Relationship of Fe and Zn Content in a Population Developed for Resistance to Iron Deficiency Chlorosis

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Introduction

Plants need an adequate supply of nutrients and minerals for normal growth and development. However, oftentimes environmental factors and genetics influence the plant's ability to acquire these nutrients, which leads to mineral deficiencies. Iron deficiency chlorosis (IDC) is one of these nutritional diseases occurring in soybean (Glycine max (L.) Merr.). Along with IDC, zinc deficiencies also occur in soybean. Both IDC and Zn deficiencies are commonly observed when plants are grown on calcareous soils that have a characteristically alkaline pH (> 7.0). This high pH increases the inability of the plants to take up the available nutrients in the soil (Cianzio et al. 1979; Cianzio et al., 2008). Consequently, this leads to a reduction in seed quality and yield loss with an average of 20% loss for each unit increase of chlorosis score (Mortvedt, 1991; Froehlich and Fehr, 1981). In severe cases, death can occur, which has been estimated to cost producers a minimum of $120 million dollars annually (Hansen et al., 2003; Naeve and Rehm, 2006).

Previous research has identified QTL associated with IDC scores and chlorophyll content in both field conditions and nutrient solutions, and also confirmed the genetic mechanisms responsible for IDC resistance (Lin et al, 1997 and 1998). However, there is still a wide variability in susceptibility among soybean genotypes with IDC and zinc deficiencies. Furthermore, environmental and genetic components of Zn deficiency in plants are not fully understood (Cianzio et al., 2008). Being that variety selection is the most important management practice for producers on chlorosis prone soils (Hansen et al., 2003), more information is needed to efficiently select superior genotypes. Determination of seed Fe and Zn mineral contents provide a logical approach to identifying mineral efficient genotypes. Symptoms are commonly seen during the early growing stage when mineral stores of the embryo and cotyledon have been translocated to the new growth. As a result, genotypes with higher mineral content could possibly outgrow deficient conditions.

Objective

To identify QTL for iron and zinc content in a soybean breeding population previously used to identify QTL associated with IDC efficiency.

Materials and Methods

To test the hypothesis that elevated mineral content can be used as a selection method for resistance and/or mineral-efficiency, 120 lines from the Anoka x A7 mapping population, Pride B216 (Fe-inefficient), A15 (Fe-efficient), Clark (Fe-efficient), IsoClark (Fe-inefficient) and Williams 82 (Fe-inefficient) were selected for evaluation.

Phenotypic Analysis

Five grams of seed from each line and check were ground using a Foss Cyclotec mill (1093 Sample Mill, Foss, Eden Prairie, MN) equipped with a 1-mm screen. A 0.5 g subsample was taken from each genotype and dry-ashed in a muffle furnace in the following sequence:
200 C for 1 hour, 350 C for 1 hour, and 500 C for a minimum of 4 hours. Afterwards, samples were allowed to air cool for a minimum of 2 hours, and then digested in a dilute acid solution (300 ml HCl, 100 ml HNO3 in 1 L of ddH2O) and brought to a final volume of 10 ml. In the field, leaf samples were taken from each line from the second fully expanded trifoliate. Leaf tissue was then stored in liquid nitrogen, before being lyophilized for analysis. A 0.5 g subsample of leaf tissue ground with mortar and pestle was dry-ashed and digested using the previous procedure. Fe and Zn content was then quantified at the Agronomy Soil and Plant Testing Facility using the Inductive coupled plasma-optical emission system (ICP-OES).

The experiment was conducted as a randomized complete block design with 3 replications and two years (2008-2009) of data. All effects were considered random. Data were analyzed using standard ANOVA procedures with the Jmp statistical package (JMP, Version 8. SAS Institute Inc., Cary, NC, 1989-2007). Tukey's HSD multiple comparison was implemented to determine significant differences. Zinc values were log transformed. Iron and zinc content was determined by multiplying each concentration (ppm=µg/g) by 100 seed weight (g).

Genotypic Analysis

A genetic map was constructed with 224 SSR loci using Mapmaker 3.0 (Lander et al., 1987). Linkage groups were determined with the "group" command using a LOD of 4.0 and maximum Haldane distance of 50 centiMorgans (cM). Map order was determined using the "three point" command followed by "order", "framework", and "place"

Linkage maps were imported into QTL Cartographer 2.5 (Wang et al., 2007), and QTLs located using composite interval mapping with the default LOD threshold of 2.5. The results presented in this poster are for combined analyses only.

Trait Evaluated	Mean ± SD	Min	Max	Tukey HSD	p value
100 Seed Weight (g)	15.723 ± 0.931	10.717	18.233	2.3779	<.0001
Seed Zinc (ug/g)†	3.708 ± 0.139	3.542	3.968	0.3556	0.017
Seed Iron (ug/g)	76.915 ± 9.596	64.140	94.133	24.5214	<.0001
Zinc content (mg)†	0.0586 ± 0.004	0.047	0.068	0.0102	<.0001
Iron content (mg)	1.234 ± 0.167	0.798	1.581	0.4277	<.0001
Leaf Zinc (ug/g)	33.086 ± 6.210	24.118	41.497	15.8696	0.3155
Leaf Iron (ug/g)	599.199 ± 269.853	379.910	1089.660	689.554	0.9410

Table 1. The seven traits evaluated for the F2:4 Anoka x A7 mapping population. The means are presented with the maximum and minimum values for each trait. Significance of α=0.05 was used.
† indicates data for this trait was log transformed

Results

Each of the seed traits evaluated indicated highly significant differences among the F2-derived lines (Table 1.) However, leaf zinc and iron were not significantly different within the population. One hundred twenty-two SSR markers formed linkage groups spanning 2399.79 cM with average marker distance 19.7 cM (Figure 2). A total of 18 QTL on 10 chromosomes were identified for the seed traits (Table 2.) Three QTL on chromosomes 5, 8, and 13 for average seed weight were identified with LOD scores of 3.5, 4.3, and 3.6 (Table 2; Figure 1A.). The QTL on chromosome 13 explained the most variation of 26%. Satt187 in previous research has been shown to be highly associated with seed weight (www.soybase.org). Two QTL for zinc concentration were identified on chromosomes 3 and 8. The LOD scores were 3.1 and 3.6, respectively (Figure 1C). Four QTL for seed Fe concentration were indentified. Two of those QTL were on chromosome 14 with LOD scores of 2.9 and 2.5 (Figure 1B). Markers Satt534, Satt070, Satt556, Satt304, Sat_268, and Satt239 have previously been mapped to regions with known Fe-efficiency QTL (www.soybase.org). Additionally, the QTL on chromosome 4 had not any QTL associated with Fe-efficiency.

Additionally, nine other QTL were identified for zinc and iron content, which took into account seed weight for each genotype. Two QTL for zinc content on chromosome 8 had LOD scores of 3.4 and 2.5 and account for 36% of the variation. For both iron and zinc content, one QTL was identified in the marker interval Satt424-Satt187 with LOD scores of 3.8 and 3.5, representing 16% and 18% of the variation, respectively.

Traits	Marker Interval	LG/Chrom	LOD	R2	Interval Distance (cM)	Misc Marker(s) in interval
Avg. Seed Weight	Sat_265-Satt591	A1/5	3.54	20%	21	
	Satt424-Satt187	A2/8	4.34	20%	14.1	
	Satt146-Satt423	F/13	3.65	26%	54.9	
Avg. Seed [Zinc]	Sat_236-Sat_033	N/3	3.62	27%	39	
	Satt233-Sat_392	A2/8	3.1	15%	16.6	
Avg. Seed [Iron]	Sat_416-Sat_311	C1/4	2.65	16%	37.7	Satt713
	Satt534-Satt070	B2/14	2.98	20%	53.2	Satt474
	Satt556-Satt304	B2/14	2.56	20%	15.3	
	Sat_268-Satt239	I/20	3.25	19%	39.2	
Avg. Zinc Content	Sat_265-Satt155	A1/5	3.48	17%	23	Satt591
	Satt327-Sat_250	A2/8	3.46	36%	21.9	
	Sat_250-Satt409	A2/8	3.2	36%	41.5	
	Satt424-Satt187	A2/8	3.55	18%	14.3	
	Satt325-Satt423	F/13	3.21	23%	47.1	Satt586
	Satt071-Sat_160	E/15	2.56	21%	11.4	
Avg. Iron Content	Satt155-Satt042	A1/5	2.66	13%	16.4	Satt471
	Satt424-Satt187	A2/8	3.86	16%	14.3	
	Satt588-Satt273	K/9	2.61	16%	104.3	

Table 2. QTL intervals for the five seed traits traits evaluated for the F2:4 Anoka x A7 mapping population. Markers within interval along with linkage groups, LOD score, and R2 values. Some QTL also had markers that were in the middle of the interval

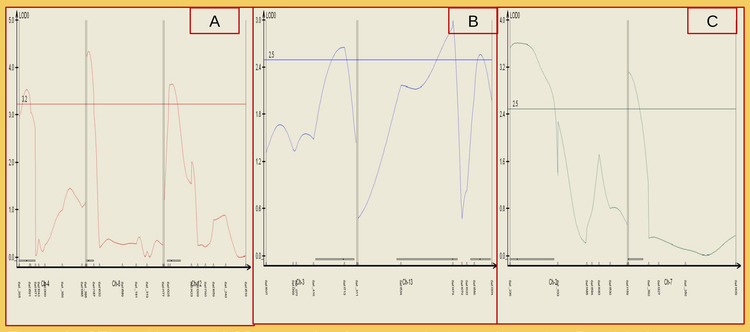

Figure 1. QTL positions and LOD score plots for composite interval mapping. Panel A. Average seed weight. Panel B. Average seed [iron]. Panel C. Average seed [zinc]. Peaks indicate QTL positions for each trait.

Future Directions

- Screen population for ferritin content and phytate content to determine the bioavailability of Fe
- Add additional markers to the map to decrease marker interval distance
- Determine correlation between QTL identified and existing iron QTL previously identified in field

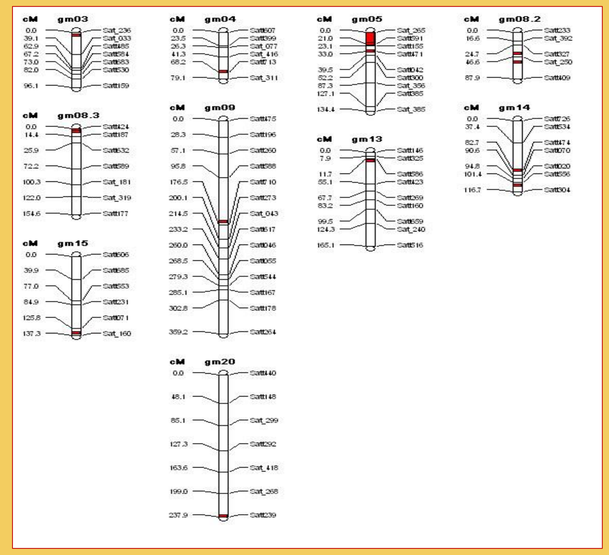

Figure 2. Chromosome map showing positions of all QTL identified (red bars). Numbers above each chromosome indicate chromosome number.

Traits	Sd Zn (µg/g)	Sd Fe (µg/g)	Lf Zn (µg/g)	Lf Fe (µg/g)
100 Sd wt (g)	0.470	0.602	0.232	0.339
Sd Zn (µg/g)		0.707	0.199	0.611
Sd Fe (µg/g)			0.420	0.562
Lf Zn (µg/g)				0.354

Table 3. Multivariate analysis was conducted to determine correlations of the traits evaluated. Seed Fe had strong correlations with Sd Zn and 100 Sd wt. All correlations were significant p= 0.05.

Conclusions

- **We identified nine QTL for Fe and Zn content in this research. Three QTL on chromosomes 9, 13, and 15 were novel with the others mapping to chromosomes and positions of previously identified Fe efficiency QTL**
- **SSR markers that have been mapped in known regions of Fe efficiency QTL also identified QTL in the Anoka x A7 population for seed Fe concentration and seed Zn concentration with one QTL landing on chromosome 4 which does not have any known Fe efficiency QTL**
- Seed Fe concentration had strong correlations with seed weight and seed Zn concentration (Table 3)
- Higher seed Fe concentration can be possibly used as a predictor and selection criteria for IDC resistance and content used in breeding for human consumption

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USDA United States Department Of Agriculture Agricultural Research Service

NCSRP NORTH CENTRAL SOYBEAN RESEARCH PROGRAM