Biofortification of winter wheats by incorporating low phytate and *GPC-B1* traits

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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, consumption of staple crops with low tissue mineral concentrations and/or high concentrations of antinutrients such as oxalate, tannins or phytic acid (IP_6). While Fe fortification is widely recommended, less than half of the world's countries fortify flours (Fig.1). To effectively alleviate this situation, efforts have been undertaken to develop wheat varieties with higher grain Fe and Zn concentrations, and low phytic acid (LPA) content. Additionally, *Gpc-B1*, a gene encoding a sequence-specific DNA-binding protein (NAM-B1), has been associated with increased grain protein and Zn and Fe concentrations (Uauy *et al.* 2006).

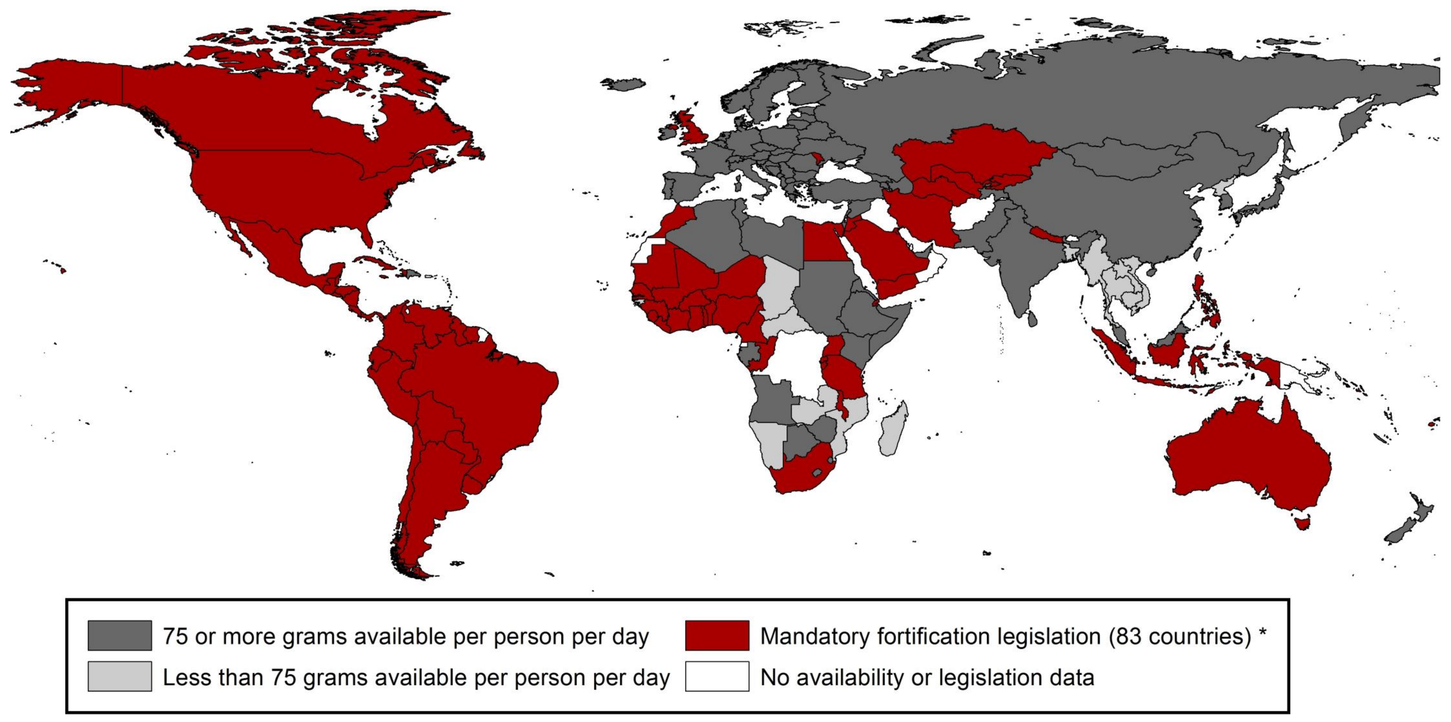


Figure 1. Wheat availability and fortification legislation.
Source: Flour Fortification Initiative, 2015

CREATING AND CHOOSING THE POPULATIONS

MATERIALS AND METHODS

For this study, two types of recombinant inbred lines (RILs) populations were created: (1) one population from *Gpc-B1*/LPA straight crosses, and (2) ten populations from *Gpc-B1*/LPA//adapted cultivars three-way crosses, in which F_1 derived from the initial *Gpc-B1*/LPA crosses were mated with Nebraska-adapted winter wheat materials to enhance agronomic adaptation to the target growing environments. After F_4, all RILs were classified as either wild type (WT) or LPA using the high inorganic phosphate (HIP) protocol (Fig.2).

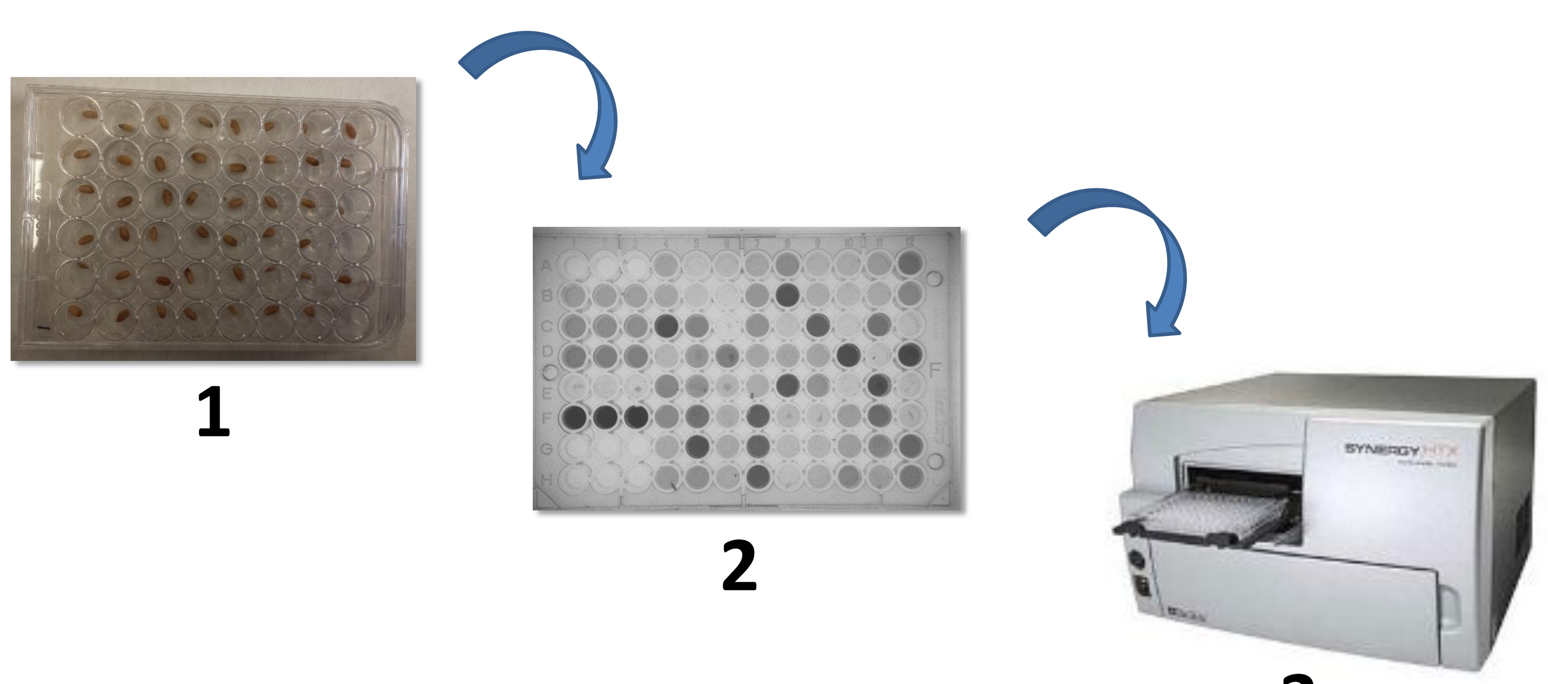
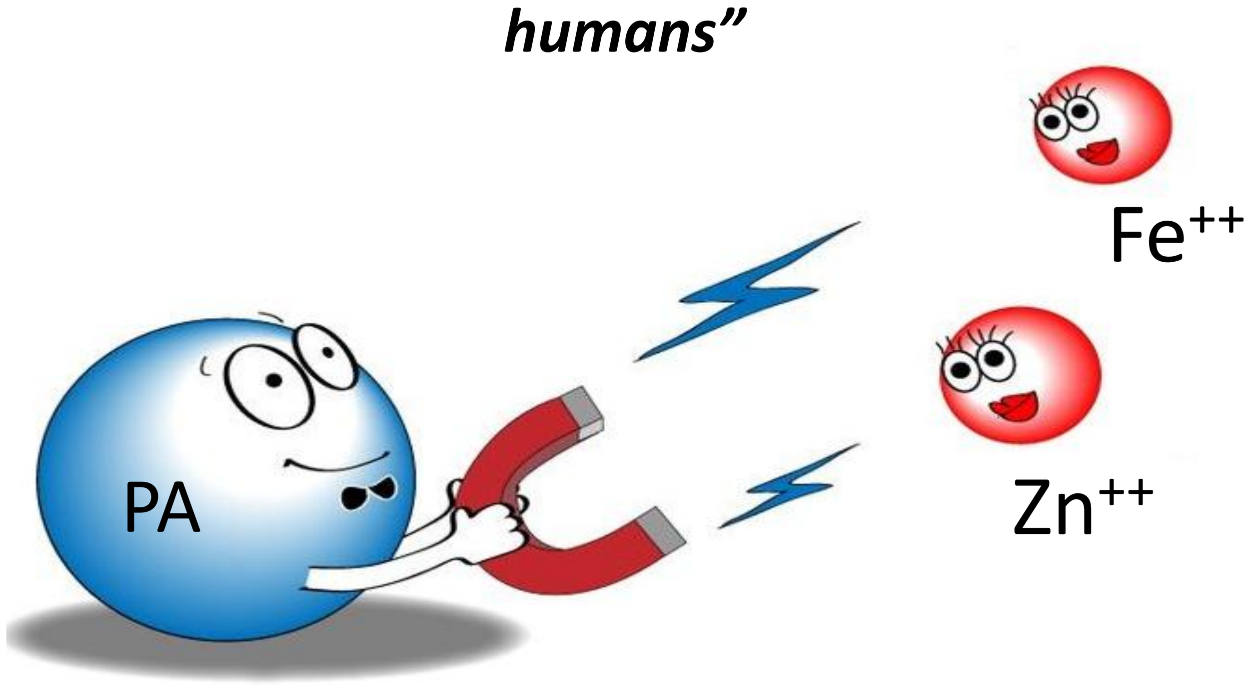


Figure 2. Colorimetric HIP protocol steps. (1) Crushing, (2) P*i* precipitation, and (3) Absorption plate reading.

Phytic Acid-Iron-Zinc binding:

"A love story without a happy ending… for humans"



Phytic acid is also known as an antinutrient because it is capable of chelating divalent cations (eg. Fe, Zn, Ca, etc. Fig.3). This tight bond reduces the bioavailability of these nutrients in the diet of monogastric animals, including humans. There are two ways to reduce micronutrient deficiencies in grain diet: flour fortification or reducing phytic acid concentration in grains through breeding. Several low phytic acid mutants have been created in different crops such as corn, barley, rice and wheat. These mutants have 50-95% less phytic acid than their non-mutant counterparts, increasing the bioavailability of Zn and Fe.

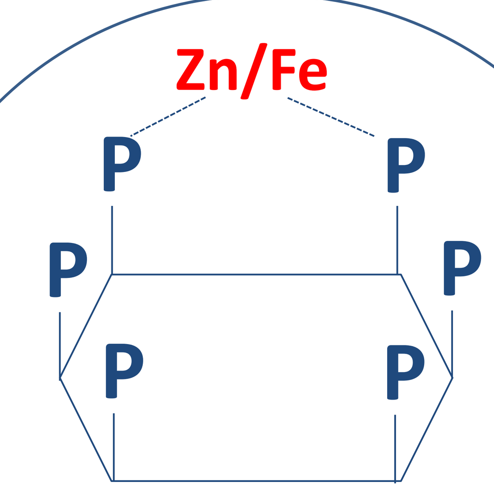


Figure 3. Phytic acid-Iron-Zinc chelation.

RESULTS AND DISCUSSION

HIP results from the two- and three-way cross populations showed differences in the amount of LPA RILs in both crossing methods. Fifty LPA RILs out of 400 total lines were identified in the two-way cross population and twenty-four LPA RILs out of 200 total lines were identified in one of the families from the three-way cross populations (Figure 4). The observed segregation suggests that the trait is controlled by two or more genes. Several single LPA mutations have been isolated in other crops, namely maize, barley and rice. This study confirms the results of a previous segregation analysis using a different population and the polygenic inheritance of the wheat LPA mutation (Guttieri *et al*. 2004).

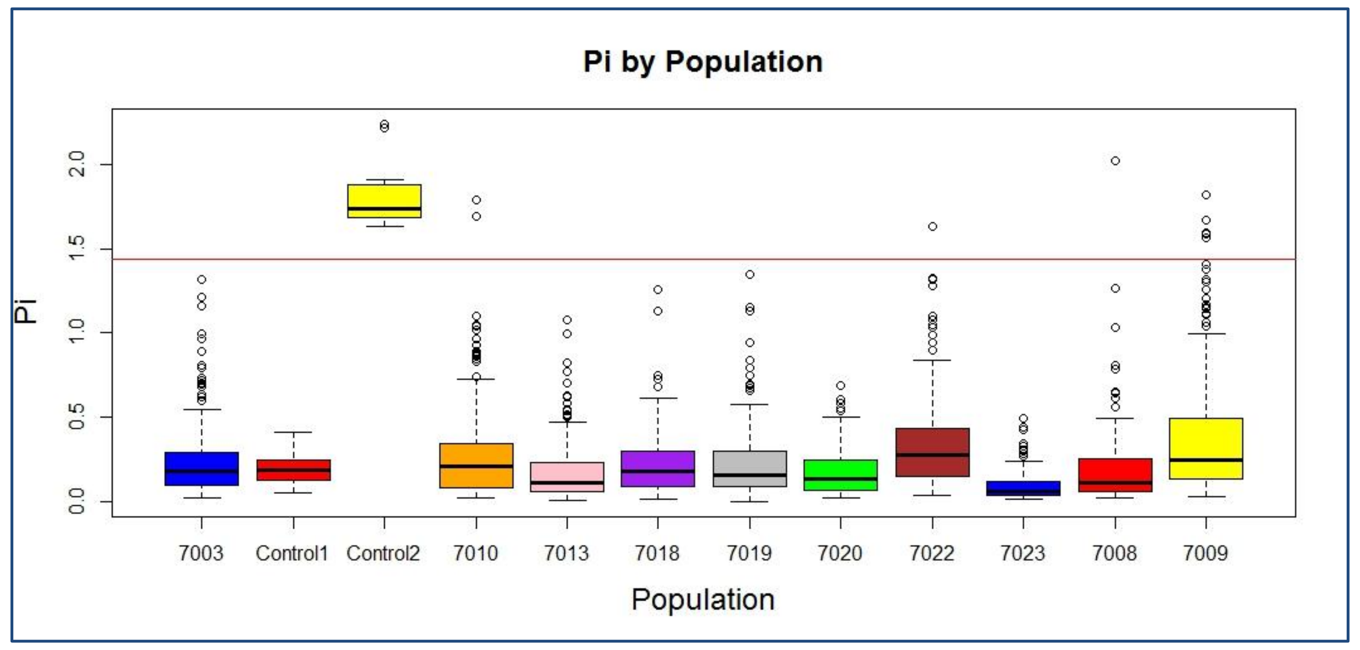


Figure 4. Three-way cross populations showing differences in the amount of LPA RILs.

HYPOTHESIS

Combining low phytic acid and high protein (*Gpc-B1*) traits in Great Plains adapted wheats will increase Fe, Zn and P concentration and bioavailability without negatively affecting grain yield.

ONGOING EXPERIMENTS

A set of 69 RILs of the two-way cross population were planted in September and October 2015 in four different locations of Nebraska (two western and two eastern). This set contains LPA+GPC, LPA+WT (wild type), WT+GPC, or WT+WT genotypes. Grain protein, grain ash content, Fe and Zn concentrations will be determined by a commercial laboratory after harvesting in 2016. Grain bioavailability analysis will be performed to determine the "real" Fe and Zn absorption in the presence of phytate.

FUTURE EXPERIMENTS

Molecular Marker development

Bulked Segregant Analysis (BSA) using Genotyping-by-Sequencing (GBS) will be performed on homozygous LPA and WT RILs. BSA samples will be saturated with markers to create a robust SNP library. Selected markers will be validated in new LPA populations. Validated markers will be converted to RT-PCR markers for analysis of large LPA populations as a cost-effective method of Marker Assisted Selection (MAS).

REFERENCES

- **Guttieri, M.J., Bowen, D., Dorsch, J.A., Raboy, V., and Souza, E.J. 2004.** Identification and characterization of a low phytic acid wheat. Crop Science 44:418-424.
- **Uauy, C., Distelfeld, A., Fahima, T., Blechl, A. E., and Dubcovsky, J. 2006.** A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science 314(5803):1298-301.