Low phytate QTL mapping in winter wheat (*Triticum aestivum* L.) using Genotyping-by-Sequencing

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Abstract

The phytate molecule in grains and legumes reduces the bioavailability and absorption of minerals in the gut due to its capacity to chelate divalent elements such as iron and zinc. In wheat (*Triticum aestivum* L.), a low phytate mutant (lpa1-1) is now available and has been reported to reduce phytate in wheat kernels by up to 35%. However, little is known about the genetic control of low phytate trait and its gene location in the wheat genome. To identify quantitative trait loci (QTLs) associated with the low phytate trait in wheat kernels and develop molecular markers tightly linked to these QTLs, we developed 171 recombinant inbred lines (RILs) from a hexaploid winter wheat cross 'Danby' (a Kansas elite variety) x 'AO2568WS-A-12-10' (a low phytate donor parent). This population was phenotyped for inorganic phosphate concentration, a predictor of phytate levels, and genotyped using genotyping-by-sequencing (GBS). A total of 2,509 high quality SNPs was used to construct a linkage map and two major QTLs were identified on chromosomes 4D and 5A for the inorganic phosphate trait, which accounted for 43% of the total phenotypic variation. Sequence analysis of the flanking markers for the two QTLs identified two candidate genes. One candidate gene synthesizes a polygalacturonase-like enzyme that would interfere in the synthesis of phytic acid. Several SNP markers associated with these major QTLs were converted to Kompetitive Allele Specific PCR (KASP) markers that can be used in marker-assisted breeding to reduce phytate in wheat.

Introduction

- Approximately 60% of the world's population are iron (Fe) deficient and over 30% are zinc (Zn) deficient. This situation is attributed to production areas with low mineral phytoavailability, and consumption of staple crops with low tissue mineral concentrations and/or high concentrations of antinutrients such as oxalate, tannins or phytate.
- A low phytic acid (LPA) mutant in wheat has been developed and germplasm containing this mutation is available for breeding uses. However, little is known about the genetics controlling the LPA trait in wheat and the location of quantitative trait loci (QTLs) affecting this trait.
- For this study, our objectives were to (i) construct a high-density SNP map using GBS and a winter wheat population of 181 RILs; (ii) identify QTLs related to the low phytate trait; (iii) predict candidate genes underlying mapped QTLs that may be involved in genetic regulation of the low phytate trait in wheat; and (iv) develop Kompetitive allele-specific PCR (KASP) markers for rapid genotyping of LPA materials.

Materials and Methods

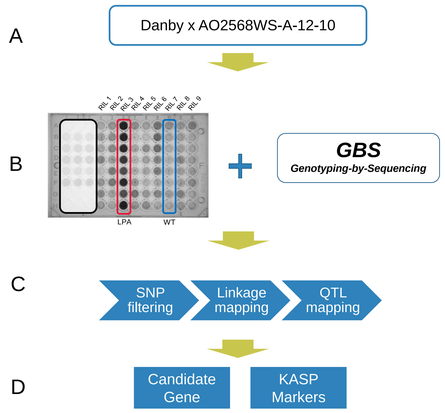

Figure 1. Experimental approach. Cross between parent Danby and LPA donor parent AO2568WS-A-12-10; (B) phenotyping LPA by P*i* (Inorganic phosphorus) determination and GBS sequencing; (C) SNP filtering pipeline in TASSEL, and linkage map development, and QTL identification using ICI Mapping v4.1; and (D) candidate genes identification and Kompetitive allele-specific PCR (KASP) markers development.

Results

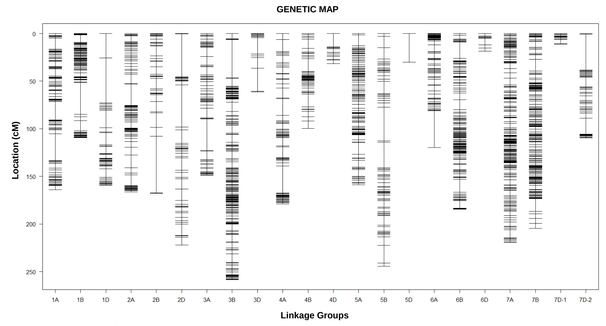

Figure 2. High-density linkage map constructed using a recombinant inbred population derived from the parents Danby x AO2568WS-A-12-10. A total of 2,509 high quality SNPs were used to construct a linkage map covering 21 linkage groups and spanning 3,067 cM with an average distance of 1.22 cM between adjacent markers.

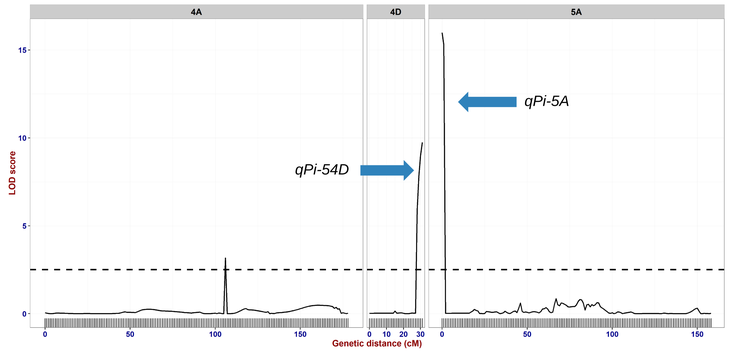

Figure 3. Location of QTLs of the P*i* trait on linkage groups 4A, 4D, and 5A. QTL identification was performed on linkage map of Danby x AO2568WS-A-12-10 mapping population. Major QTLs *qPi-4D* and *qPi-5A* explained 15.78% and 27.33% of the P*i* phenotypic variation respectively.

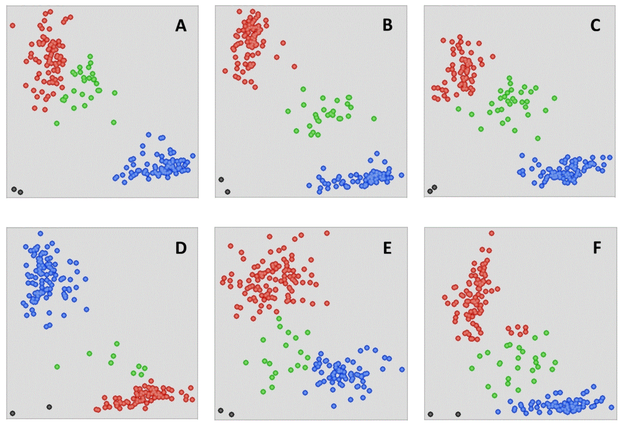

Figure 4. KASP markers amplification and validation. Markers (A) chr4D_467639736, (B) chr4D_473161612, (C) chr4D_481885135, (D) chr5A_595373329, (E) chr5A_596599151, and (F) chr5A_639292843 were tested in 181 recombinant inbred lines and the two parents of population Danby x AO2568WS-A-12-10. P*i* genotypes (low phytate) are represented in blue, wild-type genotypes (high phytate) are represented in red, heterozygous genotypes are represented in green, and water controls are represented in black.

Results

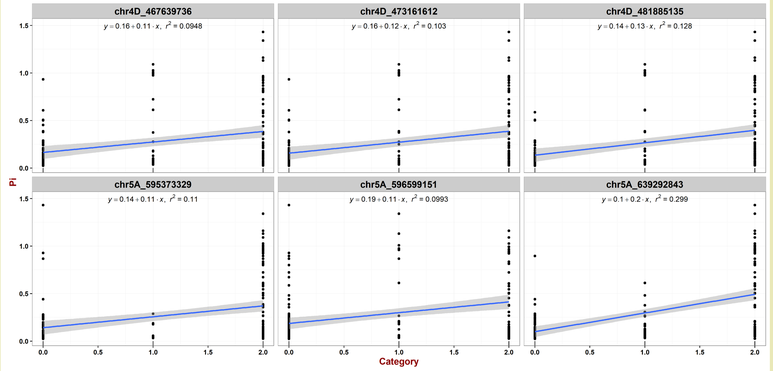

Figure 5. Relationship of KASP marker categories (genotyping) and P*i* phenotyping of 181 recombinant inbred lines. The regression models for all markers were highly significant (P < 0.00001). Category 0: wild-type genotypes (high phytate); category 1: heterozygous genotypes; category 2: P*i* genotypes (low phytate). Shaded areas represent the 95% confidence intervals of the linear models.

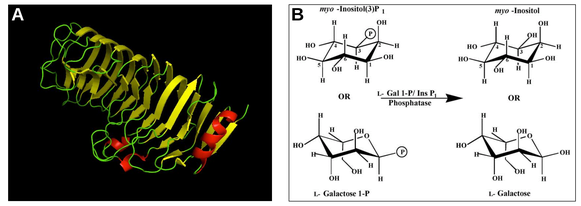

Figure 6. Candidate gene identification. Marker chr5A_639292843 had a high DNA sequence similarity with polygalacturonase ADPG2-like predicted sequence of *Aegilops tauschii* subsp. tauschii. Polygalacturonases are hydrolyzing enzymes that could interfere with the galactose-inositol phosphate synthesis, and therefore phytic acid levels. (A) structure of polygalacturonase (Woo Cho *et al.*, 2011); (B) potential dual functionality of galactose in the inositol phosphate pathways (Raboy, 2009).

Conclusions

- This is the first report of two major QTLs, *qPi-4D* and *qPi-5A*, linked to the P*i* trait in wheat, which is correlated with the low phytate attribute.
- Based on sequence alignment, *qPi-5A* QTL appears to play a key metabolic role in the phytic acid formation pathway.
- The KASP markers generated in this study will provide the international wheat community with a time- and cost- effective tool for breeders who aim to develop low phytate cultivars.

REFERENCES:

- Raboy V. 2009. Approaches and challenges to engineering seed phytate and total phosphorus. Plant Science 177, 281–296.
- Woo Cho S, Lee S, Shin W. 2001. The X-ray structure of *Aspergillus aculeatus* polygalacturonase and a modeled structure of the polygalacturonase-octagalacturonate complex. Journal of Molecular Biology. 311: 863–878.

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