

Genetic Mapping of QTL Associated with Seed Macronutrients Accumulation in ‘MD 96-5722’ by ‘Spencer’ Recombinant Inbred Lines of Soybean

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Abstract

Research of quantitative trait loci (QTL) for macronutrient accumulation in soybean seed is limited. Therefore, the objective of this research was to identify QTL related to macronutrients (N, C, S, P, K, Ca, and Mg) in seeds in 92 F_{5,7} recombinant inbred lines (RILs) developed from a cross between MD 96-5722 (MD) and Spencer using a total 5,376 Single Nucleotide Polymorphism (SNP) markers. A genetic linkage map based on 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 8 QTL for K (qPOT001-qPOT008) were identified on LGs D1b (Chr 1), N (Chr 3), A1 (Chr 5), O (Chr 10), F (Chr 13), B2 (Chr 14), and J (Chr 16). Four QTL for Mg (qMAG001-qMAG004) were identified on LGs N (Chr 3), A1 (Chr 5), J (Chr 16), and G (Chr 18). One QTL for P (qPHO001), one for C on LG J (Chr 16), one for N (qNIT001) and S (qSUL001) on the same LG J (Chr 16), and one QTL for Ca (qCAL001) on LG G (Chr 18). K and Mg QTL were clustered together on LG A1 (Chr 5) with a peak position of 9.50 cM and LOD support interval of 8.50-9.50 cM. Similar observation was noticed for P, K, Mg, C, N, and S, where the QTL were clustered on LG J (Chr 16) with peak position of 11 cM for K, P, and S, and 10

cM for C and N, and 12 cM for Mg. The LOD support intervals for all these clustered QTL were between 8.90 and 12.30 cM. The QTL clustering of these nutrients suggests possible common physiological and genetic relationships, suggesting possible similar metabolic processes and pathways for these nutrients. The inverse relationships between N:S ratio and all nutrients suggest possible use of N:S ratio as a measure for higher nutrients accumulation in seed. Since most of QTL identified in this study were not previously reported, this research will further help breeders to improve nutrient accumulation in seeds and contribute to our understanding of the physiological and genetic bases of seed nutrition quality.

Keywords: SNPs markers, RILs, nutrients, minerals, genetic mapping, seed composition.

Introduction

Macronutrients such as N, C, S, P, K, Mg, and Ca are essential for plant growth, development, and production, and their deficiency in soil leads to yield loss, disease infection, and poor seed quality. Metabolic and physiological roles of these nutrients were well documented (Mengel and Kirkby, 1982; Barker and

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Pilbeam, 2007; Marschner, 2012). For example, N is involved in protein synthesis and protein storage, nucleic acid (DNA and RNA), phytohormones, co-enzymes, and energy transfer systems. Sulfur is involved in enzymes, co-enzymes, S-containing amino acids such as cysteine and methionine (two amino acids that are deficient in soybean cultivars). Phosphorus role in plants includes its involvement in cell membrane, lipid synthesis, energy transfer as ATP and NADP-H, and phosphorylation reactions, nucleic acids (DNA and RNA), carbohydrate metabolism, and nutrient active uptake processes. Magnesium is important for chlorophyll molecule, osmoregulation, cell development, activation of ATPase and enzyme molecules (Balke and Hodges, 1975), activation of ribulose biphosphate carboxylase (Lilley and Walker, 1975), and CO₂ assimilation and carbohydrate production. Calcium is an important mineral for membrane permeability, cell structure, cell membrane integrity, and stabilization. Calcium deficiency leads to a leaky and impaired membrane (Marinos, 1962; Van Steveninck, 1965). It has a role in germination and growth of pollen, cell nucleus matrix (Wunderlich, 1978), interaction with protein bound-membrane enzymes to make Ca-protein. The process of Ca-protein complex formation is regulated by cellular flux of Ca. Potassium is essential for osmoregulation, stomatal closure, carbohydrate movement, nutrient mobility and uptake, photorespiration and CO₂ fixation, activation of enzymes, translocation and mobilization of stored metabolites, and ATPase activity stimulation.

Macronutrient accumulation in seeds determines the nutritional value of seeds. For example, the major storage form for P in seeds of plants is myo-inositol-1,2,3,4,5,6-hexakisphosphate (IP6, or phytic acid), and this compound often chelates metal ions such as Ca, Mn, Zn, and Fe (Raboy, 1997), and the relationship between the accumulation of P in seed and minerals such as Ca, Mg, Fe, Mn, Cu is not well known. Calcium in seeds determines the quality of soyfoods such as tofu, natto, and miso (Mullin and Xu, 2001; Zhang et al., 2009). The accumulation of nutrients in seed involves complex physiological and metabolic process starting from their uptake through translocation and distribution, to their movement from leaves to seed. In spite of the enormous efforts devoted to plant nutrition research, mechanisms controlling the movement of nutrients within plants and from leaves to seeds are still not well understood. This is due to a complex process of nutrient metabolism that are affected by several biotic and abiotic stress factors such as drought, heat, disease, and nutrients levels in the soil and their availability. Therefore, understanding the physiological and genetic bases of nutrients accumulation in seeds and genetic factor controlling the accumulation of nutrients in seeds is critical for breeding selection for higher seed nutrition qualities.

Mapping QTL on the genome is becoming increasingly important in modern breeding programs through marker-assisted selection (MAS) and gene discovery (Price, 2006). There are many successful examples of using QTL to facilitate crop cultivar development, especially for disease resistance (Beaver and Osorno, 2009; Swarbrick et al., 2009) and quality improvement (Blair et al., 2009; Sabouri, 2009). However, information on genetic mapping for macronutrients accumulation in seeds is scarce (Zhang et al., 2009), and what is available deals with other

species such as seed P QTL in Andean bean (Cichy et al., 2009); K QTL related to K efficiency in wheat (Kong et al., 2013); nutrients QTL in Arabidopsis (Chardon et al., 2014); QTL associated with P in seed in mungbean (Sompong et al., 2012); QTL related to P in seed in common bean (Blair et al., 2009); QTL in seed minerals in Brassica napus (Ding et al., 2010) mostly deals with macronutrients and their status in soybean leaves, shoot, or roots, but not in seeds. For example, QTL of P nutrition in relation to root morphology and physiology have been reported (Beebe et al., 2006; Li et al., 2007; Chen et al., 2009; Cichy et al., 2009; Li et al., 2009). Liang et al. (2010), using a population of 106 F₉ recombinant inbred lines (RILs) derived from a cross between BD2 and BX10 and 296 simple sequence repeat (SSR) markers, found a total of 31 QTL controlling root traits and P efficiency in soybean. Among them 13 QTL were found for root traits and 18 for P efficiency parameters. Yuste-Lisbona et al. (2014) studied the epistatic QTL using a population of 185 RILs of a cross involved an adapted common bean (PMB0225 parent) and an exotic nuña bean (PHA1037 parent), and they found 59 QTL on all linkage groups (LGs), 18 of them had individual additive effects, 27 had epistatic effects, and 14 had both effects. It was also found that the detected QTL explained from 8 to 68 % and 2 to 15 % of the additive and epistatic effects, respectively.

Zhang et al. (2009) studied QTL associated with seed calcium content (hardness) in 178 F_{2:3} and 157 F_{2:4} lines derived from a cross of SS-516 (low calcium) x Camp (high calcium) using 148 simple sequence repeat markers (SSR). They found four QTL (Ca 1, Ca 2, Ca 3, and Ca 4) on LGs A2, I, and M, identified by both single-marker analysis and composite-interval mapping, and found that the QTL accounted for 10.7%, 16.3%, 14.9%, and 9.7% of calcium content variation, respectively. Hard seeds do not absorb water during the soaking process and cause problems for soybean sprouting and natto manufacturing (Mullin and Xu, 2001) and hard seed determine soyfoods texture (Trumbull, 1992). Studying Ca levels in seeds determines the quality of tofu, natto, and miso, which make up nearly 90% of the food quality soybean market (Maughan et al., 2000). Also, it was found that Ca in the seed coat to be positively correlated with water absorption (Saio et al., 1973; Saio, 1976). Three QTL for calcium uptake were identified and accounted for 51.8% of the phenotypic variation, and 3 survivorship QTL were found to explain 78% of the calcium uptake (Lexer et al., 2003).

Because of the limited information on genetic mapping of soybean seed minerals QTL (Zhang et al., 2009; King et al., 2013; Ramamurthy et al., 2014), the objective of this research was to identify QTL for macronutrient concentrations in seed in 92 F_{5:7} recombinant inbred lines developed from a cross between MD 96-5722 and Spencer using a total 5,376 Single Nucleotide Polymorphism (SNPs) markers. The genetic mapping of seed nutrients by identifying QTL controlling seed nutrient levels in seeds and their associated molecular markers on the genome could lead to an efficient soybean breeding to select for appropriate seed nutrient levels using marker-assistant selection (MAS) (Chang et al., 2009). The information obtained from this research will provide a fundamental basis for future research on genetic improvement of soybean seed nutrition qualities.

Materials and Methods

Plant Material and Growth Conditions

A recombinant inbred lines (RILs) population of 92 $F_{5.7}$ was developed by a cross between MD 96-5722 (MD) and Spencer to obtain phenotypic and genotypic data. The cross was made in 2004 by Southern Illinois University at Carbondale (SIUC) Breeding Program and advanced to the $F_{5.7}$ generation by single-pod descent method. Soybean lines were 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^{-1} . No fertilizers were added to soil and no insecticide applications were made. The soybean population was developed as previously reported by Akond et al. (2013). Soybean seed were collected at maturity (R8) for macronutrient (N, C, S, P, K, Mg, P, and Ca) quantification as described below.

Phosphorus Measurement

The concentrations of P in seeds were determined at seed maturity (R8). Phosphorus concentration was measured spectrophotometrically using the yellow phosphor-vanado-molybdate complex according to Cavell (1955). Briefly, a dried ground seed sample of 2 g was ashed to completely destroy organic matter, and after ashing, 10 ml of 6 M HCl was added and the sample was placed in a water bath to evaporate the solution to dryness. After drying, 2 ml of 36% v/v HCl were added; the sample was boiled under the heat. Then, 10 ml of distilled water was added, and the solution was then boiled for a few seconds, transferred to a 50-mL volumetric flask, diluted to 50 mL with distilled water, and filtered. A volume of 2 ml of filtrate was first discarded and the remainder was kept for P analysis. A volume of 5 ml of 5 M HCl and 5 ml of ammonium molybdate–ammonium metavanadate reagent were added to 5 ml of the filtrate, and the solution was diluted with distilled water to 50 ml. The solution was then allowed to stand for 30 minutes before measurement. Ammonium molybdate–ammonium metavanadate was made by dissolving 25 g of ammonium molybdate and 1.25 g of ammonium metavanadate in 500 ml of distilled water. The standard curve was made from P standard solutions (0–50 $\mu g ml^{-1}$ of P) using dihydrogen orthophosphates. Phosphorus concentrations were determined using a Beckman Coulter DU 800 spectrophotometer at 400 nm.

Seed Minerals, N, S, and C Analysis

Seed samples were collected at maturity stage (R8) and were analyzed for minerals, N, S, and C by digesting 0.6 g of dried, ground plant materials in HNO_3 in a microwave digestion system. Samples were ground using a Laboratory Mill 3600 (Perten, Springfield, IL USA), and the concentration of K, Ca, Mg was determined using inductively coupled plasma spectrometry at The University of Georgia's Soil, Plant, and Water Laboratory, Athens, GA, and detailed by Bellaloui et al., 2011, 2014. For N, C, and S measurements, a 0.25 g ground-dried sample was combusted in an oxygen atmosphere at 1350 °C, convert-

ing elemental N, S, and C into N_2 , SO_2 , and CO_2 , respectively. These gases were then passed through infrared cells and N, S, and C are determined by an elemental analyzer using thermal conductivity cells (LECO CNS-2000 elemental analyzer, LECO Corporation, St. Joseph, MI USA) at The University of Georgia's Soil, Plant, and Water Laboratory, Athens, GA, as detailed by Bellaloui et al. (2011, 2014).

Genetic Map Construction and QTL Identification

The genetic linkage map of the MD 96-5722 by Spencer was constructed using SoySNP6K Illumina Infinium BeadChip array (Akond et al., 2013) and used to identify QTL for seed macronutrients quantification. The RILs were genotyped using 5,376 Single Nucleotide Polymorphism (SNP) markers using the Illumina Infinium SoySNP6K BeadChip array (Akond et al., 2013). A genetic linkage map was constructed based on 537 polymorphic SNP markers (Akond et al., 2013). The analysis of QTL was conducted using the Composite Interval Mapping (CIM) of WinQTLCart 2.5 (<http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>) (Wang et al., 2014). 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 WinQTLCart (Wang et al., 2014). Permutations of 1,000 were used as threshold. Analysis of Means (CV, maximum and minimum values, and SD) were carried out using Proc Means in SAS. Coefficient of correlation was conducted by SAS using PROC REG.

Results and Discussion

Variation and Correlations

The range of variations of nutrient accumulation in seeds in the population is dependent on the nutrient type (Table 1). The lowest coefficient of variation was noticed for C and N, and the highest was in Ca and S, K, Mg (Table 1). The concentrations of nutrients in MD parent fell within the range of the population lines. However, for the parent Spencer, the nutrient accumulation of Ca, K, Mg, and P fell within the range of the population lines, but N, C, and S did not. Minerals (Ca, K, Mg, and P) were significantly ($P < 0.0001$) correlated compared with non-minerals (C, N, and S) (Table 2), and the pattern of this correlation was positive either for minerals or non-minerals (Figures 1 and 2).

Table 1. Mean, standard deviation (SD), minimum and maximum values of macronutrient concentrations, and coefficient of variation (CV) of soybean RIL population and its parents (Maryland, MD, and Spencer).

Nutrient	RILs					Parents		
	Mean	SD	Minimum	Maximum	Differences (%)	CV	MD	Spencer
Ca	0.39	0.06	0.23	0.56	143.5	14.13	0.54	0.53
K	1.77	0.19	1.12	2.17	93.8	10.73	1.58	1.56
Mg	0.25	0.03	0.16	0.33	106.3	10.33	0.26	0.25
P	0.52	0.05	0.32	0.68	112.5	9.86	0.60	0.63
C	50.84	0.63	49.4	52.20	5.63	1.24	49.22	49.2
N	6.29	0.31	5.53	6.79	22.8	4.92	6.74	6.82
S	0.25	0.03	0.16	0.31	93.8	14.09	0.29	0.33

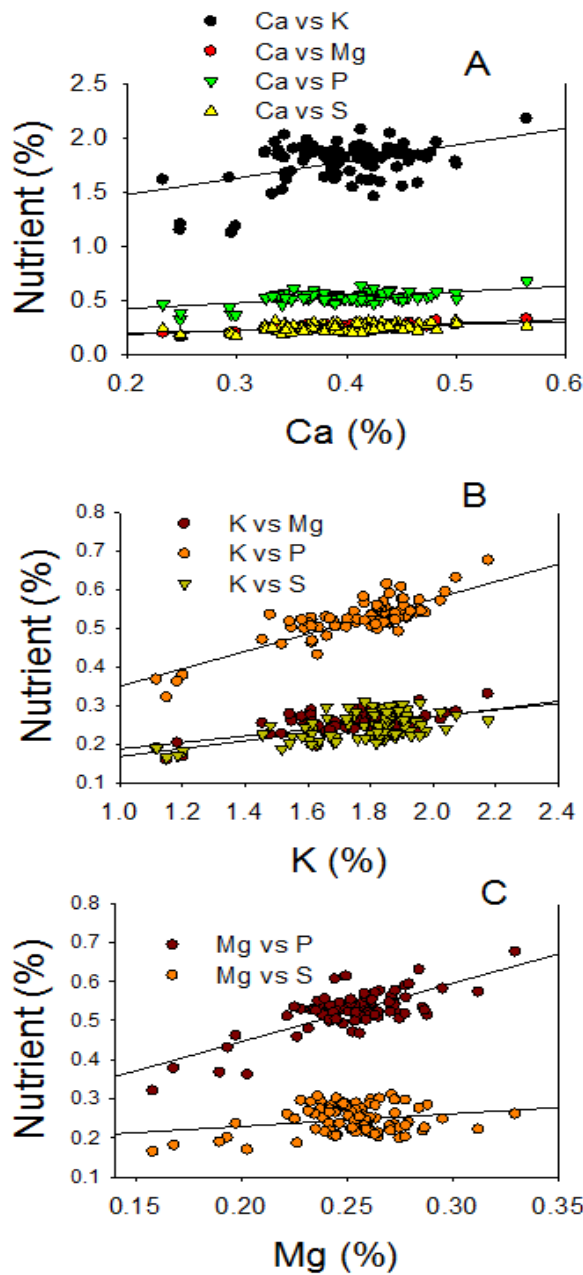


Figure 1. Patterns of correlation between seed macronutrient and Ca (A), K (B), and Mg (C) concentrations in a population of 92 $F_{5,7}$ recombinant inbred lines (RILs).

Frequency distribution showed, generally, a normal distribution (Figures 3, 4). The wide range of nutrient concentrations in seeds of individual lines was expected as the accumulation of nutrients in seed was reported to be affected by genotype differences (White and Broadley, 2009; Bellaloui et al., 2011), and could be due to the uptake, transport, accumulation, and distribution of nutrients within the plant. The wide range of nutrient accumulation would give breeders an opportunity to select for more efficient genotypes for macronutrients. The differences of coefficient of variation between nutrients in the population

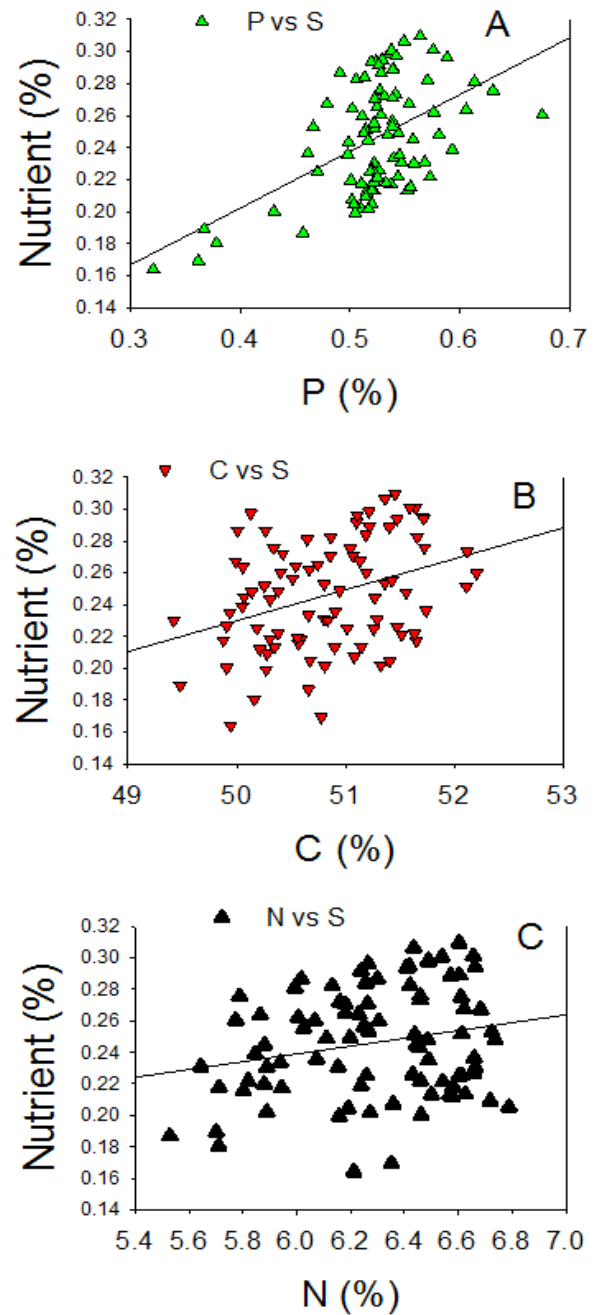


Figure 2. Patterns of correlation between seed macronutrient and P (A), C (B), and N (C) concentrations in a population of 92 $F_{5,7}$ recombinant inbred lines (RILs).

indicated the different level of sensitivity of each nutrient to intrinsic and extrinsic factors.

Genetic analysis (Figure 5) showed that 8 QTL for K (qPOT001-qPOT008) were identified on LGs D1b (Chr 1), N (Chr 3), A1 (Chr 5), O (Chr 10), F (Chr 13), B2 (Chr 14), and J (Chr 16). Four QTL for Mg (qMAG001-qMAG004) were identified on LGs N (Chr 3), A1 (Chr 5), J (Chr 16), and G (Chr 18). One QTL for P (qPHO001), N (qNIT001), and S (qSUL001) on the same LG J (Chr 16), and one QTL for Ca (qCAL001) on LG G (Chr 18) (Table 3). QTL for K and Mg were clustered together

Table 2. Correlation between seed nutrient concentrations in 92 F5:7 recombinant inbred lines developed from a cross between Maryland (MD) 96-5722 and Spencer. Level of significance was at $P \leq 0.05$.

Nutrients	Ca	K	Mg	P	C	N	S	N:S Ratio
Ca	1							
K	0.451 <.0001	1						
Mg	0.733 <.0001	0.500 <.0001	1					
P	0.550 <.0001	0.83489 <.0001	0.749 <.0001	1				
C	-0.0066 NS	0.0946 NS	-0.0049 NS	0.12531 NS	1			
N	0.06546 NS	-0.03978 NS	-0.0216 NS	-0.0660 NS	0.2097 NS	1		
S	0.43941 <.0001	0.56612 <.0001	0.25681 0.0157	0.5341 <.0001	0.340 0.001	0.221 0.039	1	
N:S Ratio	-0.452 <.0001	-0.640 <.0001	-0.329 0.0017	-0.623 <.0001	-0.291 0.006	0.129 0.231	-0.924 <.0001	1

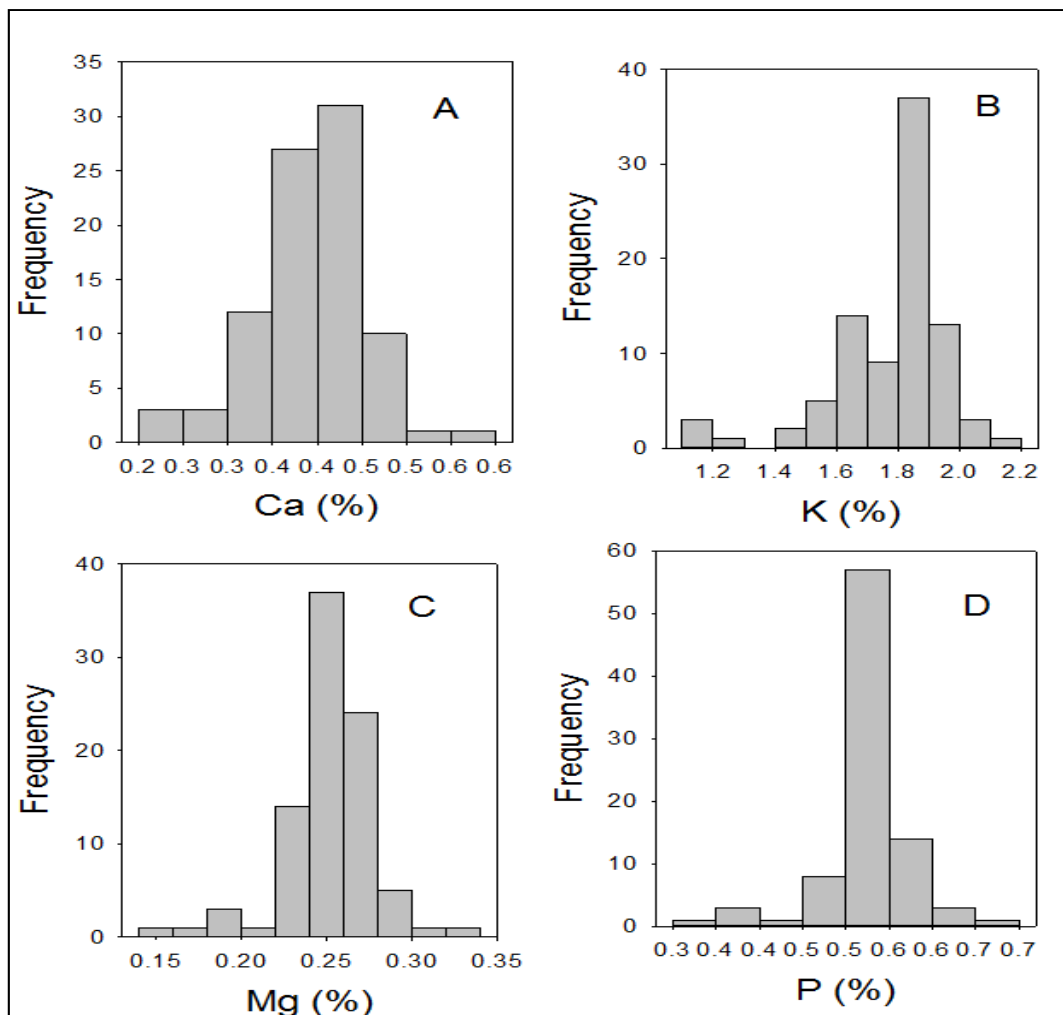


Figure 3. Frequency distribution for seed Ca (A), K (B), Mg (C), and P (D) in the MD 96-5722 by 'Spencer' RIL population in soybean.

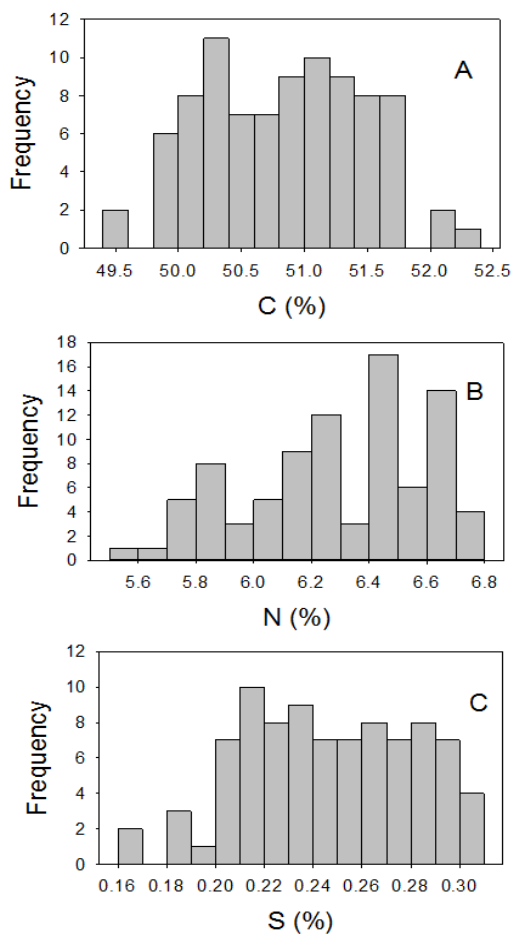


Figure 4. Frequency distribution for seed C (A), N (B), and S (C) in the MD 96-5722 by 'Spencer' RIL population in soybean.

on LG A1 (Chr 5) with a peak position 9.50 cM and LOD support interval of 8.50-9.50 cM. Similar observation was noticed for P, K, Mg, C, N, S where the QTL were clustered on LG J (Chr 16) with peak position of 11 cM for K, P, and S, and 10 cM for C and N, and 12 cM for Mg. The LOD support intervals for all these QTL were between 8.90-12.30 cM (Table 3).

The identification of QTL for macronutrients is very limited, and almost non-existent for nutrient accumulation in soybean seed. Most of the researchers identified QTL associated with leaves, roots, and shoot, but very few on seeds. Searching GRIN Soybean base, it was found that Zhang et al. (2009) detected four QTL for seed calcium (Ca 1-Ca 4) on A2, I, M with the optimum position of 16.4 on A2; 9.2 cM on I; 96.7 on M, and 92.7cM on M, with the position of flanking markers on the consensus map positioned at 116.3-154.11 cM on A2; 36.59-46.22 cM on I; 5.6-1.0 cM on M; and 75.6-1.0 cM on M. For example, Li et al. (2005) found two QTL for leaf P in soybean on LGs F at genomic map position between 11.37-16.08 and 0-3.86 cM. Liang et al. (2010) found 7 QTL associated with shoot and root P in soybean on LGs D2, B1, and F; Specht et al. (2001) found 5 QTL related to carbon isotope for drought tolerance in soybean on LGs C2, D2, F, and I; and Panthee et al. (2004), using

a population of 101 $F_{6,8}$ recombinant inbred lines (RIL), found 17 QTL for soybean seed N on LGs A2, B2, D1b, E, G, M, D1a, and G. They found 3 QTL on LGs A2, 2 on B2, 2 on D1b, 5 on E, 3 on G, 1 on M, and 1 on D1a. They found that phenotypic variation explained by an individual QTL ranged from 5 to 11.6% and concluded that these QTL and markers associated with them may be useful for soybean seed protein improvement. The QTL reported by Panthee et al. (2004) were detected at developing stages of seeds (7 QTL were detected at R5; 3 QTL at R6 stage; and 7 QTL at R7 stage). In the current study, one QTL was detected on LG J (Chr 16) and it is different from those reported by Panthee et al. (2004) and Ramamurthy et al. (2014) as QTL found by Panthee et al. were detected at R5, R6, R7 stages, which characterize developing seeds and not mature seed at R8 stage at which the full seed-fill and seed physiological maturity was reached. In addition, the QTL for N, reported here, was found clustered with S, Ca, P, C, Mg, and K, suggesting close relationships between these nutrients and N. The close relationship between these minerals was reflected with the highly significant positive correlation (Table 2) as the increase pattern indicated the increase of one nutrients leads to the increase in the other (Figures 1, 2). The QTL clustering of these nutrients suggests similar physiological and metabolic processes. Ramamurthy et al. (2014) found 2 QTL for S on LG J (16) and LG G (Chr 18). We detected one QTL for S on LG J (Chr 16) at a peak position of 11.00 cM with LOD support interval of 9.90-11.10 cM and marker interval of ss248983974- ss248977568. The current QTL for S could be different from that detected by Ramamurthy et al. (2014) as the peak position of their QTL was 66.3 cM.

Zhang et al. (2009) found twenty-two markers on 8 LGs associated with seed Ca content among which six markers on each of LGs A2 and I, 3 markers on LG L, 2 markers on each of LGs B1 and M, and 1 marker on each of LGs D2, F, and G, and one marker on each of LGs D2, I, and M. They reported that the QTL found in this study can be considered as preliminary research on MAS for low Ca content in natto soybean breeding. We were able to detect one Ca QTL on LG G (Chr 18) with a peak position of 13.20, interval position of 10.70-15.60 cM, and markers intervals of ss249715368- ss249830195. This QTL was not previously reported as Zhang et al. (2009) detected four QTL for seed calcium (Ca 1-Ca 4) on A2, I, M with the optimum position of 16.4 on A2; 9.2 cM on I; 96.7 on M, and 92.7cM on M, with the position of flanking markers on the consensus map of 116.3-154.11 cM on A2; 36.59-46.22 cM on I; 5.6-1.0 on M, and 75.6-1.0 on M. Also, we were able to detect four QTL for Mg on LGs N, A, J, and G, which were different from those reported by Ramamurthy et al. (2014), who found one QTL on LG N (Chr 3). Therefore, the current reported QTL for Mg are new findings. Potassium QTL were previously reported by Ramamurthy et al. (2014) who found four QTL on LGs C1, E, J, and G. We detected 8 QTL for seed K on 7 LGs (D1b, N, A1, O, F, B2, and J). Except for QTL on LG J, the rest of QTL were not previously reported, contributing to our understanding of the genetic bases of seed K nutrition. One C QTL was detected in the current research on LG J (Chr 16) with peak position of 10.00 cM and LOD support interval of 9.00-10.00 cM and marker interval of ss248983974- ss248977568. No seed C QTL were previously

Table 3. Chromosomal locations and parameters associated with the quantitative trait loci (QTL) for seed mineral accumulation in 92 F_{5,7} recombinant inbred lines developed from a cross between MD 96-5722 and Spencer.

Trait	QTL	LG /Chr	†Peak Position (cM)	‡2-LOD support interval (cM)	Marker interval	§Peak LOD	#R ² (%)	††Additive effects
Potassium	qPOT001	D1b /Chr_1	4.50	4.40-4.60	ss244503611-ss244513054	2.63	0.02	-0.04
Potassium	qPOT002	D1b /Chr_1	12.10	11.10-13.10	ss244525143-ss244552583	2.81	0.02	0.03
Potassium	qPOT003	N /Chr_3	16.40	16.00-16.40	ss245025187-ss245021991	9.88	0.04	0.01
Magnesium	qMAG001	N /Chr_3	9.00	8.10-9.10	ss244947608-ss244967332	8.26	0.91	0.13
Potassium	qPOT004	A1 /Chr_5	9.50	8.50-9.50	ss245747167- ss245786667	3.43	0.69	0.75
Magnesium	qMAG002	A1 /Chr_5	9.50	8.50-9.50	ss245747167- ss245786667	8.68	0.02	-0.02
Potassium	qPOT005	O /Chr_10	5.30	4.30-6.30	ss247085505- ss247098566	11.82	0.03	6.36
Potassium	qPOT006	F /Chr_13	2.70	2.50-2.80	ss247942156- ss247937719	8.55	0.01	0.05
Potassium	qPOT007	B2 /Chr_14	8.00	5.80-9.20	ss248293401- ss248275088	7.25	0.57	0.91
Potassium	qPOT008	J /Chr_16	11.00	9.90-12.90	ss248983974- ss248977568	3.24	0.05	0.34
Magnesium	qMAG004	J /Chr_16	12.00	11.00-12.30	ss248983974- ss248977568	8.49	0.94	0.25
Phosphorus	qPHO001	J /Chr_16	11.00	9.90-11.00	ss248983974- ss248977568	9.49	0.94	0.25
Carbon	qCAR001	J /Chr_16	10.00	9.00-10.00	ss248983974- ss248977568	10.01	0.94	24.93
Nitrogen	qNIT001	J /Chr_16	10.00	8.90-10.00	ss248983974- ss248977568	9.85	0.94	3.25
Sulfur	qSUL001	J /Chr_16	11.00	9.90-11.10	ss248983974- ss248977568	9.73	0.94	0.15
Calcium	qCAL001	G /Chr_18	13.20	10.70-15.60	ss249715368- ss249830195	2.72	0.66	0.01
Magnesium	qMAG005	G /Chr_18	2.20	2.10-2.20	ss249623816- ss249632893	8.59	0.95	0.13

†Position of peak LOD value on composite maps described previously (Coles et al., 2010);‡The positions that define the two LOD intervals around the position of peak likelihood for the QTL; §The log of odds (LOD) value at the position of peak likelihood of the QTL; #R² estimates the proportion of RIL mean variance (%) explained by the detected QTL; †† 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.

reported, hence the current QTL is new finding. It must be noted here 4 QTL associated with C isotopes related to drought trait and water efficiency use were reported (Specht et al., 2001). No QTL for seed K, Mg, or S were found in Soybean Base (<http://www.soybase.org/search/>). Recently, Ramamurthy et al. (2014) identified QTL for seed mineral concentrations in three soybean recombinant inbred line mapping populations derived from crossing Williams 82 × DSR-173, Williams 82 × NKS19-90 and Williams 82 × Vinton 81. Using a joint linkage map from these populations, Ramamurthy et al. (2014) detected forty QTL for 18 traits including seed Mg, S, K, Ca, N:S; one QTL for Mg on Chr 3, two for S on Chr 16 and 18, two for K on Chr 4, 15, 16, and 18, one for Ca on Chr 6, two for N on Chr 14, 15, 16, and 18, and three for N:S on Chr 16, 20, and 3. They found an inverse relationship between N:S ratio and most nutrients, and suggested that the N:S ratio can be used as an indirect measure of seed mineral concentration in soybean breeding programs. Our finding agreed with Ramamurthy et al. (2014) in that we found an inverse relationship (correlation) between N:S ratio and all nutrients investigated (Table 2), and QTL detected in

the current research for seed nutrients were different from theirs for the reasons previously explained above. Genetic mapping for P was reported by King et al. (2013), who identified candidate gene(s) involved in phosphate metabolism and transport, and P accumulation in soybean seed. They were able to identify one putative QTL region on Chr 12, and this region contained a phosphate transporter gene. Also, they identified two additional suggestive QTL on Chr 7 and 17, with Chr 7 having both a phosphate transport gene and a ZIP transporter gene in the region of the QTL. King et al. (2013) reported, using combined total P QTL, 6 QTL on LGs O (Chr 10), G (Chr 18), I (Chr 20), M (Chr 7), H (Chr12), D2 (17). We were able to identify one QTL for seed P accumulation on LG J (Chr 16) that was not previously reported, suggesting that the current P QTL is a new finding. No QTL for seed K, Mg, P, and S were reported in soybean Base (<http://www.soybase.org/search/>), indicating further research is needed in the area of genetic mapping for seed mineral nutrition (King et al., 2013; Ramamurthy et al., 2014).

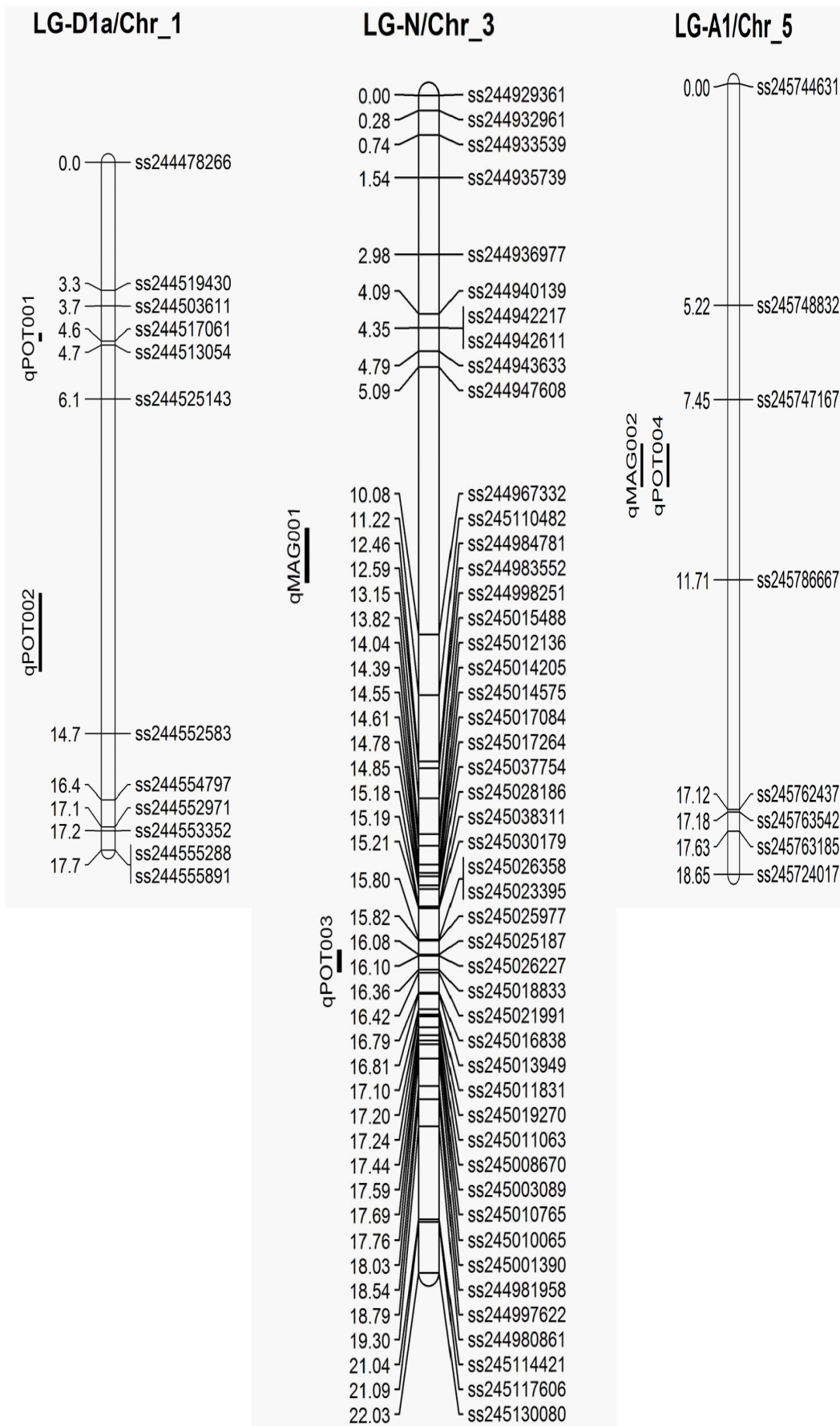


Figure 5. Locations of QTLs and SNP markers associated with seed macronutrients accumulation.

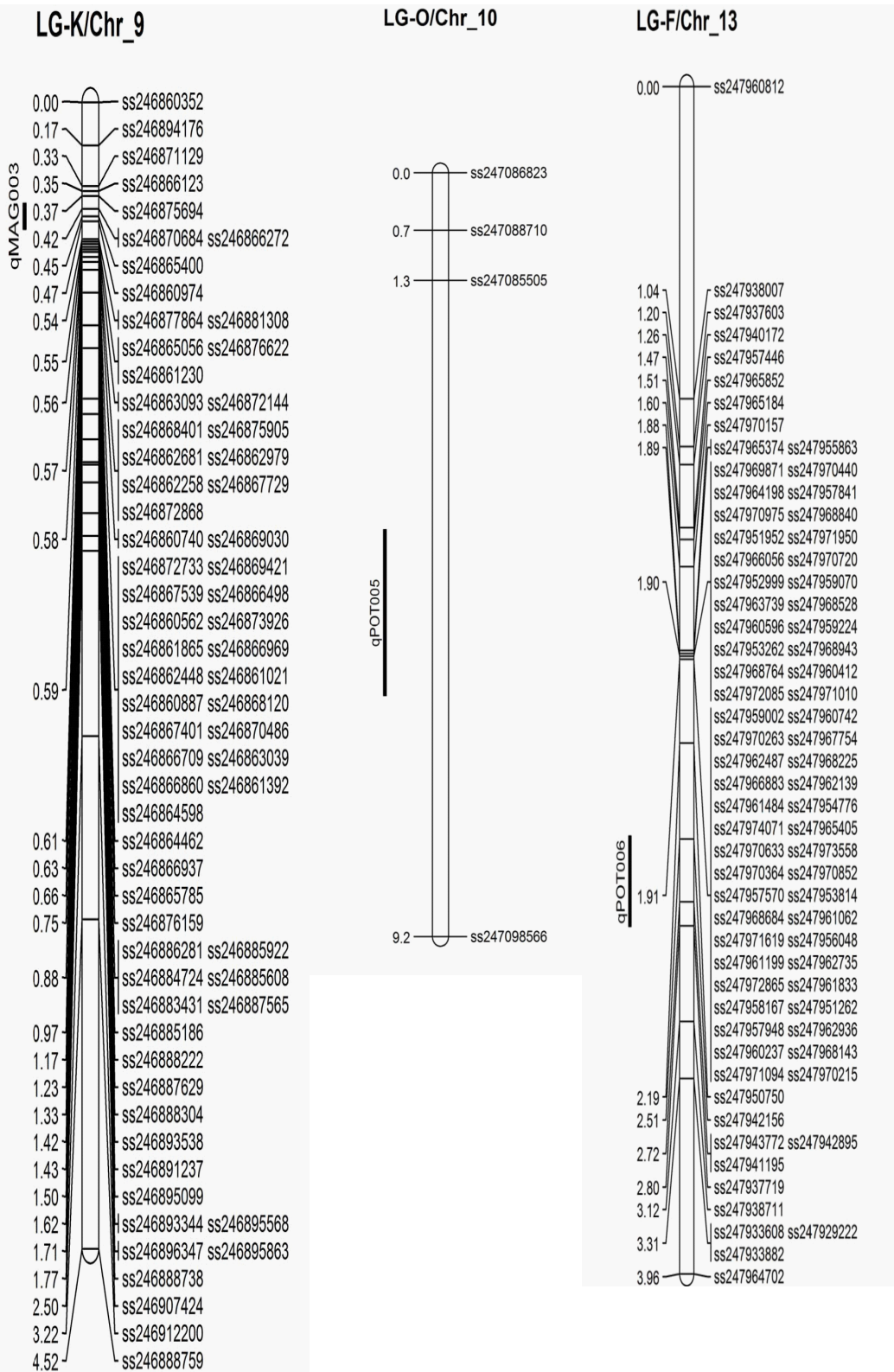


Figure 5. Continued.

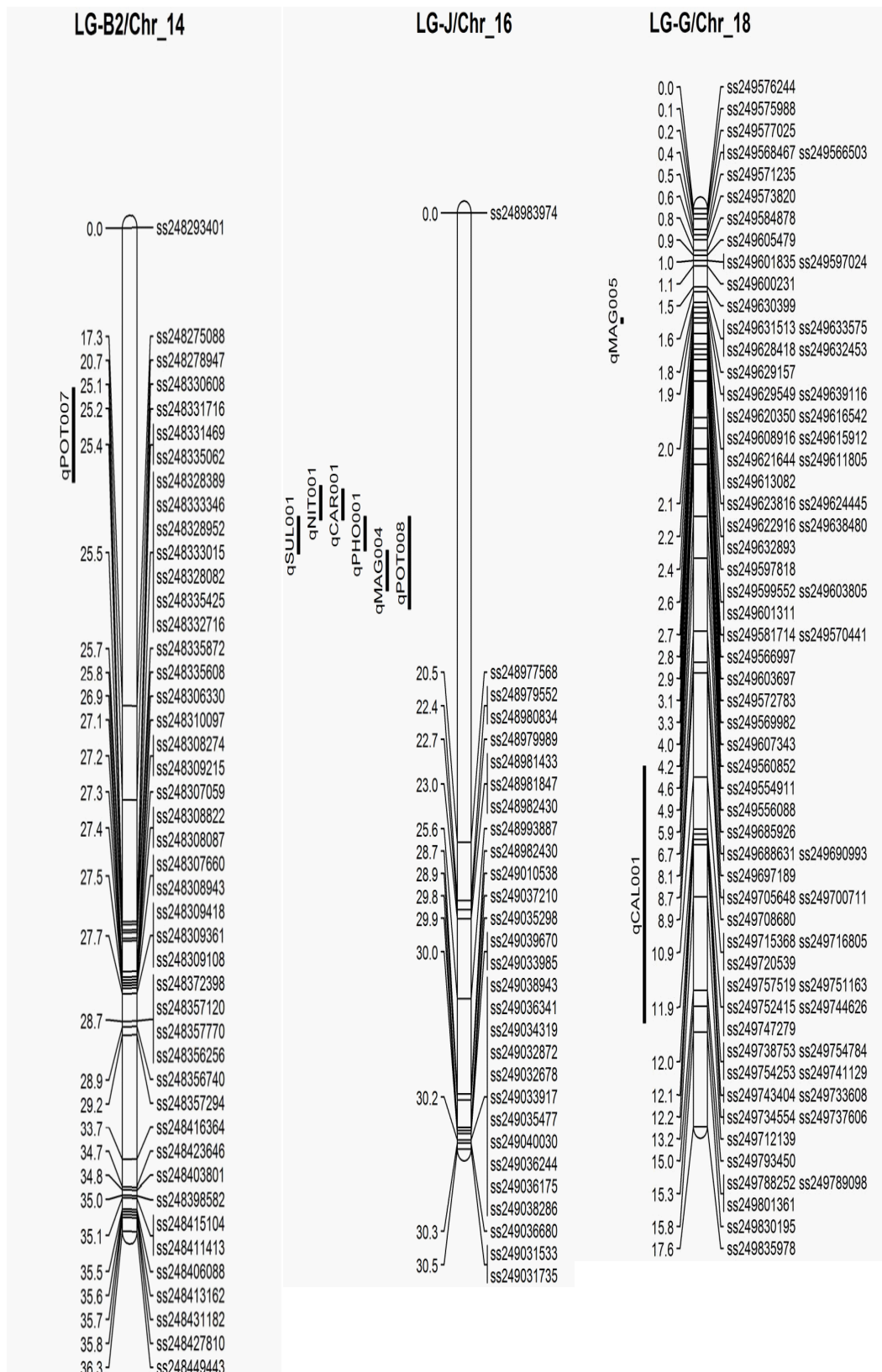


Figure 5. Continued.

Conclusions

Most of the research conducted on the genetic mapping for plant nutrition was associated with leaves, roots, or shoot, but not on nutrient accumulation in seeds. Our current research contributes to the limited available information (King et al., 2013; Ramamurthy et al., 2014) on genetic mapping of nutrient accumulation in soybean seeds. The current research showed that several QTL for macronutrients were detected. The genetic analyses resulted in 8 QTL for K (qPOT001-qPOT008), four QTL for Mg (qMAG001-qMAG004), one QTL for P (qPHO001), one QTL for N (qNIT001), one QTL for S (qSUL001), and one QTL for C (qCAL001). Most of these QTL are new, contributing to the previously reported QTL. QTL clustering between K and Mg, and between P, K, Mg, C, N, and S indicates physiological and genetic relationships between these nutrients, suggesting possible similar metabolic processes. Information obtained from this research would help breeders to select for higher seed mineral quality, and physiologist to further understand the physiological and genetics based of seed mineral accumulation.

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