Novel Sources of *aus* Rice for Zinc Deficiency Tolerance Identified Through Association Analysis Using High-Density SNP Array

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Abstract: Zinc (Zn) deficiency is a major soil constraint limiting rice crop growth and yield, yet the genetic control of tolerance mechanisms is still poorly understood. Here, we presented promising loci and candidate genes conferring tolerance to Zn deficiency and identified through association analysis using a 365 K single nucleotide polymorphism (SNP) marker array in a diverse *aus* (semi-wild type rice) panel. Tolerant accessions exhibited higher growth rate with relatively rare stress symptoms. Two loci on chromosomes 7 and 9 were strongly associated with plant vigor under Zn deficiency at a peak-stress stage. Based on previous microarray data from the same experimental plots, we highlighted four candidate genes whose expressions were accompanied by significant genotype and/or environment effects under Zn deficiency. Network-gene ontology supported known tolerance mechanisms, such as ascorbic acid pathway, and also suggested the importance of photosynthesis genes to overcome Zn deficiency symptoms.

Key words: *aus*; genome-wide association study; Zinc deficiency; rice

Zinc (Zn) deficiency is one of the most widespread soil constraints in crop and pasture production, especially for high-yielding varieties of cereal crops (Alloway, 2008). It was firstly identified as a growth-limiting factor on calcareous soils of northern India (Yoshida and Tanaka, 1969). Zn deficiency causes stunted growth and leaf bronzing that appears two to three weeks after transplanting, delays maturity, increases plant mortality and reduces grain yield (Neue and Lantin, 1994). Zn is known as a key component in the antioxidant mechanisms, thus lack of Zn in plant results in root solute leakage with membrane peroxidation and leaf bronzing/cell death with photo-oxidation (Cakmak, 2000; Lee et al, 2017). Along with high accumulation of antioxidants such as ascorbate (Frei et al, 2010), tolerant genotypes overcome Zn deficiency through rapid development of new roots and leaves for better Zn uptake and photosynthetic activity, ultimately leading to higher biomass and grain yield (Widodo et al, 2010; Lee et al, 2017). Since the high-cost Zn fertilizer is not affordable to many resource-poor farmers in developing countries, breeding tolerant varieties is the only way to overcome Zn deficiency problems (Quijano-Guerta et al, 2002; Singh et al, 2003). Although various soil and plant studies on Zn deficiency tolerance have been conducted, the genetic aspects of tolerance are still poorly understood. A couple of genetic studies identified quantitative trait loci (QTLs) associated with Zn deficiency tolerance in *indica* rice (Wissuwa et al, 2006; Lee et al, 2017b). The *aus* rice accessions mostly originate from Bangladesh and India and are grown under short-season (from March to August) (Khush, 1997). *aus* is the minor sub-population sharing similar haplotypes with wild relatives, thus is expected to possess novel sources for disease resistance and abiotic stress tolerance (Londo et al, 2006). For example, Famoso et al (2011) reported 46 *a priori* candidate genes that confer high tolerance to aluminum toxicity using *aus* accessions. This study aims to identify loci/candidate genes associated with Zn deficiency tolerance using a diverse *aus* panel.

MATERIALS AND METHODS

Rice materials and experimental design

A panel of 108 *aus* accessions, held in the T.T. Chang Genetic Resources Center at the International Rice Research Institute, the Philippines (Supplemental Table 1), were screened under Zn deficiency in the dry season of 2013. Experimental plots were prepared using concrete tanks (16.0 m × 8.0 m × 0.3 m), containing Zn-deficient soil collected from a Zn-deficient site in Tiaong, Quezon Province, the Philippines. The field has lower available Zn (0.05 mg/kg), higher pH (8.0), and higher organic matter (3.4%). For the control plots, 5 kg/hm^2 of Zn sulfate was broadcast on the soil one day before transplanting. Seeds were germinated in seedling plots in the field (normal nursery), then transplanted after 20 d at 15 cm × 18 cm in the experimental plots.

Phenotyping collection

Seed vigor was scored at the fourth week after transplanting (WAT) following the modified Standard Evaluation System for Rice (SES) with visual scoring from 1 (small plants with severe leaf bronzing symptoms) to 5 (tall with no or very rare leaf bronzing symptoms) (Lee et al, 2017a).

Data analysis

Data were analyzed using analysis of variance (ANOVA), and within each genotype, mean was tested for significant differences using HSD (honestly significant difference) at a 95% probability level using STAR v2.0.1 (International Rice Research Institute). Association between DNA sequence and phenotype was analyzed using the 700 K single nucleotide polymorphism (SNP) marker data set (McCouch et al, 2016). After filtering SNPs with minor allele frequency below 5%, a total of 365 K SNPs were used for the analysis using mixed linear model (MLM) by TASSEL 5.2.7. The significance threshold was set to $1.0 × 10^{-4}$ following McCouch et al (2016) that a significance threshold of 10% false discovery rate was calculated based on the Bonferroni-test.

RESULTS AND DISCUSSION

Zn deficiency negatively affected plant growth as evident from a pronounced increase in stunting, leaf bronzing and high mortality (Fig. 1-A). The normal plant growth with high survival rate in Zn-fertilized plots indicated that Zn deficiency was responsible for the abnormal plant growth in Zn-deficient plot. The difference in zinc availability between Zn-deficient plots (0.05 mg/kg) and the control plots (0.15 mg/kg) was highly significant. Seed vigor scores of the *aus* panel were determined at 4 WAT under Zn deficiency, and data showed normally distribution in the range of 1.7–4.5 (Fig. 1-B). The Manhattan plot using the 365 K SNP markers showed two major peaks above the threshold line on chromosomes 7 and 9 (Fig. 1-C). The -log10 (*P*-value) in the quantile-quantile plot was very close to the expected distribution, which indicated that the peaks detected were unlikely to be false positive peaks
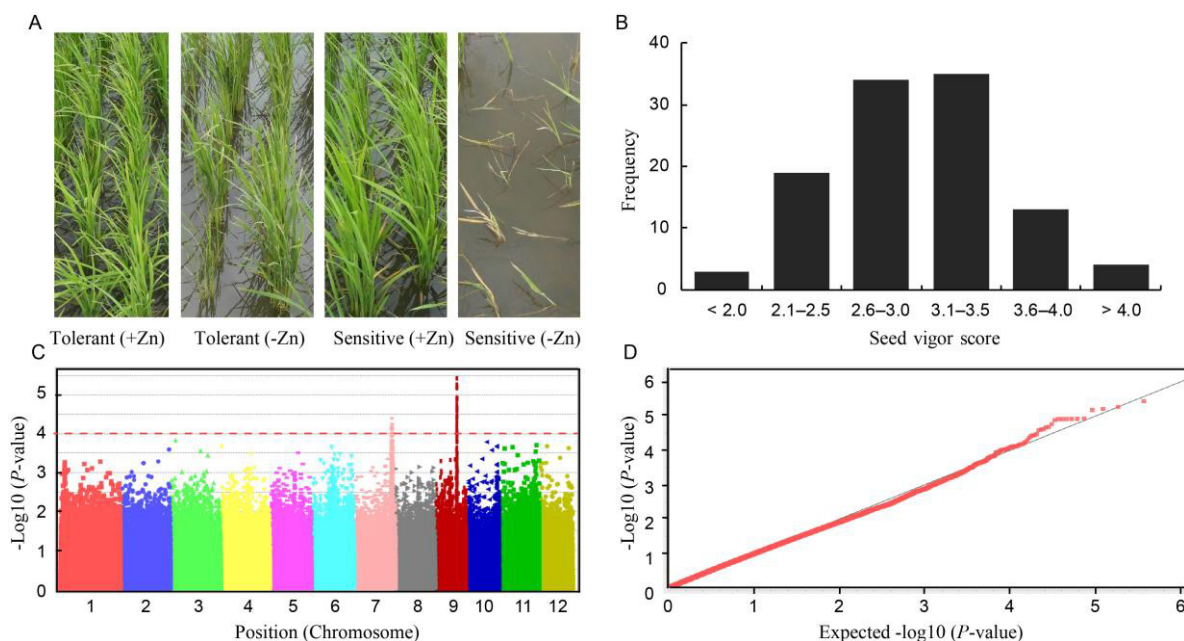

Fig. 1. Genome-wide association analysis of plant vigor of a diverse panel of *aus* accessions under Zn deficiency.
Data was collected at fourth week after transplanting during the peak-stress period.
A, Plant growth of tolerant and sensitive accessions under control (+Zn) and Zn deficiency (-Zn). B, Distribution of seed vigor scores. Higher scores indicate higher tolerance to Zn deficiency. C, Manhattan plot. Plots above the threshold line indicate significant association. D, Quantile-quantile plot.