

# Identification of QTL Underlying Seed Micronutrients Accumulation in ‘MD 96-5722’ by ‘Spencer’ Recombinant Inbred Lines of Soybean

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## Abstract

Genetic mapping of quantitative trait loci (QTL) associated with seed nutrition levels is almost non-existent. The objective of this study was to identify QTLs associated with seed micronutrients (iron, Fe; zinc, Zn; boron, B; manganese, Mn; and copper, Cu) accumulation (concentration) in a population of 92 F<sub>5,7</sub> recombinant inbred lines (RILs) that derived from a cross between MD 96-5722 (MD) and ‘Spencer’. For this purpose, a genetic linkage map based on 5,376 Single Nucleotide Polymorphism (SNP) markers was constructed using the Illumina Infinium SoySNP6K BeadChip array. The RILs were genotyped using 537 polymorphic, reliably segregating SNP markers. A total of 23 QTLs for micronutrients Fe, Zn, B, Mn, and Cu have been identified and mapped on eight linkage groups (LGs) of the soybean genome. Five QTLs were detected for Fe (qIRO001- qIRO005) on LGs N, A1, K, J, and G. Seven QTLs for Zn (qZIN001-qZIN007) on LGs D1a (Chr 1), N (Chr 3), F (Chr 5), B2 (Chr 14), J (Chr 16), A1 (Chr 5), and K (Chr 9). Two QTLs for B (qBOR001 and qBOR002) were detected on LGs N and A1. Four QTLs were detected for Mn (qMAN001-qMAN004) on LGs N, A1, K, and J, and five QTLs were detected for Cu (qCOP001- qCOP005) on LGs N, A1, K, J, and G). It was observed that the four QTLs for

Zn, Cu, Fe, and Mn on LGs N (Chr 3), LG A1 (Chr 5), and LG J (Chr 16) were clustered in a similar region of the linkage groups, suggesting possible shared physiological and genetic mechanisms. The QTLs detected in this study are novel and will contribute to our understanding of the genetic basis of seed mineral nutrition. This research would allow breeders to efficiently select for higher seed nutritional qualities to meet the seed industry and human and livestock nutritional needs.

**Keywords:** Soybean, seed minerals, trace elements, micronutrients, seed composition, RIL, genetic mapping, SNP, QTL.

## Introduction

Soybean is a major crop in the world and its seeds are considered a major source of essential nutrients for human and animal nutrition. In addition to seed protein, oil, fatty acids, isoflavones, and sugars, soybean seeds contain micronutrients (trace elements) such as Fe, Zn, Mn, B, and Cu. Micronutrients are essential for human nutrition, and unbalanced diet of these nutrients can lead to human malnutrition and health problems

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(Samman et al., 1998; Devirian and Volpe, 2003; Bouis, 2003; Fletcher et al., 2004; Lu et al., 2008). It was reported that over three billion people are suffering from malnutrition of minerals, especially iron and zinc (Welch and Graham, 2004; White and Broadley, 2009; Lu et al., 2008) in spite of biofortification (White and Broadley, 2005, 2009). Micronutrient deficiency in soil is common and leads to crop yield loss and poor seed quality (Marschner, 2012; Brown et al., 1999, 2002). Therefore, developing cultivars with higher levels of micronutrients in the seeds, especially those related to metal-binding proteins such Fe, Zn, Cu, Mo, Mn (Zhang et al., 2004; Heinemann et al., 2005; Philip and Martin, 2005) is critical. The accumulation of minerals in the seed involves several processes, including nutrients uptake, translocation, redistribution, and accumulation (Grusak and DellaPenna, 1999; White and Broadley, 2009), and most of the genetic basis of these process are not known (Ding et al., 2010).

The physiological and metabolic roles of micronutrients in plants were well documented (Marschner, 2012; Brown et al., 2002; Brown and Hu, 1996; Brown et al., 1999; Bellaloui et al., 1999; Goldbach and Wimmer, 2007). For example, B role in growth, development, carbohydrates, phenolics, nitrogen metabolism were previously reported (Marschner, 2012). Relationships between sugar-alcohols such as sorbitol was demonstrated by Brown et al. (1999); Bellaloui et al. (1999). Role of B was also reported for cell wall structure (Hu and Brown, 1994) and rhamnogalacturonan II (RG-II) cross-link in the cell wall to form dimer (RG-II-B-RG-II), which is important for both the formation and structural integrity of the cell wall (O'Neill et al., 1996; Ishii et al., 2001). Molecular function of micronutrients was also reported such as over-expression of B transporter for B efflux, BOR1, for xylem loading (Miwa et al., 2006), and a major intrinsic protein, NIP5;1, for B uptake was found under B limitation (Takano et al. 2006). Cloning BOR1-like homologs in *B. napus*: BnBOR1;3a and BnBOR1;3c, the expression of BnBOR1;1c and BnBOR1;2a induced by B deficiency (Sun et al., 2011), transcription factor gene WRKY6 for root growth under B deficiency in *Arabidopsis thaliana* (Kasajima et al., 2010) were also reported. Over-expression of AtBOR1 led to high seed yield of *Arabidopsis* under low B condition (Miwa et al., 2006), enhanced expression of AtNIP5;1 for enhancing B uptake under low B stress and increasing seed yield (Kato et al., 2009). Similarly, the physiological and metabolic roles of Fe, Zn, Cu, and Mn in plants were well documented in Mengel and Kirkby (1982) and Marschner (2012).

Quantitative trait loci (QTL) associated with mineral accumulation in seeds were identified in rice (Garcia-Oliveira et al., 2009), wheat (Peleg et al., 2009), and *Medicago truncatula* (Sankaran et al., 2009), but limited to Fe, Zn, and Mn, and no QTLs for B or Cu have been reported to the best of our knowledge. Recently, QTLs for Mn, Fe, Zn, B, and Cu have been identified in *Brassicaceae* shoots (Wu et al., 2008; Liu et al., 2009). Diers et al. (2000) identified QTLs for Fe efficiency in soybean, and such information can provide clues to researchers to identify genes related to mineral accumulation and to finally uncover genetic networks that control plant iron homeostasis. Based on the above discussion, it is clear that there is a lack of information on QTL that are associated with seed micronutrients that could be

associated with mineral efficiency (King et al., 2013). The QTL analysis for mineral accumulation in seeds may help identifying genes encoding transporters, chelators, biosynthesis enzymes, and regulatory factors including protein kinases, membrane receptors, and transcription factors (Vreugdenhil et al., 2004).

Quantitative trait loci (QTL) mapping is a powerful tool to study complex traits such as seed mineral contents (Lu et al., 2008; Paran and Zamir, 2003). It is used to identify genomic regions responsible for a trait variation based on the association between polymorphic markers and phenotypic measurements (Zeng et al., 2008), and to enlighten the genetic basis of complex traits, where knowledge is limited (Lukowitz, et al., 2000) such as in seed mineral nutrition. We noticed that the parents MD 96-5722 and Spencer have significant variation in mineral concentrations; therefore, we will be able to detect QTLs related to seed micronutrient accumulation in this population. It was reported that genetic variation is essential for achieving higher seed mineral concentrations (Wu et al., 2007; Broadley et al., 2008; Liu et al., 2009), and this natural variation was exploited for genetic improvement of crops (Graham et al., 1999; Blair et al., 2005; Gelin et al., 2007). Except for the very limited identified QTLs for Fe (Diers et al., 1992, 2000; Lint et al., 2000), Zn (King et al., 2013), and Mn (Kassem et al., 2004), there were no QTLs identified for Cu and B nutrients in soybean seeds. Therefore, the objective of the current study was to identify QTLs associated with the accumulation of micronutrients Fe, Zn, B, Cu, and Mn in 92 F<sub>5.7</sub> recombinant inbred lines (RILs) developed from a cross between MD 96-5722 and 'Spencer' using a total 5,376 Single Nucleotide Polymorphism (SNP) markers.

## Materials and Methods

### Plant Material and Growth Conditions

A population of 92 F<sub>5.7</sub> recombinant inbred lines (RILs) was developed by a cross between MD 96-5722 (MD) and Spencer to generate phenotypic and genotypic data. The cross was made in 2004 By Southern Illinois University at Carbondale (SIUC) Breeding Program and advanced to the F<sub>5.7</sub> generation by single-pod descent method. The population was grown in a field at Fayetteville State University (FSU) campus, Fayetteville, NC in 2012 with row spaces of 25 cm and seeding rate of 160,000 seeds ha<sup>-1</sup>. There was no additional fertilizer or insecticide used. Development of RIL population was previously described by Akond et al. (2013). At harvest maturity (R8), seeds were collected for micronutrients concentrations quantification.

### Seed Analysis for Zn, Cu, and Mn

Concentrations of Zn, Cu, and Mn in mature seeds at R8 stage were determined by digesting 0.6 g of dried, ground seed in HNO<sub>3</sub> in a microwave digestion system. Seed samples were ground to pass through 1-mm sieve using a Laboratory Mill 3600 (Perten, Springfield, IL). The concentrations of nutrients were determined using inductively coupled plasma spectrometry (ICP) and described in details by Bellaloui et al. (2011, 2013).

## Boron Determination

Boron concentration in mature seeds (at R8 stage) was determined using the Azomethine-H method (Lohse, 1982; Dordas et al., 2007), and as described in detail by Bellaloui et al. (2013). Briefly, a random sample of 1.0 g of ground seed was ashed at 500°C and then extracted with 20 ml of 2 M HCl at 90°C for 10 minutes and filtered. The filtered mixture was added to a buffer solution (25% ammonium acetate, 1.5% EDTA, and 12.5% acetic acid) and 4 ml of freshly prepared azomethine-H solution (0.45% azomethine-H and 1% of ascorbic acid) (John et al., 1975). Boron concentration in seeds was measured at 420 nm using a Beckman Coulter DU 800 spectrophotometer (Beckman Coulter, Inc., Brea, CA, USA).

## Iron Determination

Iron concentration in seeds at maturity (R8 stage) was measured after acid wet digestion, extraction, and reaction of the reduced ferrous iron with 1,10-phenanthroline. The measurement of iron concentration was conducted according to the methods (Bandemer and Schaible, 1944; Loeppert and Inskeep, 1966) and was detailed by Bellaloui et al. (2013). Briefly, a random sample of 2 g of dried ground seeds was acid digested. The soluble constituents were dissolved in 2 M of HCl, and an aliquot of 4 ml, containing 1 - 20 µg of iron of the sample solution was transferred into a volumetric flask of 25 ml and diluted to 5 ml using 0.4 M HCl. A quinol solution of 1 ml was added to the 5 ml diluted sample solution and mixed. Phenanthroline solution [(25% (v/v) ethanol] of 3 ml and 5 ml of the tri-sodium citrate solution (8% w/v) was added, and the solution was diluted and incubated for 4 hours. The concentrations of Fe standard curve was prepared in 0.4 M HCl and ranged from 0.0 to 4 µg·ml<sup>-1</sup> of Fe using FeSO<sub>4</sub> salt. Iron concentration in samples was determined by reading the absorbance at 510 nm using Beckman Coulter DU 800 spectrophotometer.

## Genetic Map Construction and QTL Identification

A genetic linkage map based on 5,376 Single Nucleotide Polymorphism (SNP) markers was constructed using the Illumina Infinium SoySNP6K BeadChip array. The RILs were genotyped using 537 polymorphic, reliably segregating SNP markers. The MD 96-5722 by Spencer genetic linkage map, constructed using SoySNP6K Illumina Infinium BeadChip array, was previously reported elsewhere (Akond et al., 2013) and used to identify QTL for seed micronutrients accumulation. The Composite Interval Mapping (CIM) of WinQTLCart 2.5 (<http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>) (Wang et al., 2014) was used for QTL analysis. The Model 6 with four parameters for forward and backward stepwise regression, 10 cM window size, 1 cM step size and five control markers were selected for running WinQTL-Cart (Wang, et al., 2014). The threshold with 1,000 permutations was used. Analysis of Means (CV, maximum and minimum values, and SD) were carried out using Proc Means in SAS. Correlations were conducted by SAS using PROC REG.

## Results and Discussion

The variation of phenotypic trait (micronutrient concentrations in seeds) was wide among RILs and the percentage difference in micronutrient concentrations between lines was 128, 88, 161, 130, and 103 %, respectively for B, Cu, Fe, Mn, and Zn (Table 1). Some lines had higher concentrations of micronutrients than either parent. Except for Cu, Spencer showed higher concentrations of micronutrients than MD. The average concentrations of RILs suggested that the segregation of these traits fits a normal distribution model as the skewness values were <1.0 (data not shown). Coefficient of variation for B, Fe, and Mn was higher than for Cu and Zn, may be due to genotypic differences and environmental factors effects. Frequency distribution of different minerals generally showed normal distribution (Figures 1 and 2).

Correlation analysis showed that minerals had a significant ( $P < 0.0001$ ) correlation with each other (Table 2), and the correlation pattern was positive (Figure 3). The wide variation of seed minerals concentrations in the studied RILs reflects the effects of genotypic differences of lines in the population. These variations of nutrients concentrations among RIL individuals could be due to genotype differences in nutrients uptake, efficiency, demand, and nutrient translocation from leaves (source) to seed (sink) (Lazof et al., 1994; Nielsen and Schjorring, 1983; Marschner, 2012). The results of the current study agreed with previous reports that variation in minerals exists among soybean genotypes grown under the same conditions (White and Broadley, 2009; Bellaloui et al., 2011). Recently, it was shown that concentrations of mineral in seed varied significantly even in sets of near-isogenic lines having similar genetic backgrounds such

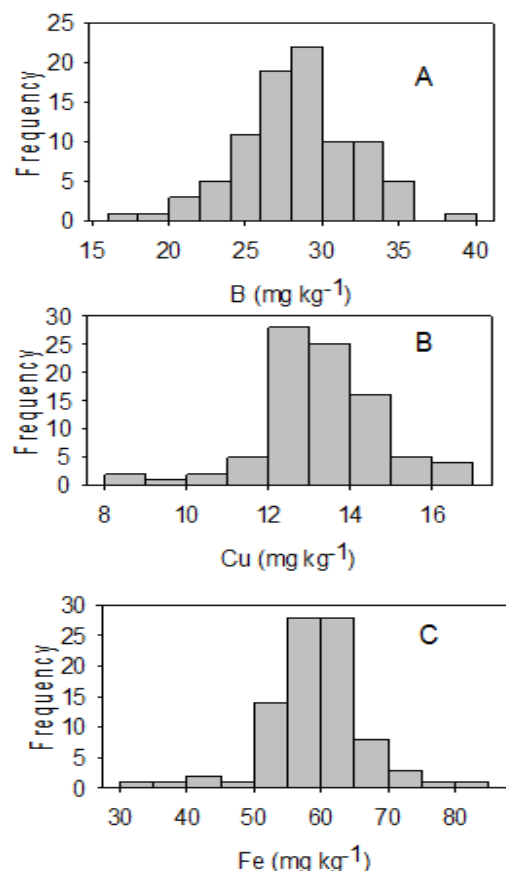
**Table 1.** Mean (concentrations, mg kg<sup>-1</sup>), standard deviation (SD), maximum (Max), minimum (Min), coefficient of variation (CV) in soybean RILs of a cross between MD 96-5722 (MD) and Spencer.

Nutrient	RILs					Parents		
	Mean	SD	Minimum	Maximum	Differences (%)	CV	MD	Spencer
<b>B</b>	28.3	3.86	17.0	38.8	128.2	13.64	32.2	37.0
<b>Cu</b>	13.2	1.43	8.6	16.2	88.4	10.87	14.9	12.4
<b>Fe</b>	59.0	7.68	32.0	83.4	160.6	13.01	57.4	72.9
<b>Mn</b>	19.1	2.71	11.2	25.8	130.4	14.16	18.7	19.1
<b>Zn</b>	51.1	5.58	32.0	65.2	103.8	10.93	57.77	62.7

**Table 2.** Pearson Correlation Coefficients (R and P values) between seed micronutrients in MD 96-5722 and 'Spencer' RIL populations of soybean†.

	<b>B</b>	<b>Cu</b>	<b>Fe</b>	<b>Mn</b>	<b>Zn</b>
<b>B</b>	1				
<b>Cu</b>	R=0.61278 P<.0001	1			
<b>Fe</b>	R=0.35389 P=0.0007	0.50086 <.0001	1		
<b>Mn</b>	R=0.66771 P<.0001	0.50607 <.0001	0.54386 <.0001	1	
<b>Zn</b>	R=0.51559 P<.0001	0.69718 <.0001	0.68122 <.0001	0.48311 <.0001	1

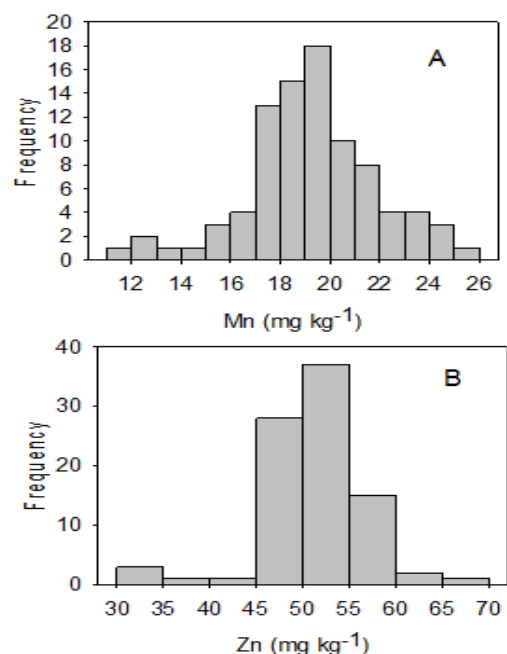
†P < 0.05 was used as level of significance.



**Figure 1.** Frequency distribution for seed boron (B) (A), copper (Cu) (B), and iron (Fe) (C) in the MD 96-5722 by 'Spencer' RIL population in soybean.

as maturity genes (Bellaloui et al., 2011). Characterizing the factors controlling the uptake system of a genotype is still complicated (Lazof et al., 1994) and further research is needed.

Genetic analysis showed that there were a total of 23 QTLs detected on eight linkage groups (LGs) for seed Fe, Zn, B, Mn, and Cu accumulation (Table 3; Figure 4). Five QTLs were detected for Fe (qIRO001-qIRO005) on LGs N, A1, K, J, and G. Seven QTLs for Zn (qZIN001-qZIN007) were identified on LGs D1a, N, F, B2, J, A1, and K. Two QTLs for B (qBOR001 and qBOR002) were detected on LGs N and A1. Four QTLs were detected for Mn (qMAN001-qMAN004) on LGs N, A1, K, and J. Five QTLs were detected for Cu (qCOP001-qCOP005) on LGs N, A1, K, J, and G. It was observed that the four QTLs of Zn, Cu, Fe, and Mn (on LG N, Chr 3; LG A1, Chr 5; LG J, Chr 16) were clustered in a similar region of the linkage group (Figure 4). For example, QTLs for Cu, Fe, Mn, and Zn on LG N (Chr 3) had peak position of 15.80 cM and LOD support intervals 15.60-15.80 cM for all of these nutrients. Similarly on LG A1 (Chr 5) with peak position of 9.50 and LOD support interval of 8.50-9.50 cM; on LG J (Chr 16, with the peak position of 12.00 cM for Fe and Mn, and 11 cM for Cu and Zn with LOD support interval 9.90-12.90 cM. Generally, the LOD support intervals and LOD values varied, depending on the linkage group and the chromosome where the QTLs are associated with the markers (Table 3).



**Figure 2.** Frequency distribution for seed manganese (Mn) (A) and zinc (Zn) (B) in the MD 96-5722 by 'Spencer' RIL population in soybean.

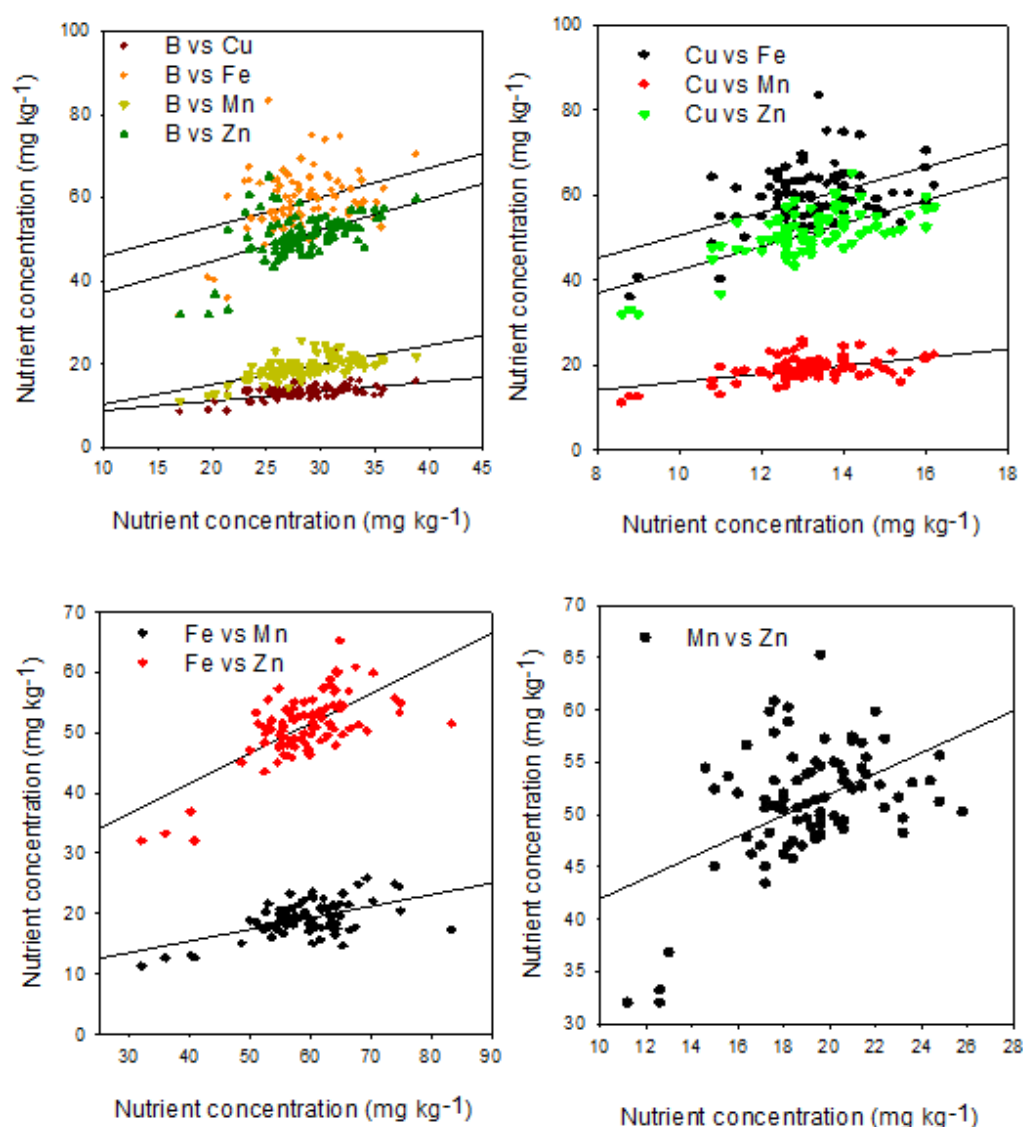
Previous research on genetic mapping associated with seed micronutrients were reported, but very limited. For example, Diers et al. (1992) studied QTLs associated with Fe efficiency in 13 F2-derived lines using restriction fragment length polymorphism (RFLP) linkage map and used 272 markers for the genetic mapping. They found that three markers were significantly ( $P < 0.01$ ) associated with Fe efficiency, two markers explained 31 and 25% of the variation for Fe-efficiency, and one marker explained 17% of the variation, although the results did not agree with the tester set population. Others reported that segregation from a cross of Fe-efficient and inefficient genotypes could be explained by a single major gene and modifying genes (Ciansio and Fehr, 1980), although it was concluded that the inheritance of Fe efficient trait was quantitative and controlled by additive gene action. Diers et al. (2000) studied the molecular characterization of iron deficiency in soybean in different populations (Pride B216 x A15; Anoka x A7) and could detect markers associated with Fe efficiency on LG B2, G, N, I, and H in Pride B216 x A15, and on LG A1, N, in Anoka x A7 population. Two QTLs were mapped on linkage groups A1 and N, and the QTL on LG A contributed 35.2% with LOD = 2.8, and the QTL on LG N contributed 72.7% with LOD = 13.1. The two QTLs associated with Fe efficiency were detected and mapped on LG I and N and explained 80.7% of the phenotypic variation, with QTL on LG N explained the largest total variation (68.8% with LOD = 7.3). Lin et al. (1997) studied the iron efficiency in soybean using total of 89 RFLP and 10 SSR markers in the Pride B216 x A15 population, and 82 RFLP, 14 SSR and 1 morphological marker in the Anoka x A7 population. They also found different QTLs related to Fe efficiency symptoms on LGs G, N, H, L, B2, and I. They suggested a polygene mechanism for QTL with



**Table 3.** Chromosomal locations and parameters associated with the quantitative trait loci (QTL) for seed micronutrient concentrations in MD 96-5722 and 'Spen-  
cer' RIL populations of soybean.

Trait	QTL	LG /Chr	<sup>†</sup> Peak position (cM)	<sup>‡</sup> 2-LOD support interval (cM)	Markers interval	<sup>§</sup> Peak LOD	#R <sup>2</sup> (%)	<sup>††</sup> Additive effects
Zinc	qZIN001	D1b /Chr_1	15.70	15.60-15.70	ss2444552583-ss244554797	9.14	0.83	-2.44
Boron	qBOR001	N /Chr_3	15.80	15.50-15.80	ss245030179-ss245023395	8.86	0.96	16.41
Copper	qCOP001	N /Chr_3	15.80	15.60-15.80	ss245030179-ss245023395	8.91	0.94	6.28
Iron	qIRO001	N /Chr_3	15.80	15.60-15.80	ss245030179-ss245023395	8.6.1	0.94	27.41
Manganese	qMAN001	N /Chr_3	15.80	15.60-15.80	ss245030179-ss245023395	8.37	0.94	9.47
Zinc	qZIN002	N /Chr_3	15.80	15.60-15.80	ss245030179-ss245023395	9.63	0.96	25.97
Boron	qBOR002	A1 /Chr_5	11.50	10.30-11.50	ss245747167- ss245786667	8.02	0.79	0.06
Copper	qCOP002	A1 /Chr_5	9.50	8.50-9.50	ss245747167- ss245786667	8.97	0.06	-0.36
Iron	qIRO002	A1 /Chr_5	9.50	8.50-9.50	ss245747167- ss245786667	8.71	0.05	-1.54
Manganese	qMAN002	A1 /Chr_5	9.50	8.50-9.50	ss245747167- ss245786667	8.38	0.05	-0.55
Zinc	qZIN003	A1 /Chr_5	9.50	8.50-9.50	ss245747167- ss245786667	9.52	0.03	-2.09
Manganese	qMAG003	K /Chr_9	0.50	0.40-0.50	ss246870684- ss246877864	8.64	0.94	0.13
Copper	qCOP003	K /Chr_9	0.30	0.20-0.30	ss246894176- ss246871129	8.94	0.94	6.28
Iron	qIRO003	K /Chr_9	0.50	0.40-0.50	ss246870684- ss246877864	8.77	0.94	26.56
Manganese	qMAN003	K /Chr_9	0.50	0.40-0.50	ss246870684- ss246877864	8.40	0.94	10.08
Zinc	qZIN004	K /Chr_9	1.50	1.40-1.50	ss246893538- ss246895099	4.16	0.02	0.30
Zinc	qZIN005	F /Chr_13	1.50	1.30-1.50	ss247940172- ss247965852	8.72	0.02	1.89
Zinc	qZIN006	B2 /Chr_14	10.90	9.90-11.10	ss248293401- ss248275088	9.09	0.94	25.99
Copper	qCOP004	J /Chr_16	11.00	9.90-12.00	ss248983974- ss248977568	8.78	0.94	6.28
Iron	qIRO004	J /Chr_16	12.00	10.90-12.20	ss248983974- ss248977568	8.58	0.94	29.05
Manganese	qMAN004	J /Chr_16	12.00	10.90-12.20	ss248983974- ss248977568	8.23	0.94	9.78
Zinc	qZIN007	J /Chr_16	11.00	9.10-12.90	ss248983974- ss248977568	9.44	0.94	25.00
Copper	qCOP005	G /Chr_18	2.20	2.10-2.20	ss249623816- ss249632893	8.69	0.95	6.28
Iron	qIRO005	G /Chr_18	2.20	2.10-2.20	ss249623816- ss249632893	8.69	0.94	26.57

<sup>†</sup>Position of peak LOD value on composite maps described previously (Coles et al., 2010). <sup>‡</sup>The positions that define the two LOD intervals around the position of peak likelihood for the QTL. <sup>§</sup>The log of odds (LOD) value at the position of peak likelihood of the QTL. <sup>#</sup>R<sup>2</sup> estimates the proportion of RIL mean variance (%) explained by the detected QTL. <sup>††</sup> A positive number in additive effect of the QTL indicates that the allele for susceptibility was derived from the line indicated and a negative number means that the allele for resistance was derived from the line indicated.

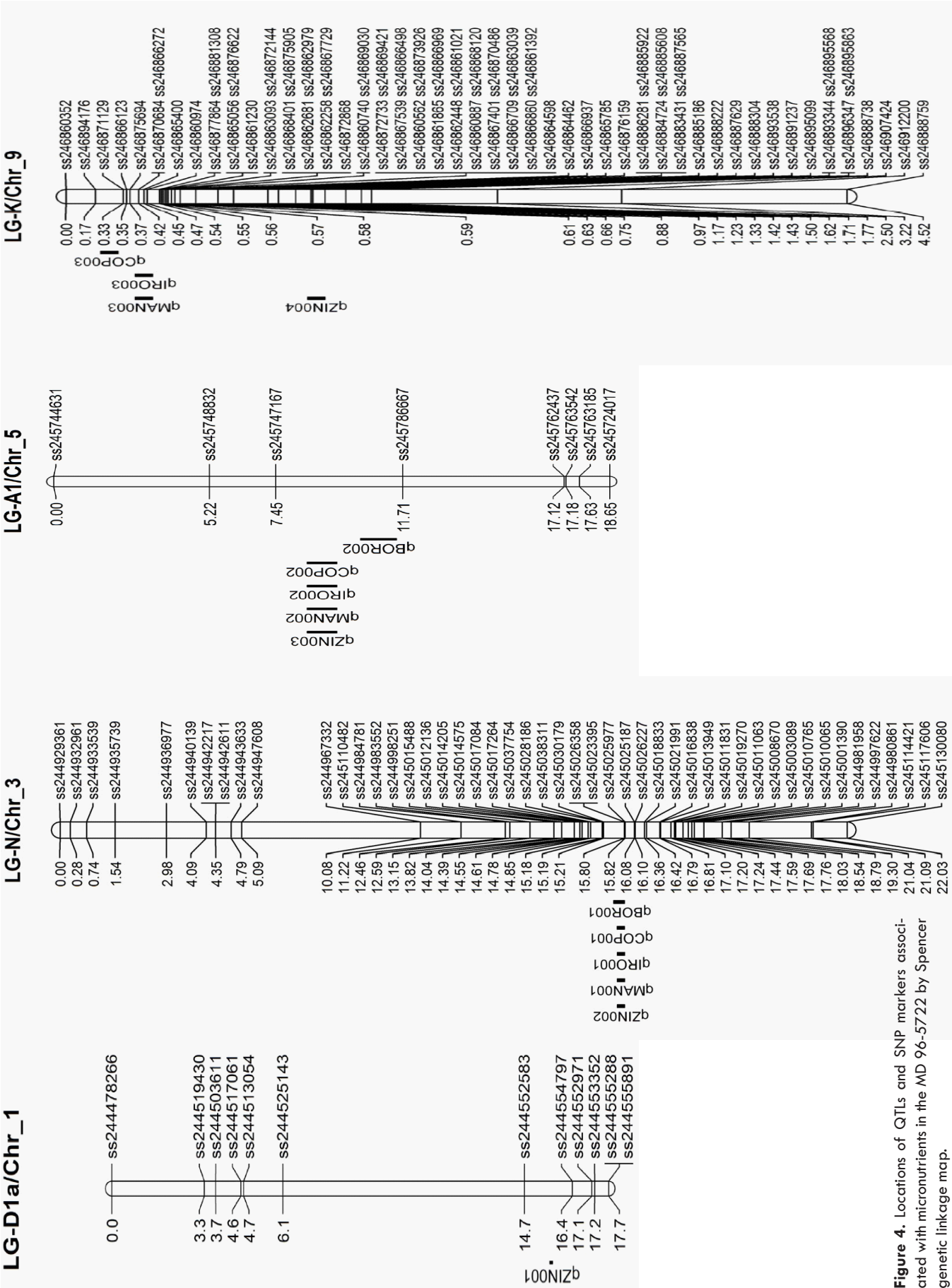


**Figure 3.** Patterns of correlation between seed micronutrients (Fe, Zn, B, Mn, and Cu) concentrations in a population of 92 F5:7 recombinant inbred lines (RILs) using 5,376 Single Nucleotide Polymorphism (SNP) markers.

minor effects on six linkage groups. It was also found in another population (Anoka x A7) that the contribution of one QTL on LG N to the visual score variation ranged on an average of 68.8-72.7%. Recently, Peiffer et al. (2012) investigated candidate genes underlying QTL related to iron efficiency in soybean. They found candidate genes underlying this QTL through molecular breeding, mapping, and transcriptome sequencing, and were able to identify the genes underlying a QTL previously identified by Lin et al. (1997), where an iron efficiency QTL on chromosome 3 responsible for more than 70% of the phenotypic variation was identified (Lin et al., 1997).

In a recent study, Mamidi et al. (2011) identified additional Fe efficiency (tolerance to Fe deficiency) QTLs using SNP-based genome-wide association mapping to detect genomic regions associated with Fe tolerance. Using two populations, they found 42 and 88 loci (with minor allele frequency >10%), and most of

these loci accounted for 74.5%-93.8% of the phenotypic variation in Fe tolerance. King et al. (2013) examined QTLs for Zn and Fe in leaves and seed that were associated with Fe efficiency. They used a population of 92 F2-derived lines, and SSR, RFLP, and BARCSOYSSR markers to construct the linkage map for mapping Fe and Zn concentrations. They were able to detect a major QTL for seed Fe accumulation on chromosome 20 that explained 21.5% of the variation, and this QTL was in the marker interval pa 515-1-Satt239. They concluded that there was a potential genetic link between Fe-efficiency and Fe accumulation in the soybean seed. King et al. (2013) found QTLs related to seed Fe concentration on LG H (chr 12), M (Chr 7), D1a (Chr 1), D2 (Chr 17), I (Chr 20). However, three Fe efficiency QTL on chromosome 1 (Diers et al., 1992) and chromosome 20 (Lin et al., 1997) were also reported. King et al. (2013) reported that a genetic link for QTL of Fe efficiency being associated to



**Figure 4.** Locations of QTLs and SNP markers associated with micronutrients in the MD 96-5722 by Spencer genetic linkage map.



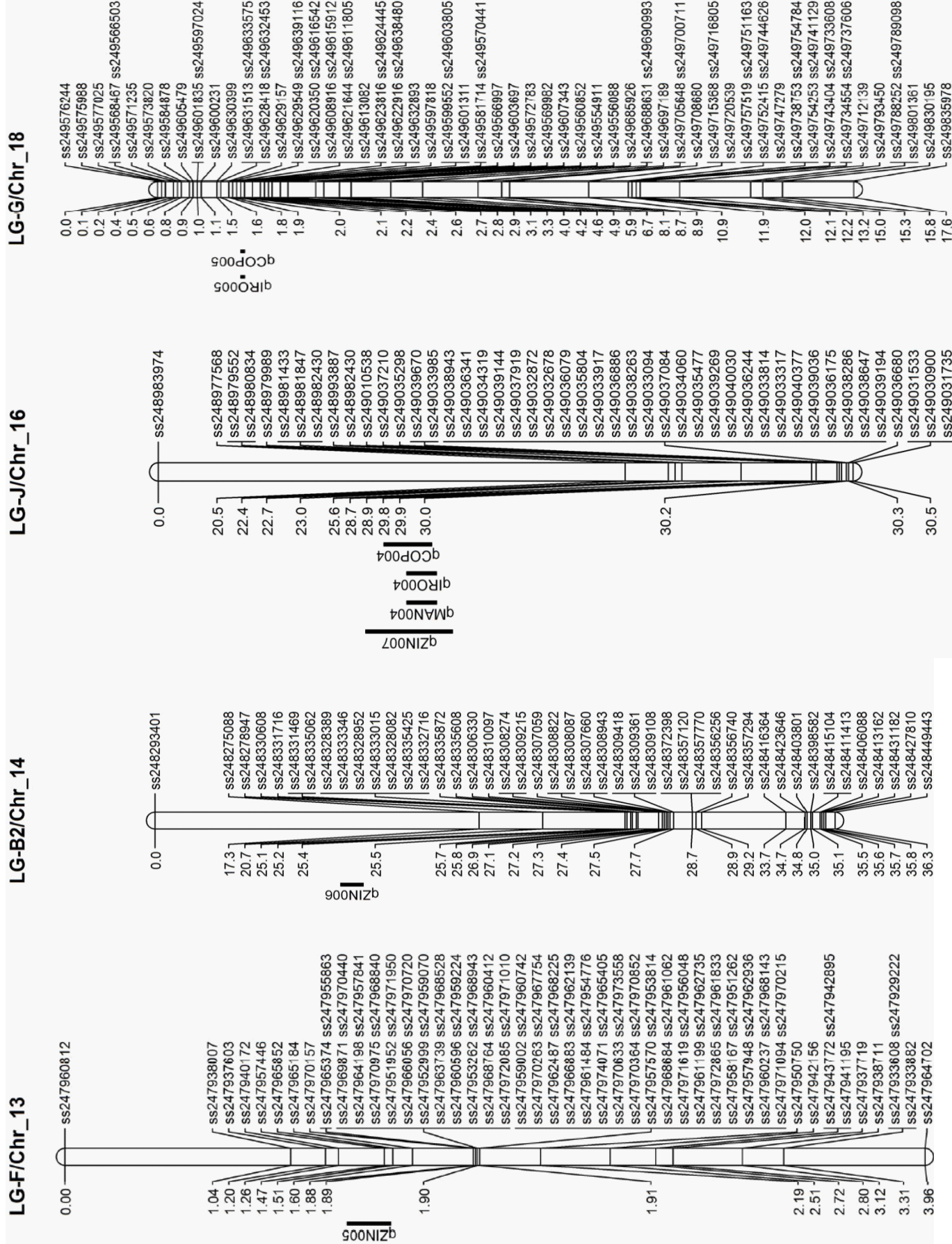


Figure 4. Continued.



QTL for Fe accumulation, and the positive allele comes from A7 with the genotypic average  $80.70 \mu\text{g Fe g}^{-1}$  compared with the inefficient parent Anoka ( $75.11 \mu\text{ Fe g}^{-1}$ ). King et al., (2013) found QTLs for Zn concentration on LGs H (Chr 12), L (Chr 19), M (Chr 7), and G (Chr 18), and they reported that possibility of seed and leaf Zn and Fe concentration had similar chromosomal regions, suggesting similar physiological and genetic mechanisms for Zn and Fe accumulation and transport (King et al, 2013; Garcia-Oliveira et al., 2009). Iron efficiency and Fe accumulation appear to be governed by similar genes, providing useful information to further advance our understanding of the genetic complexity of Fe homeostasis, transport, and mineral accumulation in soybean (Ding et al., 2013). Positive and negative interactions between nutrients within the plant were previously reported (Marschner, 2012).

Based on SoyBase, there are 39 QTLs related to Fe efficiency research were detected on 10 LG (A1, A2, D1a, G, I, N, B1, B2, H, L) (SoyBase and the Soybean Breeder's Toolbox, 2014). These QTLs are all related to either chlorosis tolerance associated with visual scoring and chlorophyll symptoms or related to different plant parts (leaves or roots), but none to seed accumulation. Our study showed that there were five QTLs on LGs N, A1, K, J, and G, and all associated with nutrients accumulation in seed, and these are new QTLs. Although there were enormous efforts devoted to develop Fe deficient chlorosis resistance cultivars, releasing cultivars with high yield have been limited (Jessen et al., 1988; King et al., 2013). In spite of the findings of King et al. (2013) regarding Fe seed accumulation in seed on LGs H (Chr 12), M (Chr 7), D1a (Chr 1), and D2 (Chr 17), our findings still represent additional QTLs that were not previously detected. For Zn nutrient, King et al (2014) detected QTLs for Zn seed concentrations on LGs H (Chr 12), L (Chr 19), M (Chr 7), and G (Chr 1). In our study, we were able to detect 7 QTLs for Zn concentrations in seeds on LGs D1b (Chr 1), N (Chr 3), A1 (Chr 5), K (Chr 9), F (Chr 13), B2 (Chr 14), and J (Chr 16), and none of these QTLs were previously identified in seeds. Searching SoyBase for QTLs for Zn revealed that there were no QTLs found.

Our finding of four QTLs for Mn (qMAN001-qMAN004) on LGs N, A1, K, and J are new additional QTLs as previous literature search revealed that there were no QTLs detected for Mn accumulation in seeds, and what is available in the literature is related to Mn toxicity using leaf and root necrosis. For example, Kilo and Lightfoot (1996) used random amplified polymorphic DNA (RAPD) markers, 100 RILs derived from the cross of 'Essex' and 'Forrest' (ExF, n=100), and identified QTLs associated with Mn toxicity resistance. In another study, Kassem et al. (2004) used 240 microsatellite markers, several RAPD markers, the same ExF RIL population, and identified four new QTLs for resistance to Mn toxicity. The QTL were additive, and three of them explained about 58% of the total variation in root resistance to Mn toxicity (Kassem et al., 2004). Search using SoyBase (2014) resulted in only six QTLs on linkage groups B2, D2, I, C2, and G, and all were not related to Mn in seed. Our findings on seed Cu and B showed that there were five QTLs were detected for seed Cu concentration (qCOP001-qCOP005) on LGs N, A1, K, J, and G, and two QTLs for seed B concentration (qBOR001 and qBOR002) were detected on LGs N and A1, and all these QTLs

are new. Previous research showed that there were no QTLs for seed Cu or B were reported in soybean in SoyBase or previously reported elsewhere, although QTLs for Cu and B in other species such rice (Garcia-Oliveira et al., 2009), wheat (Peleg et al., 2009), *Medicago truncatula* (Sankaran et al., 2009), *Brassicaceae* shoot Mn, Fe, Zn, (Wu et al., 2008), shoot B, Fe, Cu, and Zn (Liu et al., 2009) were previously reported. The QTLs clustering observation, shown in our study, for seed Zn, Cu, Fe, and Mn in similar regions of LGs may indicate common physiological and genetic mechanisms controlling the uptake and accumulation of these nutrients in seeds.

## Conclusion

Quantitative trait loci for micronutrients accumulations in soybean seed are almost non-existent. Our research detected QTLs associated with the accumulation of Fe, Zn, B, Cu, and Mn in seeds, and these QTLs are new, therefore, they are additional QTLs, contributing to further knowledge of the genetic basis of seed mineral nutrition. The clustering of QTLs associated with Zn, Cu, Fe, and Mn in similar regions of LGs suggest possible common physiological and genetic mechanisms. Further research is needed to confirm this observation by growing the population under different environments. The positive correlation between seed nutrients suggests that to obtain high need nutrition qualities, it is crucial to maintain high levels of all nutrients in seed. This research would allow to use these QTLs in marker-assistant selection to improve seed nutrition trait and help breeding programs to efficiently select for appropriate levels of micronutrients in seeds to meet human nutrition needs.

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