**	**390.31**	**38**
mlid0044708996	**6**	**20,737,946**	**C/T**	**0.37**	**1.90E-06**	**-0.0017**	**0.0239**	**0.0070**	**37**	**20,527,784-20,854,368**	**326.58**	**27**
mlid0045245146	**6**	**22,842,399**	**T/C**	**0.21**	**1.69E-05**	**0.0031**	**0.0008**	**0.0087**	**6**	**22,833,028-22,844,930**	**11.90**	**2**
mlid0045375351	**6**	**23,378,178**	**A/G**	**0.06**	**1.42E-05**	**-0.0077**	**0.0138**	**-0.0073**	**11**	**22,962,887-23,465,838**	**502.95**	**57**
mlid0045883156	**6**	**25,746,611**	**G/A**	**0.22**	**8.93E-05**	**0.0030**	**-0.0179**	**-0.0062**	**17**	**25,745,131-26,172,285**	**427.15**	**46**
mlid0046371189	6	28,083,983	T/G	0.19	1.84E-05	-0.0025	-0.0527	-0.0180	110	28,071,820-28,377,309	305.49	36
mlid0046403975	6	28,256,298	A/C	0.30	4.62E-05	0.0018	-0.0016	0.0081	28	28,256,298-28,400,201	143.90	16
mlid0047057872	**7**	**598,763**	**G/A**	**0.43**	**5.73E-05**	**0.0004**	**-0.0356**	**-0.0071**	**25**	**180,763-1,073,310**	**892.55**	**129**
mlid0047344943	7	1,976,642	T/G	0.09	6.70E-05	0.0048	0.0659	0.0040	21	1,551,405-2,248,771	697.37	111
mlid0047878419	7	4,649,020	T/C	0.09	3.05E-05	-0.0079	-0.0437	-0.0111	239	4,591,509-4,769,910	178.40	23
mlid0048486308	7	7,310,792	T/C	0.17	2.37E-06	0.0046	0.0613	0.0030	50	6,887,656-7,785,057	897.40	89
mlid0048735332	7	8,256,487	A/C	0.15	2.98E-06	0.0033	0.0631	0.0050	35	7,785,057-8,541,590	756.53	83
mlid0048878287	7	8,781,883	T/C	0.13	7.60E-05	0.0031	0.0565	0.0050	17	8,286,791-8,966,501	679.71	50
mlid0050369562	7	14,074,821	T/A	0.44	3.19E-05	-0.0071	-0.0582	-0.0099	15	13,596,994-14,513,241	916.25	35
mlid0051330148	7	17,774,245	T/C	0.11	1.71E-06	0.0058	0.0386	0.0130	14	17,590,345-18,168,663	578.32	41
mlid0051931577	7	20,503,283	T/C	0.22	8.67E-05	0.0019	-0.0259	0.0125	117	20,201,002-20,901,965	700.96	71
mlid0052704275	7	24,077,615	C/T	0.38	9.19E-05	0.0068	-0.0015	-0.0017	19	24,059,058-24,536,167	477.11	68
mlid0054110197	8	1,452,574	G/C	0.20	2.29E-05	0.0033	0.0540	-0.0007	85	1,179,366-1,538,633	359.27	35
mlid0054433657	**8**	**2,964,931**	**T/G**	**0.06**	**6.73E-06**	**-0.0079**	**-0.0148**	**-0.0117**	**14**	**2,964,931-3,305,329**	**340.40**	**43**
mlid0056084253	**8**	**9,312,470**	**A/G**	**0.08**	**7.44E-05**	**0.0095**	**-0.0187**	**0.0023**	**160**	**9,286,088-9,803,558**	**517.47**	**46**
mlid0056418718	**8**	**10,539,375**	**G/A**	**0.48**	**6.59E-05**	**0.0031**	**-0.0265**	**-0.0046**	**6**	**10,536,674-10,605,025**	**68.35**	**5**
mlid0058845647	8	19,441,251	T/C	0.08	2.34E-05	-0.0076	-0.0221	-0.0077	3	19,396,502-19,441,251	44.75	3
mlid0060144404	8	25,573,297	G/A	0.29	7.87E-05	-0.0040	-0.0535	0.0008	9	25,304,471-25,573,297	268.83	38
mlid0060879641*	**9**	**504,844**	**A/G**	**0.40**	**6.17E-07**	**-0.0113**	**-0.0397**	**-0.0135**	**2091**	**38,664-1,004,262**	**965.60**	**62**

Table S7. Continued

Index SNP	Chr.	Position	Minor/Major allele	MAF	*P*-value	ArFl06 effect size	ArFl07 effect size	TxFl09 effect size	No. of significant SNPs	Position range	Region size	No. of genes
mlid0061102684	**9**	**1,422,015**	**A/G**	**0.45**	**9.39E-05**	**-0.0082**	**-0.0274**	**-0.0079**	**161**	**935,661-1,920,658**	**985.00**	**78**
mlid0062289801	**9**	**5,946,922**	**T/C**	**0.06**	**7.67E-05**	**0.0082**	**0.0240**	**-0.0001**	**14**	**5,946,449-6,038,822**	**92.37**	**6**
mlid0062331992	9	6,115,870	A/G	0.07	7.03E-05	0.0073	0.0059	-0.0004	78	6,096,839-6,275,947	179.11	20
mlid0062863625	**9**	**8,081,414**	**T/C**	**0.18**	**3.26E-05**	**-0.0009**	**-0.0461**	**-0.0178**	**26**	**8,032,283-8,179,033**	**146.75**	**7**
mlid0063411145	9	10,212,061	A/G	0.26	6.81E-05	0.0024	0.0252	0.0090	70	10,205,318-10,381,365	176.05	16
mlid0064176730	9	13,220,840	T/C	0.29	6.58E-06	-0.0002	0.0657	0.0102	146	12,784,010-13,704,170	920.16	81
mlid0064318980	**9**	**13,755,872**	**T/C**	**0.28**	**4.17E-05**	**-0.0017**	**0.0558**	**0.0077**	**196**	**13,280,793-14,122,080**	**841.29**	**83**
mlid0065478248	**9**	**19,317,384**	**A/C**	**0.08**	**6.75E-05**	**0.0065**	**-0.0036**	**-0.0033**	**9**	**19,235,010-19,423,934**	**188.92**	**29**
mlid0067295435	10	4,051,656	C/T	0.17	8.90E-05	-0.0042	0.0131	-0.0014	7	4,049,902-4,054,829	4.93	0
mlid0068604008	**10**	**8,585,553**	**A/G**	**0.10**	**1.56E-05**	**0.0046**	**-0.0298**	**0.0026**	**3**	**8,585,553-8,600,241**	**14.69**	**1**
mlid0069452339	10	11,855,805	T/C	0.42	1.41E-05	0.0055	0.0266	-0.0003	92	11,690,846-12,019,332	328.49	40
mlid0070499360	**10**	**16,393,038**	**A/C**	**0.29**	**5.82E-05**	**0.0043**	**-0.0338**	**-0.0007**	**559**	**15,897,032-16,614,309**	**717.28**	**57**
mlid0070835331	10	17,942,694	G/C	0.38	1.92E-05	-0.0071	0.0175	-0.0001	382	17,594,989-18,133,647	538.66	51
mlid0071372105	**10**	**20,566,356**	**C/T**	**0.15**	**4.40E-05**	**0.0013**	**0.0299**	**-0.0053**	**2**	**20,559,757-20,566,356**	**6.60**	**1**
mlid0073131281	**11**	**6,224,133**	**A/C**	**0.29**	**4.76E-05**	**0.0034**	**-0.0234**	**0.0027**	**2**	**6,038,550-6,224,133**	**185.58**	**19**
mlid0073208579	11	6,521,180	T/G	0.05	3.06E-05	0.0042	-0.0551	-0.0032	3	6,514,408-6,542,506	28.10	7
mlid0074142720	**11**	**10,110,682**	**T/C**	**0.05**	**7.79E-05**	**-0.0006**	**0.0523**	**0.0126**	**20**	**10,103,980-10,149,355**	**45.38**	**4**
mlid0074403768	**11**	**11,135,039**	**G/C**	**0.38**	**2.55E-05**	**0.0019**	**-0.0768**	**-0.0131**	**508**	**10,666,049-11,634,777**	**968.73**	**67**
mlid0074604290*	**11**	**11,886,926**	**G/A**	**0.39**	**4.80E-07**	**0.0062**	**-0.0320**	**-0.0095**	**615**	**11,391,970-12,295,345**	**903.38**	**40**
mlid0074977833	**11**	**13,193,760**	**T/C**	**0.40**	**1.96E-05**	**0.0053**	**-0.0177**	**-0.0062**	**1302**	**12,702,185-13,693,545**	**991.36**	**39**
mlid0075140599	**11**	**13,761,449**	**G/C**	**0.38**	**4.06E-06**	**0.0051**	**-0.0097**	**-0.0078**	**1407**	**13,262,755-14,259,612**	**996.86**	**72**
mlid0075586567	**11**	**15,370,194**	**A/G**	**0.32**	**3.09E-05**	**-0.0001**	**-0.0429**	**-0.0118**	**1087**	**14,878,752-15,863,313**	**984.56**	**75**
mlid0077375864	**11**	**22,025,542**	**T/C**	**0.28**	**5.48E-06**	**-0.0017**	**-0.0244**	**0.0053**	**26**	**21,638,079-22,245,086**	**607.01**	**74**
mlid0077561546	11	22,736,597	A/G	0.20	1.36E-05	-0.0040	-0.0264	-0.0104	232	22,667,520-22,923,569	256.05	23
mlid0077694518	11	23,256,507	G/A	0.08	1.03E-05	0.0059	0.0284	0.0124	33	22,799,122-23,332,367	533.25	50
mlid0078166555	11	25,085,885	C/T	0.07	1.26E-05	0.0090	0.0370	0.0007	131	24,962,490-25,135,667	173.18	9
mlid0078268426	11	25,502,331	A/G	0.09	4.42E-05	0.0043	0.0151	-0.0050	535	25,433,099-25,523,426	90.33	14
mlid0078299980*	**11**	**25,621,708**	**A/C**	**0.05**	**2.24E-07**	**0.0094**	**0.0340**	**-0.0026**	**1**	**25,619,048-25,621,708**	**2.76**	**0**
mlid0078353755	11	25,818,971	C/T	0.05	6.95E-05	0.0091	0.0487	0.0041	9	25,606,178-25,849,741	243.56	26
mlid0078489434	11	26,357,777	T/C	0.23	7.73E-05	0.0056	0.0118	0.0017	4	26,306,042-26,531,387	225.35	25
mlid0078615844	**11**	**26,864,275**	**A/G**	**0.06**	**1.44E-05**	**0.0080**	**0.0458**	**-0.0001**	**6**	**26,835,209-26,864,275**	**29.07**	**0**
mlid0079958592	12	3,865,724	A/G	0.08	5.79E-05	0.0077	0.0498	0.0107	12	3,844,627-4,175,567	330.94	45
mlid0080184534	12	4,789,369	A/G	0.07	1.37E-05	-0.0002	-0.0425	0.0070	648	4,641,827-5,074,311	432.48	48
mlid0083359334	12	15,889,073	C/G	0.09	2.41E-05	-0.0082	-0.0531	-0.0063	3	15,829,690-15,889,073	59.38	5
mlid0084609216	**12**	**20,857,807**	**T/G**	**0.31**	**4.30E-05**	**0.0039**	**-0.0241**	**0.0003**	**978**	**20,365,464-21,100,322**	**734.86**	**75**

Table S7. Continued

Index SNP	Chr.	Position	Minor/Major allele	MAF	*P*-value	ArFl06 effect size	ArFl07 effect size	TxFl09 effect size	No. of significant SNPs	Position range	Region size	No. of genes
mlid0084924877	12	22,172,677	C/T	0.07	1.37E-06	-0.0103	-0.0195	-0.0104	726	21,844,726-22,664,758	820.03	73
mlid0085427253	**12**	**24,343,696**	**T/C**	**0.21**	**1.17E-05**	**0.0061**	**0.0091**	**0.0092**	**8**	**24,108,337-24,537,021**	**428.68**	**41**
mlid0085463456	**12**	**24,527,475**	**A/T**	**0.37**	**1.02E-06**	**-0.0036**	**0.0404**	**-0.0047**	**47**	**24,465,323-24,605,323**	**140.00**	**9**
mlid0085642863	12	25,419,976	T/C	0.11	5.60E-05	0.0070	0.0269	0.0092	9	25,258,977-25,816,872	557.90	73
mlid0085791065	**12**	**26,157,133**	**A/G**	**0.06**	**5.25E-05**	**0.0043**	**0.0250**	**-0.0063**	**7**	**26,157,133-26,355,570**	**198.44**	**19**

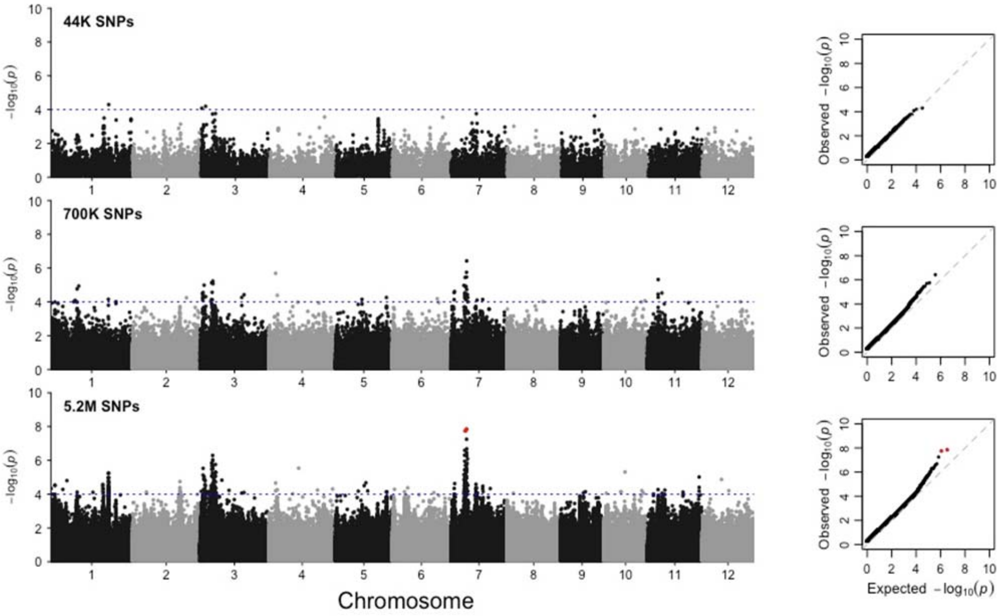

Fig. S1. Genome-wide association mapping results for grain Mn concentration in rice based on the 44K, 700K and 5.2M SNP datasets using single-trait analysis in all accessions grown in Arkansas under flooded condition in 2007. Manhattan (left) and Q-Q (right) plots are presented. The blue horizontal line represents the $-\log_{10}(P)$ threshold at 4. The red dot indicates SNP loci passed 5% FDR.

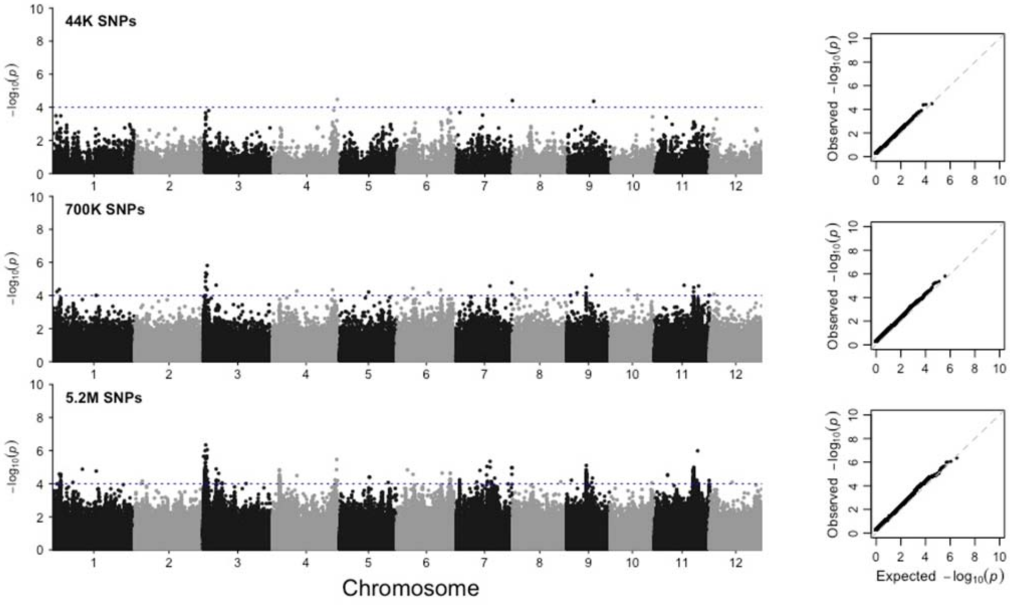

Fig. S2. Genome-wide association mapping results for grain Mn concentration in rice based on the 44K, 700K and 5.2M SNP datasets using single-trait analysis in all accessions grown in Texas under flooded condition in 2009. Manhattan (left) and Q-Q (right) plots are presented. The blue horizontal line represents the $-\log_{10}(P)$ threshold at 4. The red dot indicates SNP loci passed 5% FDR.

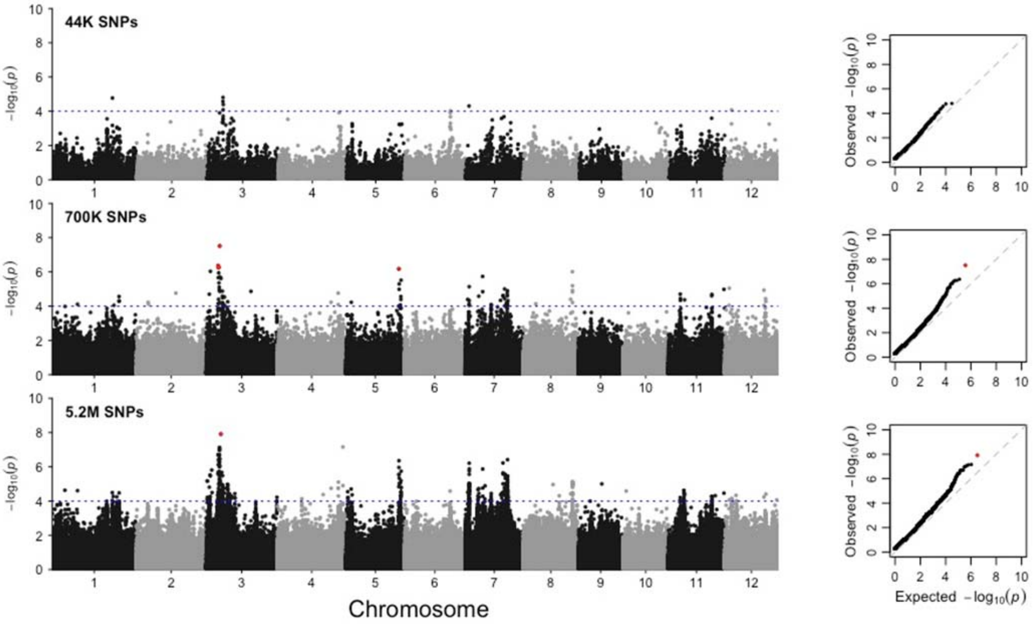

Fig. S3. Genome-wide association mapping results for grain Mn concentration in rice based on the 44K, 700K and 5.2M SNP datasets using single-trait analysis in all accessions grown in Texas under unflooded condition in 2009. Manhattan (left) and Q-Q (right) plots are presented. The blue horizontal line represents the $-\log_{10}(P)$ threshold at 4. The red dot indicates SNP loci passed 5% FDR.

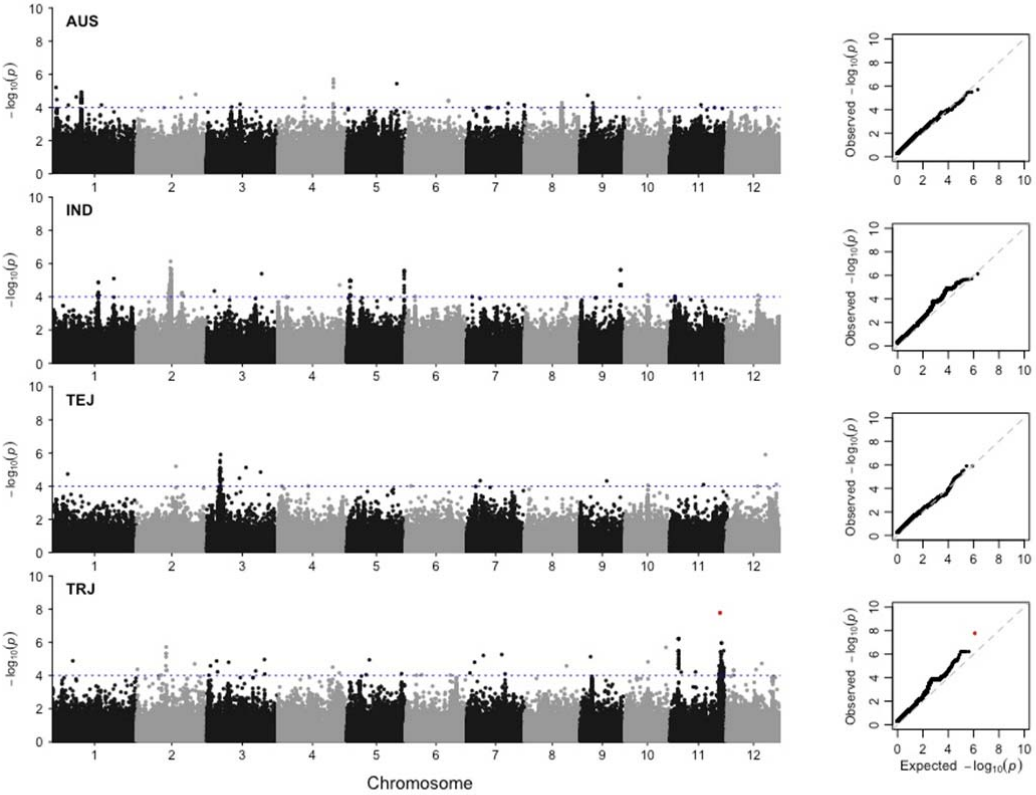

Fig. S4. Genome-wide association mapping results for grain Mn concentration in rice based on the 5.2M SNP dataset in four subpopulations grown in Arkansas under flooded condition in 2006. Manhattan (left) and Q-Q (right) plots are presented. The blue horizontal dashed line represents the $-\log_{10}(P)$ threshold at 4. The red dot indicates SNP loci passed 5% FDR. The abbreviation of subpopulations is AUS: *aus*, IND: *indica*, TEJ: *temperate japonica* and TRJ: *tropical japonica*.

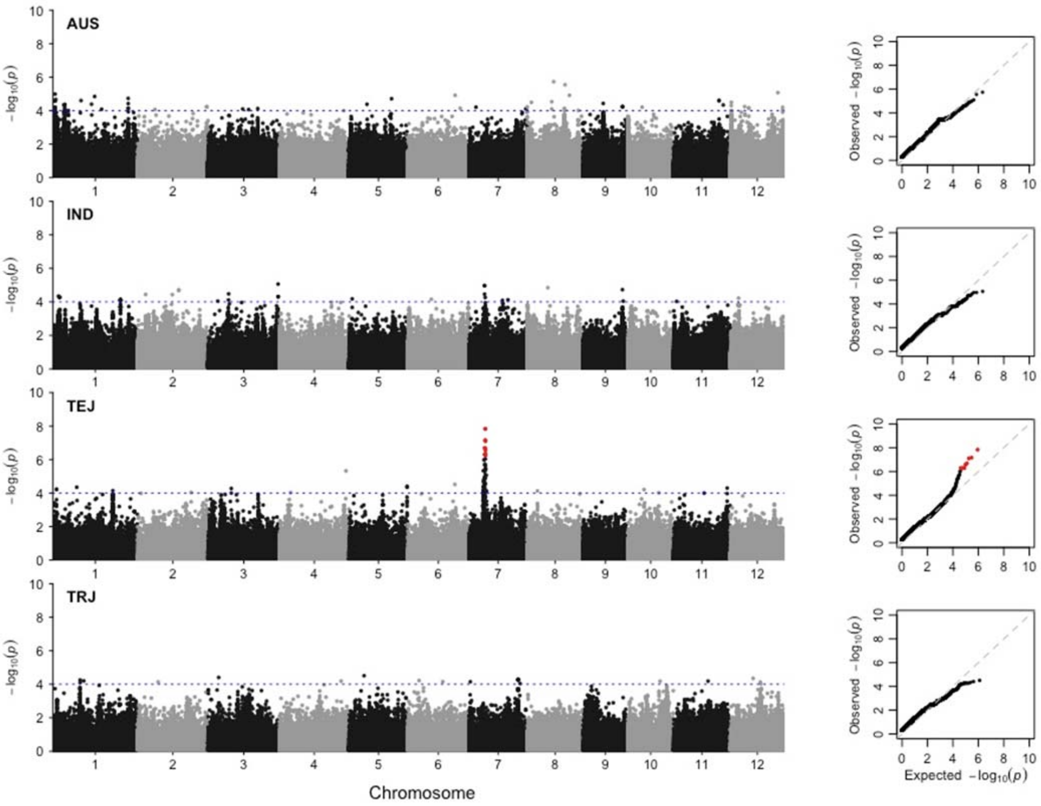

Fig. S5. Genome-wide association mapping results for grain Mn concentration in rice based on the 5.2M SNP dataset in four subpopulations grown in Arkansas under flooded condition in 2007. Manhattan (left) and Q-Q (right) plots are presented. The blue horizontal dashed line represents the $-\log_{10}(P)$ threshold at 4. The red dot indicates SNP loci passed 5% FDR. The abbreviation of subpopulations is AUS: *aus*, IND: *indica*, TEJ: *temperate japonica* and TRJ: *tropical japonica*.

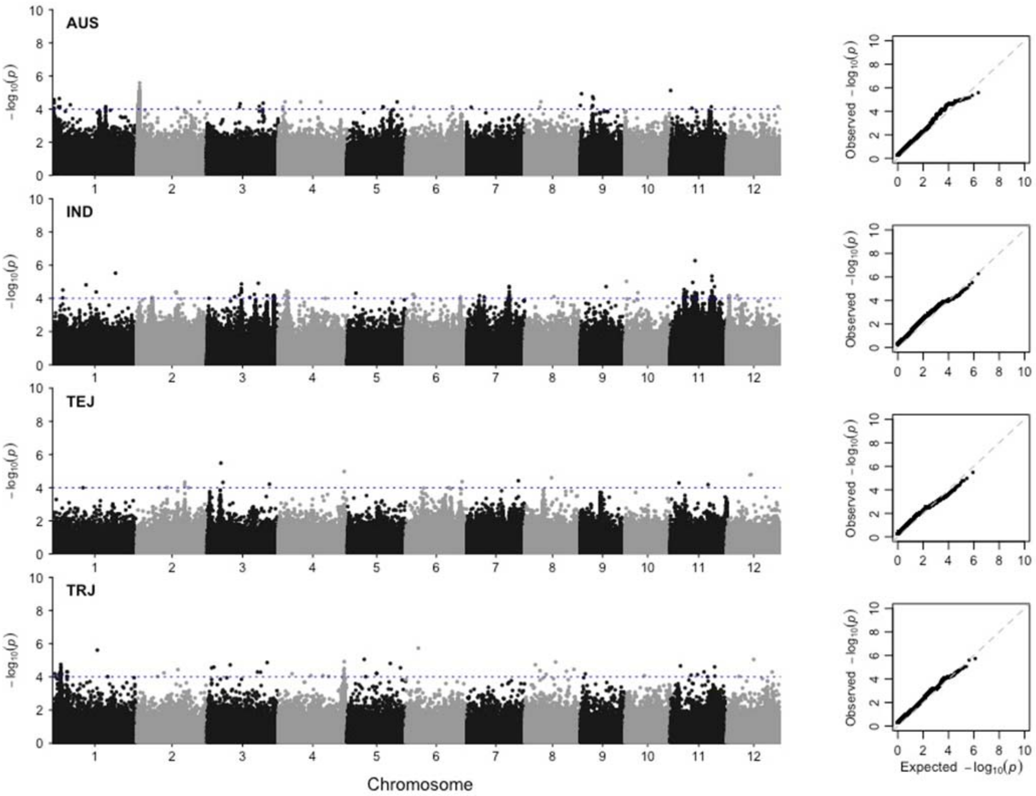

Fig. S6. Genome-wide association mapping results for grain Mn concentration in rice based on the 5.2M SNP dataset in four subpopulations grown in Texas under flooded condition in 2009. Manhattan (left) and Q-Q (right) plots are presented. The blue horizontal dashed line represents the $-\log_{10}(P)$ threshold at 4. The red dot indicates SNP loci passed 5% FDR. The abbreviation of subpopulations is AUS: *aus*, IND: *indica*, TEJ: *temperate japonica* and TRJ: *tropical japonica*.

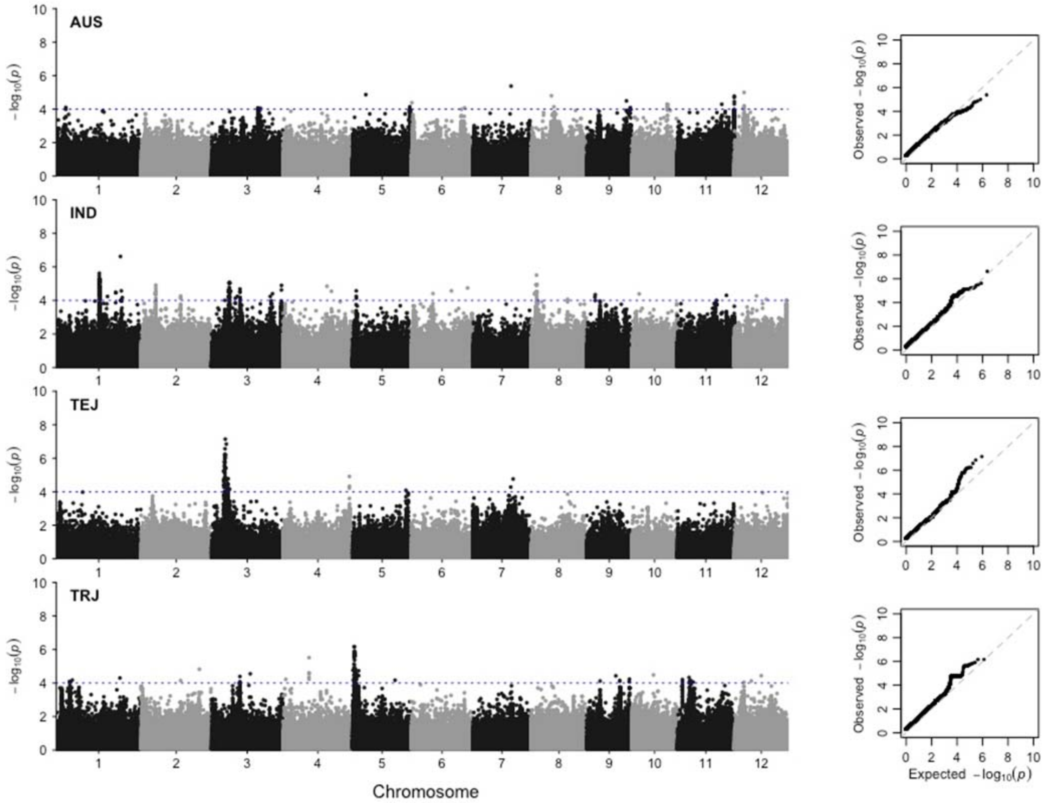

Fig. S7. Genome-wide association mapping results for grain Mn concentration in rice based on the 5.2M SNP dataset in four subpopulations grown in Texas under unflooded condition in 2009. Manhattan (left) and Q-Q (right) plots are presented. The blue horizontal dashed line represents the $-\log_{10}(P)$ threshold at 4. The red dot indicates SNP loci passed 5% FDR. The abbreviation of subpopulations is AUS: *aus*, IND: *indica*, TEJ: *temperate japonica* and TRJ: *tropical japonica*.

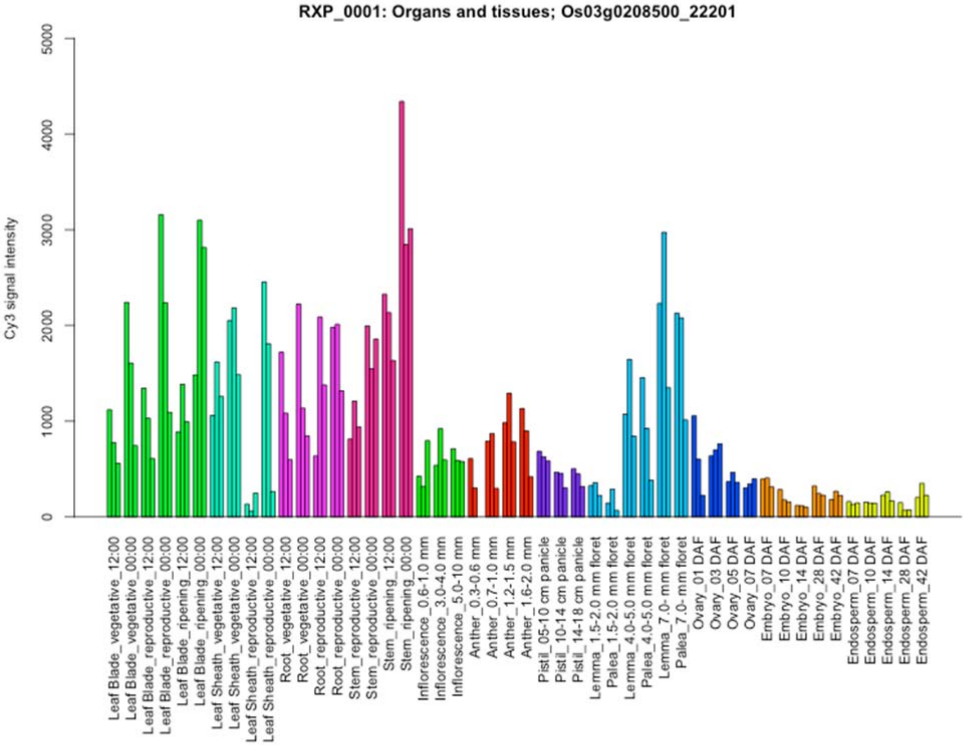

Fig. S8. Pattern of LOC_Os03g11010 (*OsNRAMP2*) expression in each rice tissue from the RiceXPro database with the feature number as 22201.

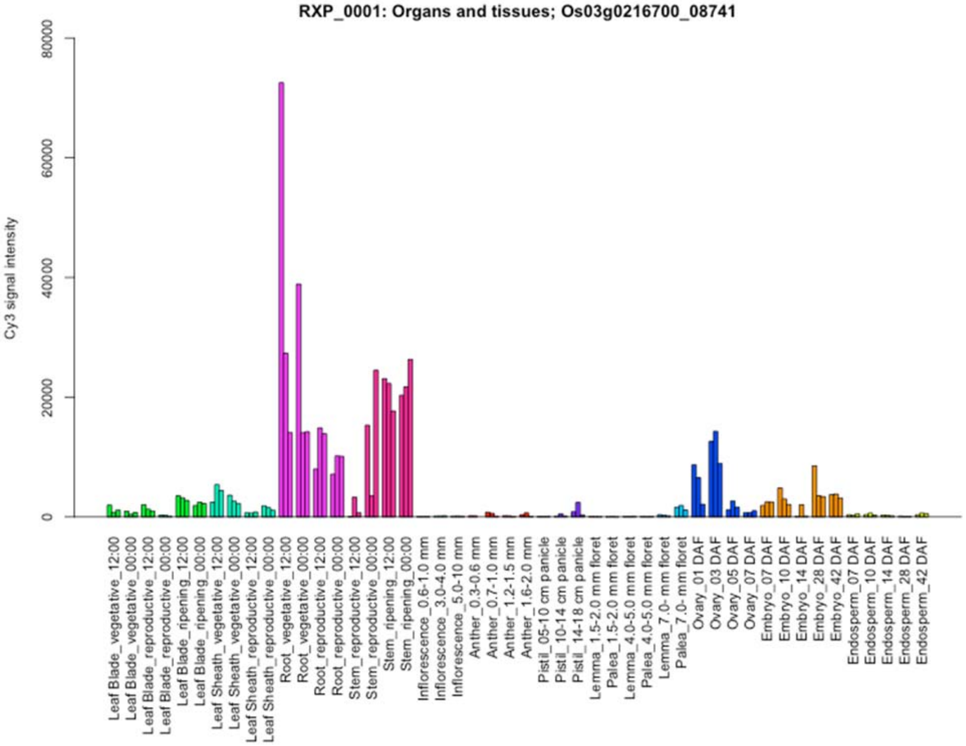

Fig. S9. Pattern of LOC_Os03g11734 (*OsFRDL1*) expression in each rice tissue from the RiceXPro database with the feature number as 08741.

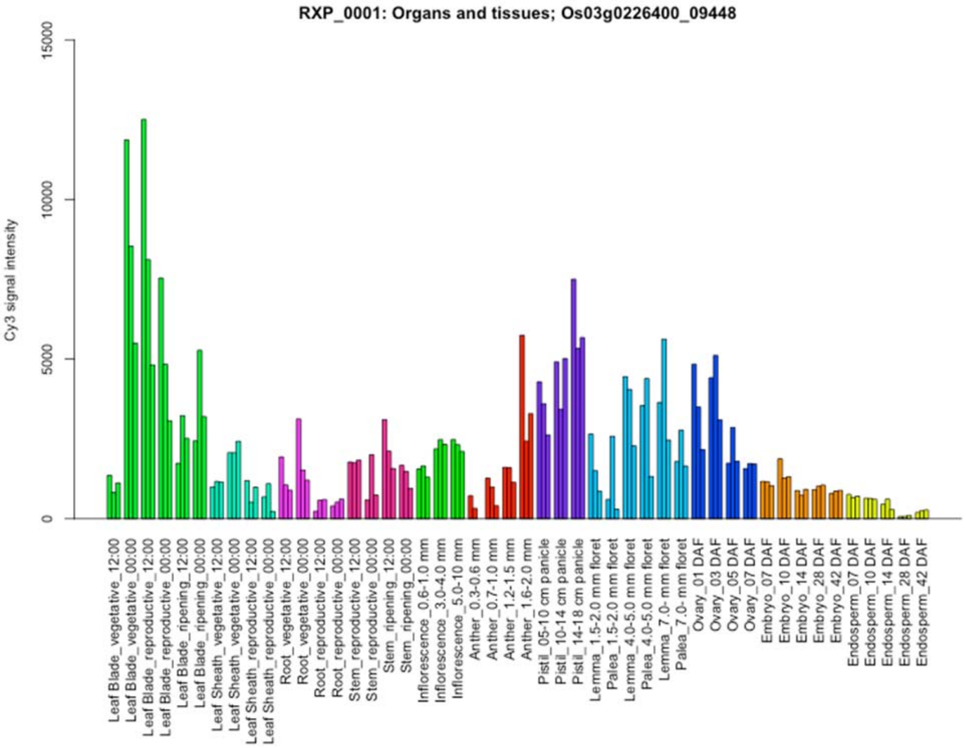

Fig. S10. Pattern of LOC_Os03g12530 (*OsMTP8.1*) expression in each rice tissue from the RiceXPro database with the feature number as 09448.

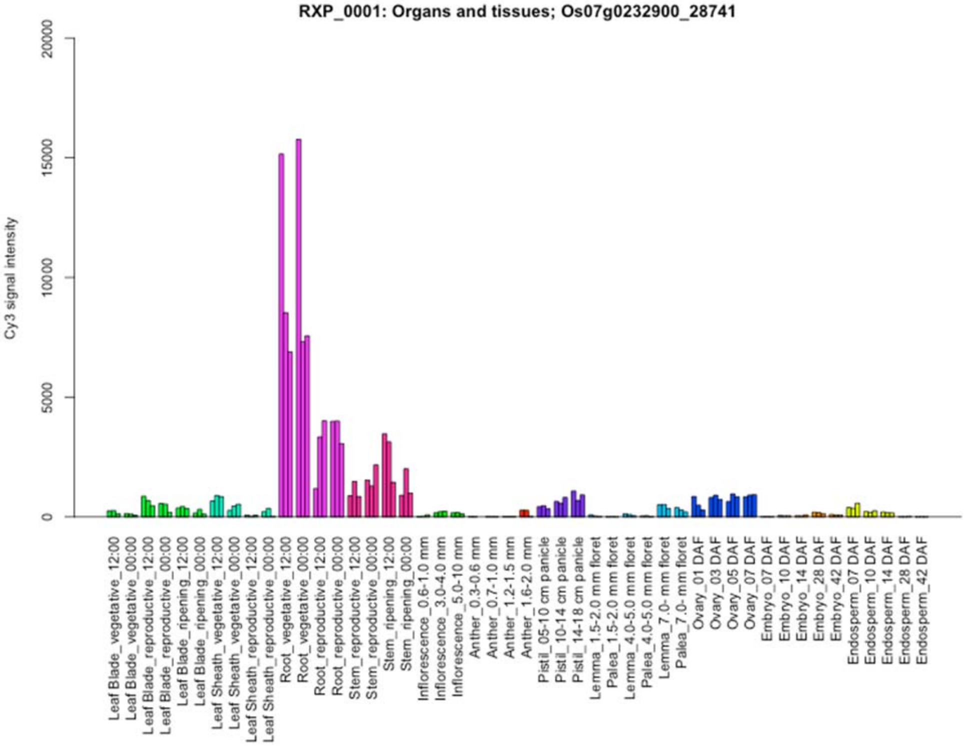

Fig. S11. Pattern of LOC_Os07g12900 (*OsHMA3*) expression in each rice tissue from the RiceXPro database with the feature number as 28741.

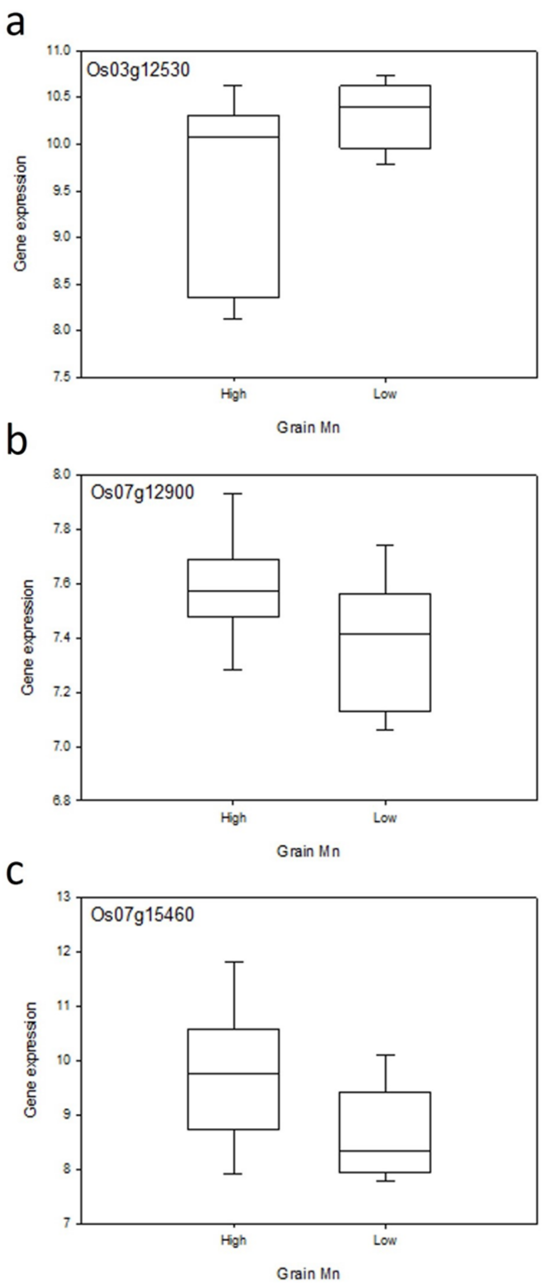

Fig. S12. Gene expression differences between cultivars with high grain Mn concentration and low grain Mn concentration. a) shoot gene expression levels for LOC_Os03g12530 (*OsMTP8.1*), b) shoot gene expression levels for LOC_Os07g12900 (*OsHMA3*) and c) shoot gene expression levels for LOC_Os07g15460 (*OsNRAMP1*). Expression data from Campbell et al., 2020.

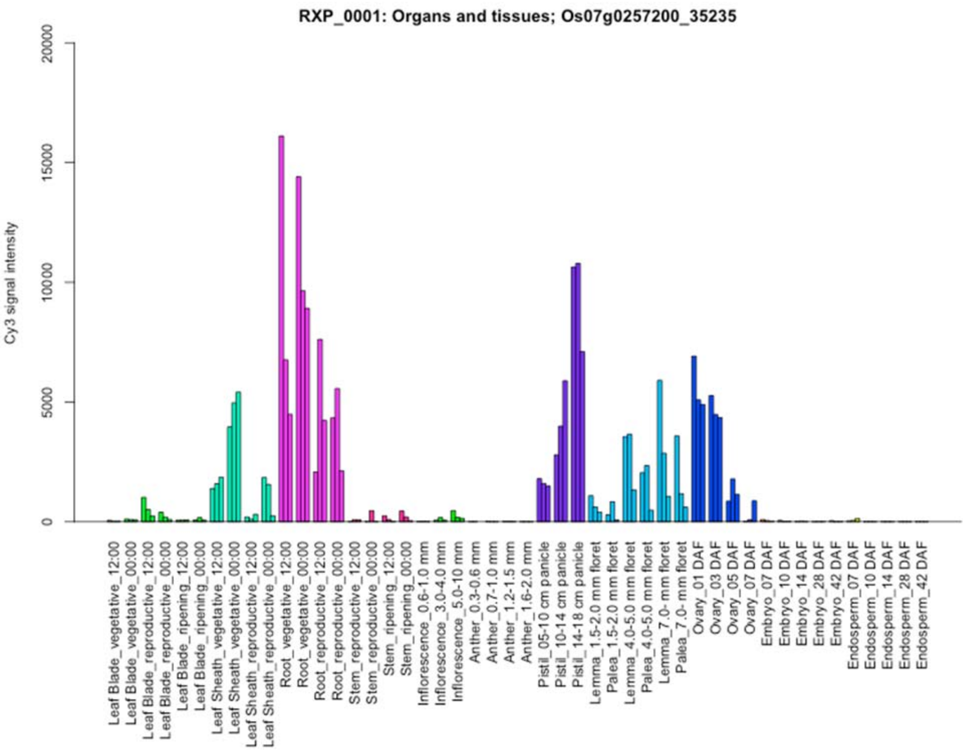

Fig. S13. Pattern of LOC_Os07g15370 (*OsNRAMP5*) expression in each rice tissue from the RiceXPro database with the feature number as 35235.

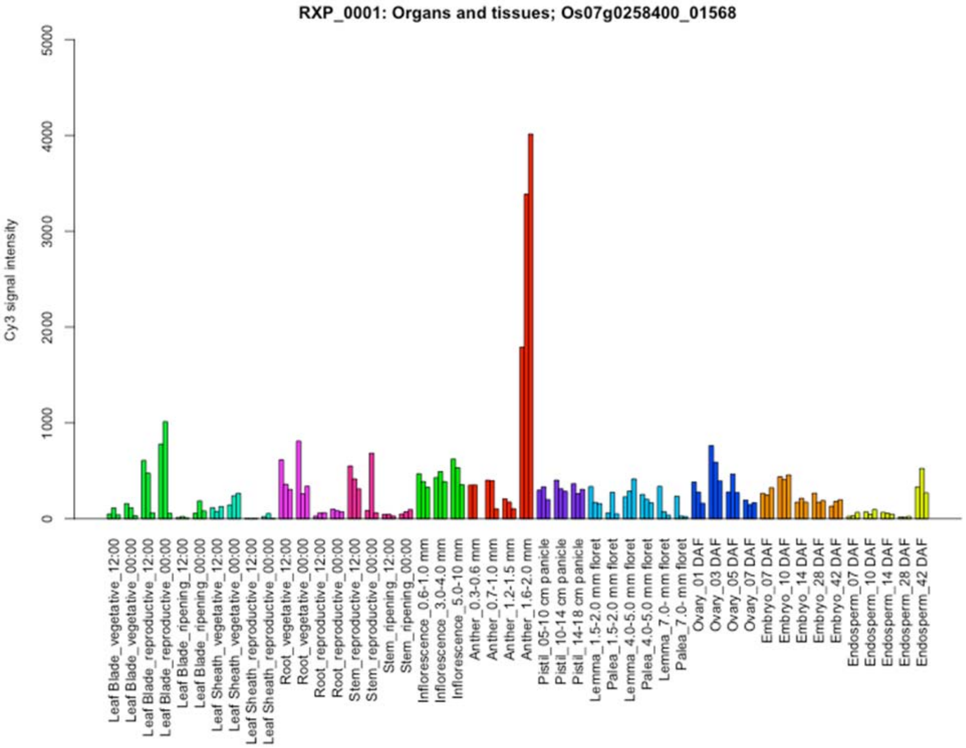

Fig. S14. Pattern of LOC_Os07g15460 (*OsNRAMP1*) expression in each rice tissue from the RiceXPro database with the feature number as 01568.

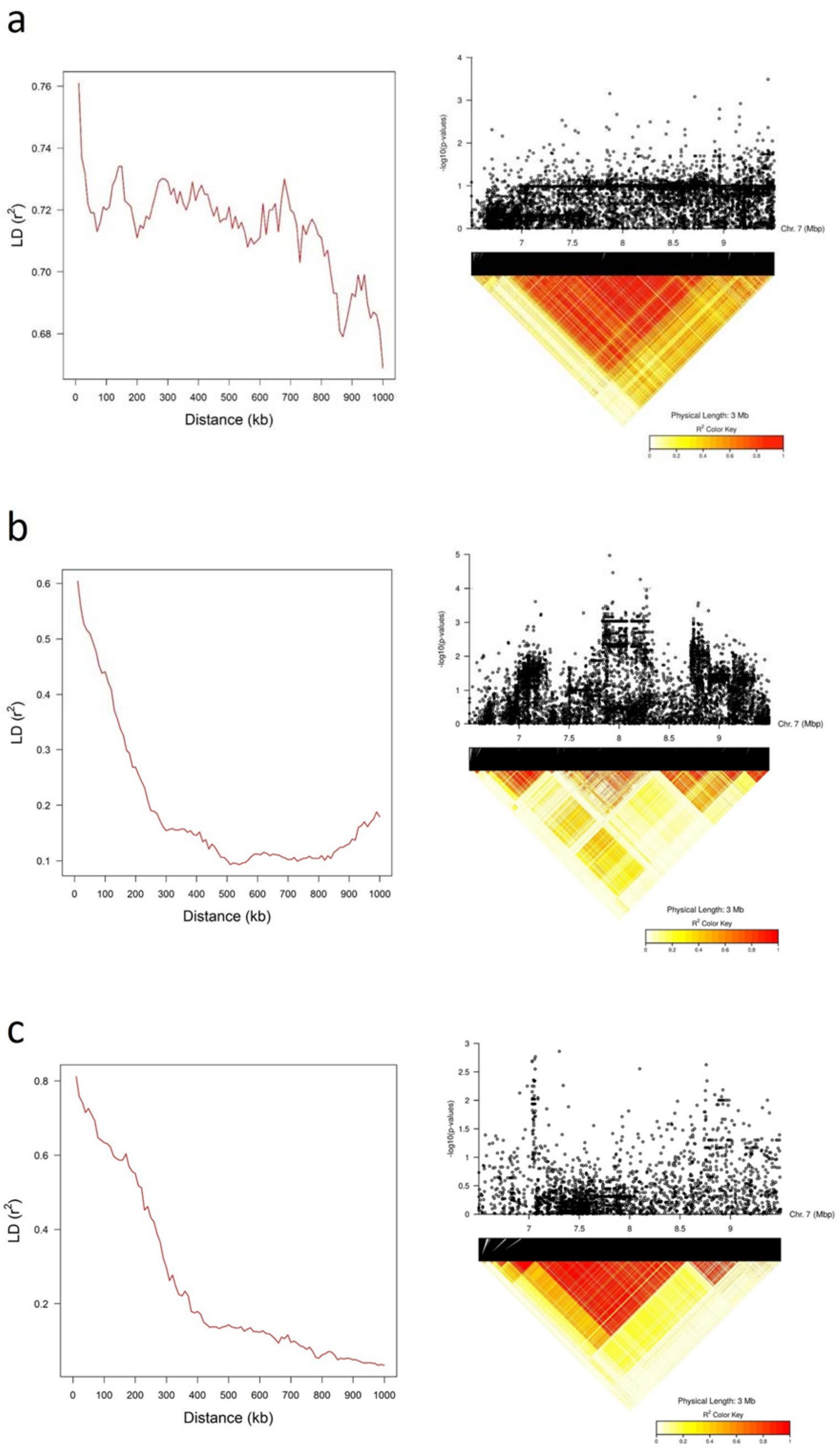

Fig. S15. LD decay (left) and LD heatmap (right) for grain Mn concentration in the Arkansas flooded 2007 experiment in *aus* (a), *indica* (b) and *tropical japonica* (c) subpopulations on chromosome 7 at 7–9 Mbp (left) and 6.5–9.5 Mbp (right), respectively.