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Identification of new QTLs for seed mineral, cysteine, and methionine concentrations in soybean [*Glycine max* (L.) Merr.]

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Abstract
Increased concentrations of important nutrients in edible parts of plants could result in biofortified foods. Soybean [*Glycine max* (L.) Merr.] is a major legume crop and an important source of certain nutrients, including protein and minerals, in human and animal diets. Understanding the underlying genetic basis of seed composition is crucial to improving seed nutrient composition. In this study we used three soybean recombinant inbred line mapping populations derived from the crosses Williams 82 × DSR-173, Williams 82 × NKS19-90 and Williams 82 × Vinton 81, and constructed a joint linkage map from these populations. Forty quantitative trait loci (QTLs) were detected for 18 traits: seed weight, seed magnesium, sulfur, calcium, manganese, potassium, iron, cobalt, nickel, copper, zinc, selenium, molybdenum, cadmium and arsenic concentrations, total nitrogen:total sulfur (N:S) ratio, cysteine, and methionine concentrations. Using the joint linkage map, we detected nine QTLs that were not identified in the individual populations. We identified several candidate genes that might contribute to these traits, including transporters and genes involved in nitrogen and amino acid metabolism. Some strong QTLs had no obvious candidate genes, offering the possibility that subsequent confirmation of these QTLs may result in identification of new genes affecting seed nutrients in soybean. Seed weight and seed mineral concentrations were not highly correlated, suggesting the possibility of improving seed mineral concentrations without significant changes in seed weight. An inverse relationship between N:S ratio and most other minerals suggests the possibility of using N:S ratio as an indirect measure of seed mineral concentration in soybean breeding programs.

Keywords: nutrient concentrations, minerals, cysteine + methionine, N:S ratio, mapping

Introduction

Plants are a primary or sole source of nutrients in human and animal diets. A well-diversified plant-based diet can provide all essential nutrients required for human nutrition (Grusak and DellaPenna 1999). However, compared to animal-derived foods, plant-derived foods are low or deficient in some required mineral nutrients

and protein, or have unbalanced amino acid composition in total protein. Diets in developing and impoverished regions are often based on staple crops and can lead to malnutrition (Waters and Sankaran 2011). For this reason, there is great interest in increasing mineral concentrations in edible tissues of plants. The enrichment of edible tissues with mineral nutrients, biofortification, is considered a sustainable and cost-effective approach to alleviating malnutrition and related health problems (Cakmak 2008; Welch and Graham 2004).

Gaining an understanding of the pathways and rate-limiting steps in accumulation of various seed nutrients is a major challenge for biofortification. Initial efforts in biofortification of seeds have focused on overexpression of single genes that affected mineral uptake, transport or storage (Goto et al. 1999; Ramesh et al. 2004; Vasconcelos et al. 2003, 2004, 2006). Analysis of these plants suggested that seed accumulation of minerals is tightly regulated, and overexpression of a single gene was typically not sufficient to increase the accumulation of minerals in seeds (Goto et al. 1999; Ishimaru et al. 2007, 2010; Qu et al. 2005; Vasconcelos et al. 2003, 2004, 2006). It is likely that multiple genes at different steps of translocation or biosynthetic pathways will need to be simultaneously overexpressed to increase seed mineral concentrations (Waters and Sankaran 2011).

Seeds are primary staple food sources. In general, legume seeds have higher concentrations of essential minerals and protein than cereals (White and Broadley 2009). Studies on seed mineral accumulation in legumes have reported significant positive correlations between some of the important minerals, suggesting the possibility of simultaneous improvement of these traits by plant breeding (Raboy et al. 1984; Sankaran et al. 2009). Soybean [*Glycine max* (L.) Merr.] is one of the most widely grown legume crops in the world. Soybean has a fully sequenced genome (Schmutz et al. 2010) and results from soybean studies may be translated to other legumes due to extensive synteny (Bordat et al. 2011; Lucas et al. 2011; McClean et al. 2010; Severin et al. 2011). Soybean seed has approximately 40% protein in commercial cultivars (Kim et al. 2012). However, soybean protein has an unbalanced amino acid composition for monogastric diets with respect to the sulfur (S)-containing amino acids cysteine (Cys) and methionine (Met) (Kim et al. 2012; Panthee et al. 2006a, b). Methionine is an essential amino acid, whereas cysteine is considered semi-essential, because farm animals and humans can convert methionine to cysteine (Kim et al. 2012). The ratios of total nitrogen (N) to total S (N:S ratio), is an indicator of proportional methionine and cysteine concentrations in soybean seeds (Sexton et al. 1998).

Significant intra-species genetic variation for overall seed mineral composition, Cys and Met concentrations may exist in soybean germplasm (Zhang et al. 2010; Panthee et al. 2006a, b; Raboy et al. 1984 and Kleese et al. 1968), which could be exploited for biofortification. Significant variation in seed calcium (Ca) concentrations, ranging from 0.21 to 0.46%, were reported in a diversity study of soybean consisting of 54 US and 51 Asian food-grade cultivars and breeding lines (Zhang et al. 2010). Approximately 1.5- fold differences in seed total phosphorus (P), zinc (Zn), Ca, and magnesium (Mg) were reported in a mineral variability study among lines of *G. max* and *G. soja* (Raboy et al. 1984). Variations in seed P, Mg, Ca, and manganese (Mn) in 10 different varieties of soybean were approximately 1.2- to 1.4-fold (Kleese et al. 1968). Approximately 1.7-fold differences in seed iron (Fe) concentration were observed in a diversity study consisting of 27 soybean genotypes (Moraghan and Helms 2005). A few studies have reported variation in seed Cys and Met (Panthee et al. 2006a, b; Sharma et al. 2011) in soybean. Variation in seed Met and Cys concentrations were reported for eight soybean genotypes, where varieties differed by 2.7-fold for Met and 1.2-fold for Cys concentrations (Sharma et al. 2011).

A comprehensive understanding of the genetics underlying overall seed nutritional composition is lacking in soybean. Studies on understanding genes and processes to improve seed nutritional composition by identifying quantitative trait loci (QTLs) were limited to a few minerals or nutrients in soybean (Jegadeesan et al. 2010; King et al. 2013; Panthee et al. 2006a; Zhang et al. 2009). Four QTLs for seed Ca concentration were detected, one of which accounted for up to 16.3% of variation (Zhang et al. 2009). A major QTL accounted for 57.3% of variation for low cadmium (Cd) in soybean (Jegadeesan et al. 2010). A major QTL for seed Fe concentration explained 21.5% of the phenotypic variation (King et al. 2013). Panthee et al. (2006a, b) reported total S-containing amino acids (Cys + Met) with a population average of 1.26/100 g, in addition to detecting three QTLs, one of which explained up to 12.3% of total

variation. Genomic loci governing seed Cys + Met concentrations in soybean are largely unknown.

One of the knowledge gaps in soybean genetics is the lack of understanding of genes and processes that affect seed composition of nutritionally relevant minerals and/or nutrients. The goal of this study was to identify QTLs for accumulation of 15 minerals, Cys and Met in soybean seeds, in order to eventually understand the genes and processes underlying these traits. We are not aware of any previous studies that focused on identification of QTLs for most of the mineral traits included in this study. We used three soybean recombinant inbred line (RIL) mapping populations to (1) identify QTLs associated with N:S ratio in soybean seeds, (2) identify QTLs that affect accumulation of minerals in soybean seeds and (3) test the association between seed weight and seed mineral concentration of soybean.

Materials and methods

Plant materials

Three RIL populations were developed using three soybean lines DSR-173 (Dairyland Seed Co., Inc, West Bend, WI, USA), NKS19-90 (Novartis Seeds, Inc., Minneapolis, MN, USA) and Vinton 81 (Fehr et al. 1984), and a common parent Williams 82 (Bernard and Cremeens 1988). For ease of referencing the populations, they will be referred to by the variety mated with Williams 82. Three F5-derived populations, Williams 82 × DSR-173 (afterwards called DSR), Williams 82 × NKS19-90 (afterwards called NKS) and Williams 82 × Vinton 81 (afterwards called Vinton) consisted of 93, 100, and 95 individual RILs, respectively (Arahana et al. 2001). The availability of a reference genome sequence for the Williams 82 parent (Schmutz et al. 2010) allowed comparison of QTLs reported on SoyBase (Grant et al. 2010). Vinton 81 is a high-protein line suitable for tofu production. The soybean cultivars Vinton 81, NKS19- 90, and DSR-173 were previously reported to be less susceptible to *Sclerotinia* stem rot (Arahana et al. 2001). The RIL populations were grown in October 2010 at the East Campus Nurseries of the University of Nebraska–Lincoln Agronomy Research Farm. For each population, all RILs and their parents were grown in a single replicate in a completely randomized design with 30 seeds planted per plot. Plots were 0.91 m long followed by a blank alley of 0.61 m with a row spacing of 0.76 m. The cropping system is a corn– soybean rotation, with soybean research plots following a uniform crop of corn. Fertilizers are applied to the uniform bulk crop part of the rotation as needed based on soil test results. No fertilizer application was made to the soybean research plots for this study. Seeds were harvested from each plot in bulk.

Mineral analysis and seed weight

A sample of approximately 50 seeds representing each line were ground in a Knifetec 1095 Sample Mill (FOSS Tecator, S-26321, Hogana, Sweden) for 20 s to obtain flour with uniform particle size prior to wet-ashing digestion. This digestion approach was slightly modified from a previously described method (Waters et al. 2009). Ground soybean seed samples of 0.75 g were weighed into glass tubes and digested in 6 ml nitric acid (trace metal grade, Fisher Scientific, Pittsburgh, PA, USA) overnight, then at 100°C for 1 h. Three ml of 30% hydrogen peroxide was added and samples were digested at 125°C for 1.5 h. The temperature was then increased to 150°C until the samples were evaporated to dryness. Residues were dissolved in 10 ml of 1% nitric acid prepared with water filtered through a MilliQ system (Millipore, Billerica, MA, USA) to 18 MX resistivity. A minimum of two and a maximum of three technical replicates were evaluated for each line. Concentrations of Mg, P, S, Ca, Mn, potassium (K), Fe, cobalt (Co), nickel (Ni), copper (Cu), Zn, selenium (Se), molybdenum (Mo), Cd, and arsenic (As) were determined by inductively coupled plasma–mass spectrometry (ICPMS). A single measurement of weight of 100 seeds was recorded for parents and RILs for all populations.

Total nitrogen, cysteine and methionine concentrations

Samples of ground soybean seeds were sent to University of Missouri Agricultural Experiment Station, Columbia, Missouri, USA for analysis of seed Cys and Met concentrations (AOAC Official Method 982.30 E(b), 2006) and crude protein concentrations (AOAC

Official Method 990.03, 2006). Total nitrogen was determined using combustion analysis (LECO). Cys and Met concentrations were determined using performic acid oxidation/acid hydrolysis.

DNA isolation

Leaf tissue was collected from all RIL and parental lines approximately 45 days after planting. One leaflet from each plant in a row was collected and bulked to retain genetic variability information within a line. Leaf tissue was kept on ice, then frozen at –80°C, then lyophilized. DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The DNA quantity and purity were assessed using spectrophotometry. Stock DNA samples were diluted to 100 ng/µl and stored at –80°C until genotyping.

Genotyping and linkage analysis

RILs from individual populations and parental lines were genotyped with single nucleotide polymorphism (SNP) markers, using the GoldenGate assay for a Universal Soy Linkage Panel (USLP 1.0) consisting of 1,536 SNP markers that are well distributed throughout the 20 chromosomes (Hyten et al. 2010). The 1,536 SNP assays were run by Dr. Perry Cregan's USDA lab in Beltsville, MD, USA. The soybean Consensus 4.0 map was used for positioning SNP markers.

Joint linkage map

Individual genetic maps of the DSR population, the NKS population and the Vinton population, consisting of 350, 391, and 400 polymorphic markers, respectively, were used to construct a joint linkage map consisting of 766 polymorphic markers using Merge- Map software (Wu et al. 2011). The order of markers was largely consistent with the individual population genetic maps.

Data analysis and QTL mapping

Mean mineral concentrations of two or three (a third replicate was performed if the first two were greater than 20% different) technical replicates were used as quantitative values for QTL mapping in individual populations. Means of Cys and Met concentrations from two technical replicates were used for QTL mapping. Means for all the traits were calculated using the MEANS procedure in SAS software (Version 9.2; SAS Institute). Phenotypic correlations between traits were determined using the CORR procedure in SAS. Joint QTL mapping was performed by including phenotypic means and corresponding genotypes for all 288 RILs for each trait. All the data-checking steps for genotyping were performed using R/qtl, and checked data was exported to QTL Cartographer (http:// statgen.ncsu.edu/qtlcart/cartographer.html) for composite interval mapping. The standard model *Zmapqtl* function with a sliding window of 5 cM and a walk speed of 2 cM was used to identify putative QTLs. Empirical threshold LOD scores for all the traits were calculated using 1,000 permutations at the 5% probability level, and only QTLs that passed the empirical threshold are reported.

Candidate gene search

By anchoring the SNP markers around the QTL confidence interval with known positions on the physical map available from phytozome (http:// www.phytozome.net/soybean.php) through SoyBase (http://soybase.org/gbrowse/cgi-bin/gbrowse/gmax1. 01/), putative candidate genes (genes with known or predicted functions that could influence accumulation of a particular nutrient) for various nutrients were identified.

Results

S, N, and N:S ratio

Despite marginal differences in S concentration between the parents (3.5–3.6 mg/g), the range for this trait was high in RILs (up to 6 standard deviations in the NKS and Vinton populations). The distribution of trait values resembled a normal distribution pattern (Supplemental Resource 1). Transgressive segregation was observed for S in all populations (table 1). Significant positive correlations were observed for S and all macronutrients, except N which had no significant association (table 2 and Supplemental Resource 2). Significant positive associations were also observed between S and Cys and Met concentrations in two populations (table 2). Ranges for

Table 1. Descriptive statistics of various traits that affect seed nutritional composition and seed weight in three soybean RIL mapping populations and their parents

Traits	Parental means				Williams 82 × DSR-173				Williams 82 × NKS19-90				Williams 82 × Vinton 81			
	DSR-173	NKS 19-90	Vinton 81	Williams 82	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
Mg (mg/g)	2.6	2.8	2.4	2.1	2.5	0.2	1.9	2.9	2.4	0.2	1.7	2.8	2.6	0.2	2.3	3.1
P (mg/g)	6.4	6.8	6.2	6.0	6.4	0.5	5.4	8.4	6.5	0.4	4.8	7.5	7.1	0.5	6.1	8.6
S (mg/g)	3.6	3.6	3.6	3.5	3.6	0.3	3.1	4.5	3.5	0.2	2.7	3.9	3.8	0.3	3.1	4.9
K (mg/g)	21.0	21.6	20.4	20.6	20.9	1.7	16.8	27.3	20.4	1.1	16.6	22.9	21.8	1.6	18.1	27.8
Ca (mg/g)	3.5	2.7	2.6	2.3	3.2	0.4	2.5	4.5	2.6	0.4	1.5	3.4	2.9	0.4	1.9	4.4
Mn (µg/g)	33.6	29.5	30.0	29.0	33.0	3.5	24.1	44.9	30.3	2.7	22.4	38.1	32.3	3.5	27.2	42.2
Fe (µg/g)	72.4	69.3	73.7	66.4	70.3	8.9	52.2	93.0	71.1	7.1	50.6	92.0	81.6	8.2	66.3	112.8
Co (ng/g)	100	80	60	60	70	20	40	140	80	10	60	140	90	20	50	160
Ni (µg/g)	5.2	8.5	7.2	7.6	5.8	1.1	3.8	9.1	6.9	0.9	4.6	9.9	7.0	1.3	4.6	10.9
Cu (µg/g)	12.3	14.7	13.1	12.1	11.3	1.4	8.1	15.8	12.5	1.3	9.2	16.1	12.5	1.0	10.1	15.7
Zn (µg/g)	38.3	41.2	42.9	37.8	37.5	3.6	30.8	47.0	37.6	3.0	30.6	44.5	39.5	2.7	33.5	49.5
As (ng/g)	53.0	33.1	9.4	39.7	25.5	18.0	6.4	65.9	32.8	22.7	8.4	68.8	11.0	3.6	5.2	24.4
Se (µg/g)	0.23	0.31	0.16	0.24	0.23	0.09	0.13	0.84	0.29	0.08	0.16	0.82	0.30	0.08	0.18	0.57
Mo (µg/g)	3.4	4.9	3.0	4.5	7.2	5.0	2.4	24.7	9.1	5.2	3.5	30.5	9.5	5.0	2.9	24.7
Cd (ng/g)	120	270	105	69	88.8	17.4	52.2	145.3	216.3	118.2	50.8	452.9	150.0	92.0	52.0	357.0
N (mg/g)	67.2	64.1	67.1	68.8	69.6	2.2	65.5	74.8	67.7	2.1	62.2	73.3	69.3	2.2	63.3	74.2
Cys (g/100 g)	0.58	0.63	0.63	0.56	0.58	0.02	0.54	0.63	0.59	0.02	0.52	0.64	0.61	0.03	0.54	0.67
Met (g/100 g)	0.55	0.56	0.58	0.57	0.57	0.02	0.53	0.63	0.56	0.02	0.52	0.59	0.59	0.02	0.53	0.63
Cys + Met (g/100 g)	1.13	1.18	1.21	1.13	1.14	0.04	1.07	1.25	1.14	0.04	1.05	1.21	1.19	0.04	1.07	1.30
N:S ratio	18.4	18.1	17.5	19.8	19.3	1.4	14.8	23.5	19.4	1.3	16.8	27.2	18.2	1.4	13.9	22.6
Seed weight (g)	12.5	13.4	13.4	13.6	13.46	1.49	10.2	16.3	13.44	1.60	10	17.9	13.98	1.50	10	17.9

SD = standard deviation

Table 2. Phenotypic correlations between seed concentrations of minerals Cys, Met, Cys + Met, and N:S ratio in RILs derived from DSR, NKS, and Vinton populations

	Mg	P	S	K	Ca	Mn	Fe	Co	Ni	Cu	Zn	Se	Cd	N	Cys	Meth	Cys+ Meth	N:S ratio
Mg		****	****	****	****	****	****	****	*	****	****	*	***					****
		****	****	****	****	****	****	**	NS	**	**	NS	NS					****
		****	****	****	****	****	****	***	NS	****	***	**	*					****
P	**0.73**		****	****	***	****	****	***	****	****	****	**	***			NS	NS	****
	0.57		****	****	***	****	****	****	*	****	****	NS	NS			NS	NS	****
	0.57		****	****	**	***	****	****	*	***	****	***	NS			*	*	****
S	**0.64**	**0.87**		****	***	****	****	*	***	****	****	**	*		NS	NS	NS	****
	0.56	**0.8**		****	****	****	****	*	NS	***	****	NS	NS		****	*	***	****
	0.53	**0.87**		****	NS	**	****	**	*	**	****	***	NS		***	**	***	****
K	**0.7**	**0.94**	**0.86**		**	****	****	**	***	****	****	*	**					****
	0.54	**0.8**	**0.7**		NS	***	****	***	**	****	****	NS	NS					****
	0.52	**0.85**	**0.86**		NS	***	****	**	*	***	****	**	*					****
Ca	**0.56**	**0.36**	**0.36**	**0.27**		****	**		NS	****	**	****		NS	*		*	**
	0.57	**0.33**	**0.4**	0.16		****	**		NS	NS	NS	NS		NS	NS		NS	***
	0.68	**0.27**	0.16	0.15		****	*		*	*	NS	NS		*	NS		NS	*
Mn	**0.55**	**0.63**	**0.67**	**0.57**	**0.57**		****	****	**	****	****	****	**					****
	0.58	**0.4**	**0.48**	**0.36**	**0.7**		****	**	NS	NS	**	NS	NS					****
	0.59	**0.39**	**0.28**	**0.33**	**0.67**		***	****	NS	*	**	NS	NS					**
Fe	**0.53**	**0.64**	**0.59**	**0.6**	**0.29**	**0.45**		**	**	****	****	*	*					****
	0.6	**0.55**	**0.54**	**0.58**	**0.28**	**0.39**		***	NS	*	***	**	NS					****
	0.51	**0.56**	**0.49**	**0.6**	**0.24**	**0.38**		****	NS	****	****	NS	NS					****
Co	**0.42**	**0.38**	**0.26**	**0.31**	0.1	**0.46**	**0.33**			****	****		NS					**
	0.32	**0.4**	**0.25**	**0.35**	-0.04	**0.26**	**0.34**			**	***		****					*
	0.35	**0.41**	**0.27**	**0.31**	0.19	**0.47**	**0.56**			***	***		*					*
Ni	**0.21**	**0.42**	**0.35**	**0.36**	0.05	**0.3**	**0.31**	0.15		**	****	NS	**	NS	NS	NS	NS	**
	-0.09	**0.24**	0.18	**0.26**	-0.13	-0.02	0.14	0.12		**	****	NS	*	*	NS	NS	NS	NS
	-0.1	**0.23**	**0.23**	**0.22**	**-0.22**	-0.17	0.18	-0.18		NS	****	**	*	NS	*	*	*	NS
Cu	**0.68**	**0.67**	**0.55**	**0.6**	**0.43**	**0.49**	**0.49**	**0.6**	**0.28**		****	***						****
	0.32	**0.43**	**0.33**	**0.42**	0.06	0.11	**0.23**	**0.29**	**0.29**		****	NS						**
	0.42	**0.35**	**0.28**	**0.36**	**0.24**	**0.24**	**0.52**	**0.38**	0.19		****	NS						*
Zn	**0.57**	**0.75**	**0.71**	**0.7**	**0.29**	**0.65**	**0.6**	**0.45**	**0.67**	**0.62**		*	****	NS	NS	NS	NS	****
	0.28	**0.54**	**0.46**	**0.49**	0.06	**0.28**	**0.34**	**0.37**	**0.66**	**0.38**		NS	NS	*	NS	NS	NS	**
	0.36	**0.55**	**0.45**	**0.5**	0.07	**0.27**	**0.61**	**0.34**	**0.46**	**0.51**		NS	NS	NS	**	**	**	***
Se	**0.25**	**0.28**	**0.31**	**0.21**	**0.49**	**0.4**	**0.21**	0.13	0.06	**0.39**	**0.23**			*				NS
	-0.01	-0.1	0.02	**-0.19**	0.02	-0.07	**-0.32**	0.02	0.07	0.09	0.06			NS				NS
	0.27	**0.33**	**0.36**	**0.27**	0.14	0.14	0.13	0.09	**0.27**	0.15	0.16			NS				***
Cd	**0.38**	**0.35**	**0.21**	**0.28**	0.15	**0.27**	**0.21**	0.1	**0.32**	0.19	**0.41**	-0.16		*				
	0.03	0.08	0.01	0.07	-0.18	-0.01	0.14	**0.5**	**0.23**	0.06	0.12	0.14		NS				
	0.21	0.18	0.19	**0.21**	0.02	0.04	0.17	**0.25**	**0.21**	0.16	0.12	0.15		NS				
N	-0.1	-0.03	0.09	-0.1	0.12	0.13	0.04	-0.15	0.14	-0.1	0.2	**0.2**	**0.21**			****	***	**
	-0.08	0.03	0.05	-0.01	0.1	-0.01	0.11	0.02	**0.22**	0.01	**0.24**	-0.04	0.11			*	NS	****
	-0.15	-0.06	0.05	0	**-0.24**	-0.11	0	-0.05	0.04	-0.01	0.14	0.01	-0.05			****	**	***
Cys	-0.04	0.01	0.19	-0.05	**-0.21**	0.04	-0.04	0.1	0.11	0.01	0.12	-0.09	0.13	0.11		****	****	NS
	0.07	0.2	**0.39**	0.08	0.08	0.05	0.16	-0.1	0.18	0.12	0.12	0.03	0.03	0.01		****	****	***
	0.11	0.2	**0.34**	0.14	-0.13	-0.14	0.1	0.04	**0.22**	0.15	**0.27**	0.11	-0.01	0.1		****	****	**
Meth	-0.17	-0.09	0.11	-0.1	-0.17	0.05	0.02	-0.14	0.02	-0.17	0.09	0.18	0.01	**0.45**	**0.49**		****	
	-0.01	0.16	**0.23**	0.07	-0.03	-0.06	0.13	-0.1	0.13	-0.03	0.17	-0.01	0.07	**0.22**	**0.63**		****	
	0.1	**0.22**	**0.32**	0.17	-0.2	-0.15	0.05	0.01	**0.22**	0.12	**0.29**	0.17	-0.05	**0.47**	**0.76**		****	
Cys+Meth	-0.14	-0.05	0.17	-0.09	**-0.23**	0.05	0	-0.03	0.06	-0.1	0.11	0.06	0.08	**0.35**	**0.83**	**0.89**		NS
	0.05	0.18	**0.36**	0.07	0.04	0	0.16	-0.12	0.15	0.04	0.14	0.01	0.04	0.12	**0.92**	**0.87**		*
	0.1	**0.22**	**0.35**	0.17	-0.17	-0.15	0.08	0.02	**0.24**	0.14	**0.31**	0.15	-0.03	**0.29**	**0.95**	**0.92**		*
N:S ratio	**-0.66**	**-0.84**	**-0.9**	**-0.85**	**-0.29**	**-0.56**	**-0.54**	**-0.32**	**-0.27**	**-0.56**	**-0.57**	-0.2	-0.1	**0.32**	-0.12	0.09	-0.01	
	-0.55	**-0.7**	**-0.86**	**-0.63**	**-0.33**	**-0.44**	**-0.43**	**-0.22**	-0.05	**-0.28**	**-0.3**	-0.05	0.03	**0.46**	**-0.34**	-0.09	**-0.25**	
	-0.54	**-0.83**	**-0.9**	**-0.78**	**-0.23**	**-0.27**	**-0.42**	**-0.23**	-0.18	**-0.22**	**-0.34**	**-0.34**	-0.16	**0.37**	**-0.29**	-0.12	**-0.22**	

Each box in the bottom left corner of the table corresponds to Pearson's correlation coefficients in DSR, NKS, and Vinton populations, respectively (top to bottom within a box).
Significant associations are highlighted in bold letters. Each box in the upper right corner of the table corresponds to the significance at 0.05 (*), 0.01 (**), 0.001(***), and < 0.0001(****) probability levels for DSR, NKS, and Vinton populations, respectively (top to bottom within a box).
NS = not significant

N in all populations were high (approximately 6 standard deviations in Vinton population), although parental differences were minimal (64.1–68.8 mg/g) (table 1). Population means for N were shifted upwards in DSR and Vinton populations and the distribution of N resembled a normal distribution with transgressive segregation (table 1). However, N alone did not have consistently significant associations with any other nutrients except methionine, with which it had a consistent significant positive association (table 2).

Although the difference between parental means for N:S ratio was minimal, the range in the populations was considerably higher (table 1). Population means for N:S ratio fell within the range of the parents in all populations, and the distribution of N:S ratio resembled a normal distribution (Supplemental Resource 1 and table 1) with transgressive segregation. N:S ratio had significant negative associations with all macronutrients (r > –0.23 up to –0.90, table 2) except N. N:S ratio also displayed significant negative associations with several micronutrients and Cys + Met (table 2). This inverse relationship between N:S ratio and most other minerals was further explored to understand whether the increased N:S ratio was due to high N or low S concentration (Supplemental Resource 2). Lower total S concentration but not protein S (Cys + Met) concentration was contributing to increased N:S ratio.

Cys and Met phenotypes

Population means for Cys concentration fell within the range of the parents (0.56–0.63/100 g) in all populations (table 1). Population means for Met concentration fell within the range of parental means in DSR and NKS populations (table 1). Population means for Cys + Met concentrations fell outside the range of parents in the DSR population (table 1). The range for Cys + Met was high in the Vinton population (approximately 6 standard deviations) (Supplemental Resource 1 and table 1). The upper end of the ranges for Cys + Met (on a seed dry weight basis) in the Vinton population was 1.30 g/100 g, which was close to the FAO standard for combination of Cys and Met (1.40 g/100 g). The phenotypic distribution of Cys, Met, and Cys + Met resembled a normal distribution with transgressive segregation (Supplemental Resource 1). Cys, Met, and Cys + Met had significant positive association with seed S concentration in two populations (table 2).

Other minerals

Seed mineral concentrations of all populations displayed wide ranges with considerable transgressive segregation (Supplemental Resource 1). The relative ranges were lowest for Mg and highest for Mo (table 1). Population means for Mo were shifted upwards compared to parents in all populations (table 1). A similar shift in population mean was observed for Mn and Fe in the NKS population (table 1). Population means for most of the traits in all but the Vinton population fell within the range of parents (table 1). Population means for the Vinton population for all minerals except Ni, Cu, and Zn shifted upwards compared to parents (table 1). Phenotypic distributions for most of the minerals resembled a normal distribution across all populations (Supplemental Resource 1 and table 1). Most of the macronutrients except N had significant positive associations with each other (table 2). Micronutrients Mn, Fe, Co, Cu, and Zn had significant positive associations with each other in at least two populations (table 2). Seed concentrations of pollutant metals Cd and As displayed a wide range with a few transgressive segregants across all populations (table 1 and Supplemental Resource 1). Both Cd and As displayed significant positive associations with all macronutrients in at least one population (table 2 and Supplemental Resource 3).

Seed weight

Seed weight displayed a wide range in all populations with considerable transgressive segregation (table 1). Population means fell within the range of parents in all but the Vinton population (table 1), where the population mean shifted upwards compared to the parents. Phenotypic distribution of seed weight resembled a normal distribution (Supplemental Resource 1). Seed weight had significant negative association with Ni in two populations and significant positive associations with Mg, Mo, Cd, and N in at least one population (Supplemental Resource 4).

Genotyping

The distribution of the parental genotypes based on the 1,536 SNP panel in each RIL population was equal in the NKS population and nearly equal, with 1.3 and 1.2% deviations, in the DSR and Vinton populations,

Table 3. Significant QTLs for various seed nutritional traits in soybean using analysis of individual mapping populations

Trait	Chr.	Williams 82 × DSR-173				Williams 82 × NKS19-90				Williams 82 × Vinton 81			
		Peak[a]	LOD	% expl.[b]	Add.[c]	Peak[a]	LOD	% expl.[b]	Add.[c]	Peak[a]	LOD	% expl.[b]	Add.[c]
Mg	3									43.9	3.28	17	–0.08
	3									52	3.35	14	–0.07
S	16									59.5	2.94	12	–0.11
	18					66.3	3.81	14	0.07				
K	4					7	3.46	13	–0.4				
	15					28.9	3.03	12	0.4				
	16					29.4	3.85	14	–0.43				
	18	21.3	2.87	11	0.63								
Ca	6	89	3.47	14	–0.14								
Mn	2					75.3	3.84	13	–1				
	13									21.3	3.23	13	1.3
Fe	3*									53.9	3.36	13	–3.25
	20									12	3.79	22	4.17
Co	3	47.4	3.7	13	0.008								
	15									56.9	5.16	23	–0.011
	15									65.3	4.1	16	–0.01
Ni	1					2.9	3.54	12	–0.33				
	10	74.3	3.48	12	–0.39								
Cu	8									63	5.56	19	0.46
	14									48.7	3.1	10	–0.34
	18									61.2	4.18	14	0.52
Zn	3									47.5	4.35	16	–1.1
	3									57.7	4	14	–1.1
	9	16.7	3.12	13	–1.35								
Se	8	41.3	2.37	9	0.028								
	18	23.4	2.86	12	–0.03								
Mo	3					33.5	3.21	10	1.72				
	4									35.1	3.51	14	–1.88
	5									28.9	3.34	13	1.93
Cd	1	0	4.55	16	7.15								
N	14*					61.1	3.48	13	0.8				
	15*	42.9	3.65	17	–0.89								
Cys	2	35.8	5.42	21	0.01								
	2	44.1	7.92	28	0.012								
	2	51.7	7.33	38	0.014								
Cys + Met	2	44.1	4.39	17	0.016								
N:S ratio	16					29.4	3.05	10	0.43				
	20									6	3.06	28	–0.77
	3									52	3.35	14	–0.07
Seed weight	10	58.7	5.07	17	0.62								
	17	68.7	3.17	12	0.53								

Chr. = chromosome

a. The peak position of the significant QTL.

b. Percentage of variation explained by each identified QTL.

c. Additive effect. A negative value indicates that the Williams 82 allele increased the trait value.

* These QTLs support the QTLs from previous studies.

respectively, after removing the heterozygotes and missed calls from the analysis. The polymorphism rates for the NKS, DSR, and Vinton populations were 25.5, 22.8, and 26% respectively. The genetic map length for the three populations was 1,529.2 cM for the DSR population, 1,736.2 cM for the NKS population, and 1,799.6 cM for the Vinton population. Average spacing between the markers was 4.6 cM in the DSR population and 4.7 cM in both the NKS and Vinton populations (Supplemental Resource 5, 6, and 7 respectively). The joint linkage map, consisting of 766 polymorphic markers, was 1,968.4 cM in length, with an average spacing of 2.6 cM (Supplemental Resource 8).

QTL mapping

Forty QTLs were detected for 18 traits that include mineral concentrations, N, N:S ratio, Cys, Met, Cys + Met concentrations and seed weight (table 3). In 18 QTLs, the Williams 82 allele contributed to improved seed nutritional composition (i.e., higher concentration) and in the remaining 22 QTLs, other parental alleles (DSR-173, NKS19-90, or Vinton 81) contributed to higher mineral/nutrient concentrations (table 3). The amount of total phenotypic variation explained by any individual QTL for any trait ranged from 9 to 38% (table 3). In QTL mapping using the joint linkage map, nine QTLs were identified for Ni, Cd, Cys, Met, Cys + Met, and seed weight (table 4). Two QTLs each for Ni, Cys, Cys + Met, and a single QTL each for Cd, Met, and seed weight were detected. On chromosome 20, the Williams 82 allele contributed to increased Ni, Cys, and Cys + Met concentrations. On chromosome 15, the Williams 82 allele contributed to increased Met concentration. Other parental alleles contributed to increased concentrations of Ni, Cd, Cys, Cys + Met, and seed weight on chromosomes 7, 18, 20, 20, and 10, respectively (table 4).

QTLs for S, N, and N:S ratio

Two significant QTLs were associated with S with LOD scores of 2.94 in the Vinton population and 3.81 in the NKS population, explaining 12 and 14% of the phenotypic variance, respectively (table 3). Two significant QTLs were associated with N concentration with LOD scores of 3.48 in the NKS population and 3.65 in the DSR population, explaining 13 and 17% of the variance, respectively (table 3). Two significant QTLs affecting N:S ratio were detected with LOD scores of 3.0 each in the NKS and Vinton populations, explaining 10 and 28% of the variance, respectively (table 3). None of the QTLs that were reported for S alone or N alone in an individual population had any significant effect on N:S ratio (table 3).

QTLs for Cys and Met

Three significant QTLs were associated with Cys in the DSR population with LOD scores ranging between 5.42 and 7.33, explaining 21–38% of the phenotypic variance (table 3). One of the three QTLs reported for Cys (flanked by markers BARC-063497-18380 and BARC-025955-05182) had a significant effect on Cys + Met with a LOD score of 4.39, explaining 17% of the variance (table 3). We also detected two significant and common QTLs affecting Cys and Cys + Met concentrations on chromosome 20 using joint linkage mapping with LOD scores of 3.66 and 5.35, respectively, explaining 9 and 16% of the total phenotypic variance (table 4).

QTLs detected for other minerals

Twenty-eight significant QTLs were detected for other minerals (table 3). Phenotypic variance explained ranged from 9% (for Se in DSR population) to 23% (for Co in Vinton population) (table 3). A single QTL was mapped for both Ca and Cd, while two QTLs were mapped for Fe, Mg, Mn, Ni, and Se. Three QTLs were mapped for Co, Cu, Zn, and Mo, while there were four QTLs for K (table 3). Some of the QTLs for different traits were located on the same linkage group in approximately the same location. For example, QTLs for K and Se in the DSR population were located on chromosome 18 at ~22 cM. Similarly, QTLs for K and N:S ratio in the NKS population were located on chromosome 16 at 29.4 cM, and QTLs for Zn, Mg, and Fe in the Vinton population were located on chromosome 3 at ~53 cM (table 3).

QTLs for seed weight

Two significant QTLs were associated with seed weight in the DSR population with LOD scores of 3.17 and 5.07, explaining 12 and 17% of the variances on chromosomes 17 and 10, respectively (table 3). Higher seed weight was conferred by the DSR-173 allele at both loci.

Table 4. Significant QTLs for various seed nutritional traits by joint linkage mapping of three RIL populations in soybean

Traits	Chr.	Peak[a]	LOD	% expl.[b]	Add.[c]	Flanking marker start	Flanking marker end	Consensus map start[d]	Consensus map end[d]	Supporting QTLs
Ni	7	34	3.27	5	0.29	BARC-042815-08424	BARC-048517-10647	41.372	47.379	
	20	59.6	3.7	10	–0.41	BARC-038869-07364	BARC-039753-07565	55.301	63.997	
Cd	18	53.8	3.21	5	23.08	BARC-059485-15839	BARC-055557-13432	48.948	54.445	
Cys	20	56.8	5.35	16	–0.013	BARC-038869-07364	BARC-039753-07565	55.301	63.997	
	20	80.1	3.66	9	0.009	BARC-013583-01166	BARC-042897-08454	69.303	85.708	Yes
Met	15	55	3.23	6	–0.007	BARC-018461-02916	BARC-066103-17539	61.363	66.033	
Cys + Met	20	56.8	5.35	16	–0.013	BARC-038869-07364	BARC-039753-07565	55.301	63.997	
	20	80.1	3.66	9	0.009	BARC-013583-01166	BARC-042897-08454	69.303	85.708	Yes
Seed weight	10	42.6	4.16	14	0.58	BARC-051153-11022	BARC-064941-19017	46.3	53.1	

Chr. = chromosome

a .The peak position of the significant QTL.

b. Percentage of variation explained by each identified QTL.

c. Additive effect. A negative value indicates that the Williams 82 allele increased the trait value.

d. The flanking marker positions based on the consensus map from SoyBase (Grant et al. 2010) are represented.

Discussion

QTLs in each mapping population

Several QTLs were identified for seed mineral concentrations, Cys, Met, N:S ratio, and seed weight in soybean (tables 3, 4). Most minerals had multiple QTLs that explained a small proportion of the total phenotypic variance. Several QTLs for some of the minerals co-localized with each other (Supplemental Resource 9). Most striking was the co-localization of Co, Mo, Mg, Fe, and Zn on chromosome 3. Similar co-localizations were observed for S, K, Se, and Cu on chromosome 18 and Ni and Cd on chromosome 1. Co-localization of QTLs might highlight genes involved in uptake, transport, trafficking, and sequestration, which may function for multiple minerals (Clemens 2001; Vreugdenhil et al. 2004). Two QTLs out of 40 identified in this study using individual analysis of each mapping population co-located with previously detected QTLs, both for N on chromosomes 14 and 15, respectively (Panthee et al. 2004b).

Seed weight QTL in previous studies (Csanádi et al. 2001; Hyten et al. 2004; Maughan et al. 1996; Mian et al. 1996; Orf et al. 1999; Panthee et al. 2005; Specht et al. 2001) co-located with several mineral QTLs in this study, suggesting the possibility that seed mass could influence seed mineral concentration (Cakmak et al. 2000 and Imtiaz et al. 2003). However, none of our

QTLs for seed weight co-located with any of our mineral QTLs, suggesting that in these populations there was no dilution effect on seed mineral concentrations as seed mass increased.

QTLs using the joint linkage map

The advantages of combined QTL analysis of multiple mapping populations are that effects of a wide range of alleles can be compared, and the larger population size gives improved power and more precise estimates of position and effects of QTLs (Coles et al. 2010; Negeri et al. 2011). Nine QTLs that were not identified in individual populations were detected using joint linkage mapping (table 4). The additional QTLs in joint linkage mapping could be due to three factors. Firstly, allelic effects at any particular locus vary in each mapping population, and thus cumulative allelic effects may have resulted in identification of new QTLs. Secondly, most of the QTLs that were detected in individual mapping populations explained a small proportion of phenotypic variance with small additive effects, insufficient to skew the cumulative effects (in joint linkage mapping) to pass the threshold LOD score. Finally, lack of markers that distinguish the parents at the genomic region of interest in one or two mapping populations will affect the QTL mapping results in joint linkage mapping analysis. Of the nine QTLs identified using joint linkage mapping, one QTL on chromosome 20 that affected both Cys and Cys + Met (table 4) co-located with a previously identified QTL for the basic glycinin subunit (Panthee et al. 2004a). Glycinin is an S-rich fraction of the seed storage protein in soybean (Kitamura 1995). It is logical that the genomic regions that affect glycinin will affect seed Cys + Met concentrations. Two QTLs that affected Cys also affected Cys + Met concentration, possibly because Cys is an intermediate product in Met biosynthesis (Panthee et al. 2006a).

Putative candidate genes in the vicinity of QTL intervals

Based on physical location of SNP markers in the Williams 82 genome and existing knowledge of the mineral-related genes/proteins, we identified candidate genes that could potentially alter seed concentrations of nutrients (table 5). Allelic variation in nutrient metabolism genes may affect the rate of biosynthesis or final product concentration, ultimately resulting in differential seed nutrient concentrations. An allantoate amidohydrolase gene (*AAH)* was detected in the N QTL interval on chromosome 15. AAHs are localized in the endoplasmic reticulum, and they hydrolyze the ureide allantoate to produce ureidoglycolate, CO_2 and two molecules of ammonium (Werner et al. 2008). Allantoate degradation is required for recycling of purine-ring N in all plants. In some tropical legumes such as soybean, fixed N is transported through allantoin and then through allantoate in shoots, where it serves as a general N source (Werner et al. 2008). On chromosome 20, homocysteine *S*-methyltransferase (HMT3) was present in the QTL interval for Cys + Met. HMT3, otherwise called methionine synthase, is located in the cytoplasm and plasma membrane. This protein is involved in the catalysis of the final step of methionine biosynthesis (Ranocha et al. 2001).

Polymorphisms in genes involved in the short- or long-distance transport of nutrients may alter final seed nutrient concentrations by affecting flux into seed loading pathways. An S transporter gene, *SULTR3;3,* was detected in the QTL interval for N:S ratio on chromosome 20. Group 3 sulfate transporters are predicted to be located on the plasma membrane (Kataoka et al. 2004; Zuber et al. 2010), and could be involved in sulfate translocation between seed compartments (Zuber et al. 2010). Selenate uptake in plants is mediated by sulfate transporters because of chemical similarities between S and Se (Anderson 1993). For Se, a QTL on chromosome 8 that includes the sulfate transporter *Sultr2;1* was detected (table 5). Group 2 low-affinity sulfate transporters are expressed in vascular tissues and were reported to be involved in translocation of sulfate from roots to leaves (Takahashi et al. 2000).

A predicted Mo transporter, *MOT1*, was detected in the QTL confidence intervals on chromosome 4. MOT1 is required for efficient uptake and translocation of molybdate in *Arabidopsis* (Tomatsu et al. 2007), and is expressed in both roots and shoots in plasma membranes and vesicles. A candidate gene for Fe on chromosome 20 is a Yellow-stripe-like (YSL) gene, *YSL7*. YSL family members are involved in root to shoot transport of Fe bound to nicotianamine (Curie et al. 2009). *Brassica juncea YSL7*-overexpressing plants had higher Fe concentrations in the seeds (Wang et al. 2013). AtYSL2 in *Arabidopsis* was proposed to be involved in the lateral movement of metals in plant vasculature (DiDonato et

Table 5. Candidate genes detected within QTL intervals across different populations categorized by their biological role

Population	Chr.	Trait	Soy gene ID	*Arabidopsis* gene ID	Gene homolog	Gene name
Transporters						
Vinton	4	Mo	Glyma04g07690.1	AT2G25680.1	MOT1	Molybdate transporter 1
DSR	8	Se	Glyma08g14700.1	AT5G10180.1	AST68/SULTR2;1	Sulfate transporter 2;1
Vinton	20	Fe	Glyma20g00690.1	AT1G65730.1	YSL7	Yellow stripe like 7
Vinton	20	N:S ratio	Glyma20g02080.2	AT1G23090.1	AST91/SULTR3;3	Sulfate transporter 91
Metabolism						
DSR	15	N	Glyma15g16870.1	AT4G20070.1	AAH/ATAAH	Allantoate amidohydrolase
Joint analysis	20	Cys + Met	Glyma20g28720.1	AT3G22740.1	HMT3	Homocysteine *S*-methyltransferase 3

Chr. = chromosome
The proposed biological role is given in *italics*

al. 2004). The *ysl1ysl3* double mutant of *Arabidopsis* had lower concentrations of Fe, Zn, and Cu in seeds compared to the wild type (Waters et al. 2006; Waters and Grusak 2008).

Diversity in seed accumulation of various nutrients

Genetic diversity in mapping populations is essential for understanding the underpinnings of seed nutrient accumulation using the QTL mapping approach. Since genotype is an important contributor to phenotype, genetic differences could result in phenotypic differences in seed nutrient concentrations. A significant phenotypic diversity for various minerals has been reported in wheat, rice, brassica, and common bean (Beebe et al. 2000; Chatzav et al. 2010; Ding et al. 2010; Gregorio et al. 2000; Peleg et al. 2009). Segregation and transgressive segregation in RIL populations for most of the traits (table 1, Supplemental Resource 1) indicate that they are quantitatively inherited. The upper end of the ranges for Cys + Met concentrations in our study suggests that genetic improvement in Cys + Met is possible through selection and/or breeding in soybean, in order to achieve a soybean protein composition that more closely conforms to the FAO standard for combination of Cys + Met (1.4 g/100 g).

Correlations between traits

Our results suggest that indirect selection for various nutritional traits is possible in soybean. Trait correlation in segregating populations could result from factors such as linkage, pleiotropy, and environment (Aastveit and Aastveit 1993; Paterson et al. 1988). Significant positive or negative correlations for various seed nutrients were found in this study, similar to previous studies (Beebe et al. 2000; Peleg et al. 2009; Sankaran et al. 2009; Vreugdenhil et al. 2004; Waters and Grusak 2008), suggesting the possibility that improvement in the concentration of one nutrient might improve others (Ozkan et al. 2007; Sankaran et al. 2009). Seed Met concentration had a significant positive association with total N in two populations. However, seed Cys was not correlated with total N, supporting a previous study (Panthee et al. 2006a). A striking negative association of N:S ratio with most of the macro- and micronutrients across populations suggests that N:S ratio is an indicator of concentrations of several minerals in soybean seeds. N:S ratio was driven by S concentration, and S concentration had no significant association with N concentration, suggesting the possibility of selecting for soybean with high S (low N:S ratio) and high N concentrations. Moderate positive association between S and Cys + Met in two populations suggests that total S might not be a strong indicator of protein S (table 2).

In conclusion, there are several results from this study that may be important for soybean breeding. First is the detection of a strong inverse relationship of N:S ratio with most of the mineral traits, suggesting that the N:S trait could be used as an indirect selection index in breeding for increased mineral concentrations in soybean. This could reduce the cost of screening by reducing phenotyping of mineral traits other than N

and S. Second is the identification of a new major QTL for Cys (and Cys + Met) on chromosome 2 in the DSR population and a second QTL for Cys (and Cys + Met) on chromosome 20 identified from the joint linkage analysis, suggesting alleles for improved protein composition in soybean. Third, association between seed weight and seed mineral concentrations exhibited no consistent significant correlations in most cases, suggesting that increased seed mineral concentrations are not secondary effects of seed size. A number of QTLs that affect soybean nutritional composition were identified in this study, suggesting that existing breeding populations that were designed and grown for other traits (such as yield, drought tolerance and disease resistance) could also be exploited for QTL mapping of seed nutritional composition in soybean.

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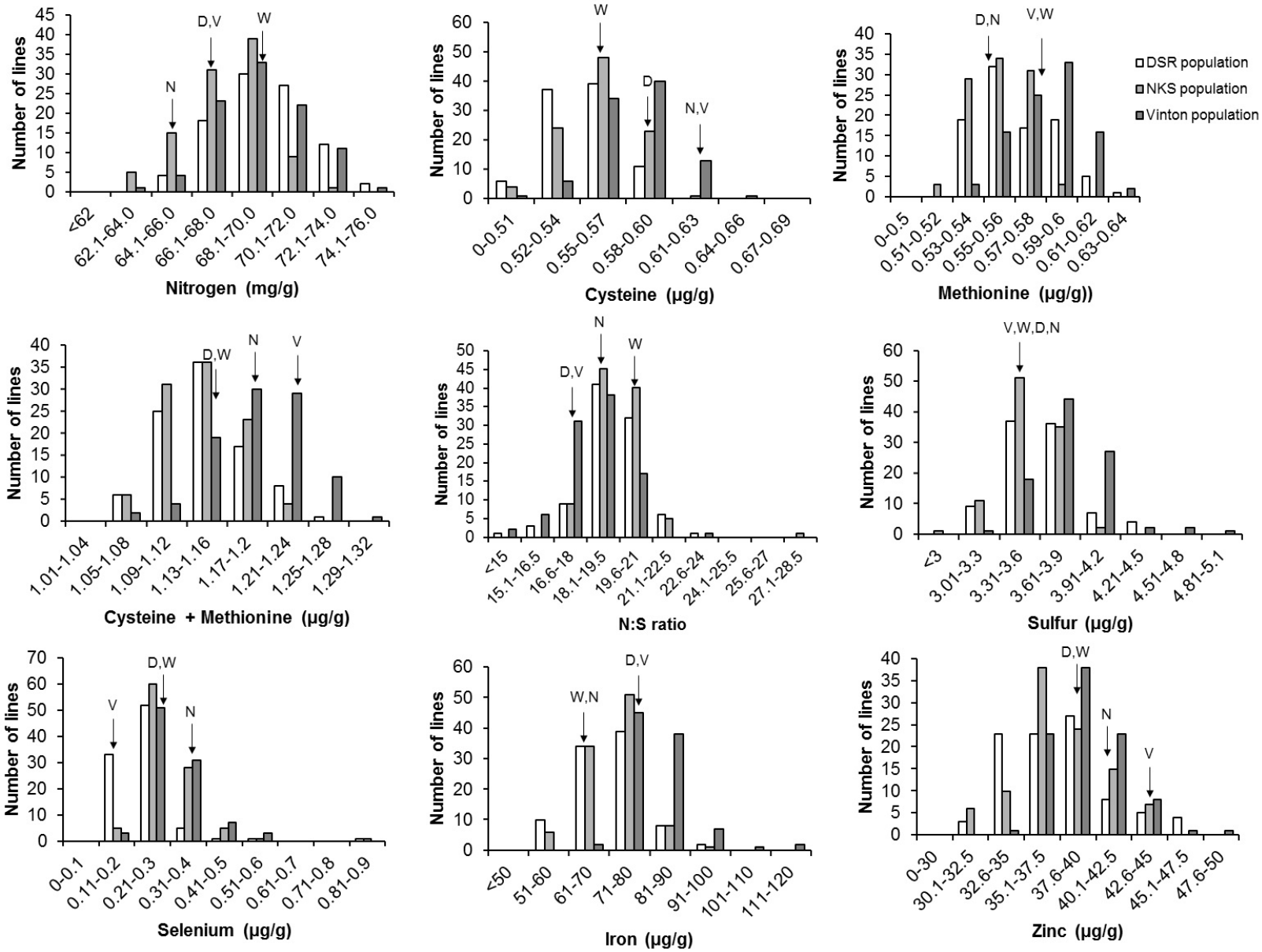
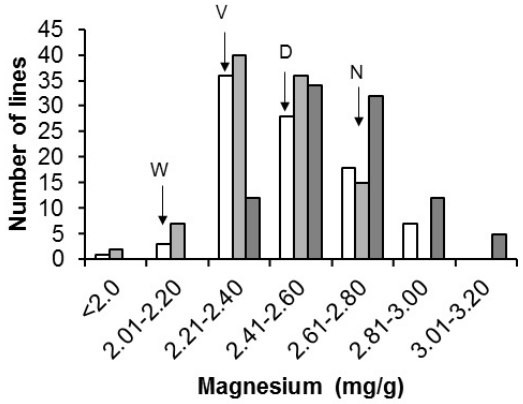
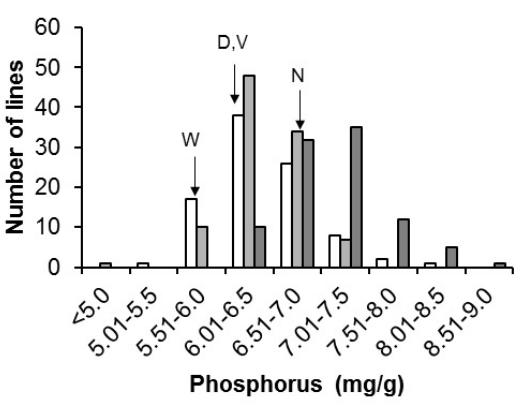
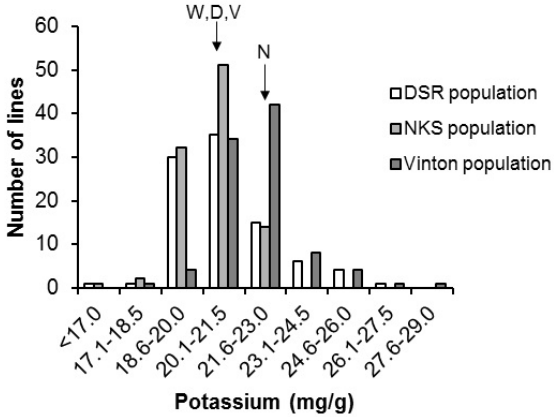
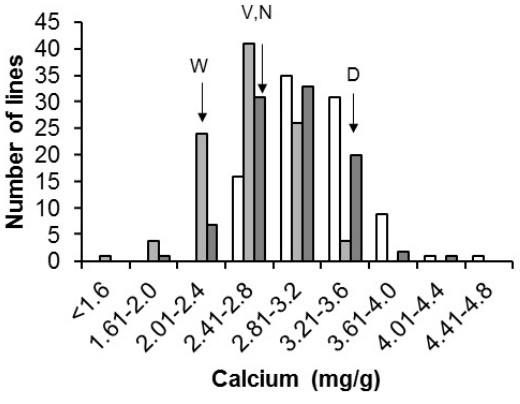
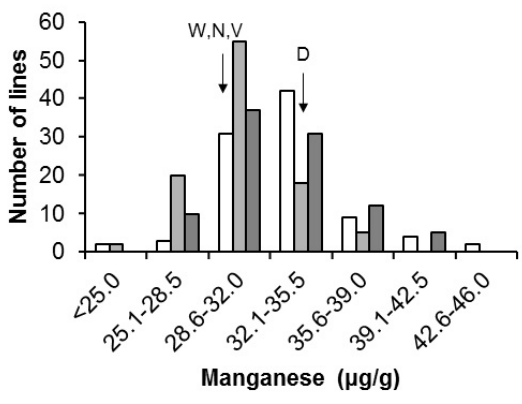

Number of lines
Nitrogen (mg/g)
<62
62.1-64.0
64.1-66.0
66.1-68.0
68.1-70.0
70.1-72.0
72.1-74.0
74.1-76.0
N
D,V
W
Cysteine (µg/g)
0-0.51
0.52-0.54
0.55-0.57
0.58-0.60
0.61-0.63
0.64-0.66
0.67-0.69
D
N,V
Methionine (µg/g))
0-0.5
0.51-0.52
0.53-0.54
0.55-0.56
0.57-0.58
0.59-0.6
0.61-0.62
0.63-0.64
D,N
V,W
DSR population
NKS population
Vinton population
Cysteine + Methionine (µg/g)
1.01-1.04
1.05-1.08
1.09-1.12
1.13-1.16
1.17-1.2
1.21-1.24
1.25-1.28
1.29-1.32
D,W
N
V
N:S ratio
<15
15.1-16.5
16.6-18
18.1-19.5
19.6-21
21.1-22.5
22.6-24
24.1-25.5
25.6-27
27.1-28.5
D,V
N
W
Sulfur (µg/g)
<3
3.01-3.3
3.31-3.6
3.61-3.9
3.91-4.2
4.21-4.5
4.51-4.8
4.81-5.1
V,W,D,N
Selenium (µg/g)
0-0.1
0.11-0.2
0.21-0.3
0.31-0.4
0.41-0.5
0.51-0.6
0.61-0.7
0.71-0.8
0.81-0.9
V
D,W
N
Iron (µg/g)
<50
51-60
61-70
71-80
81-90
91-100
101-110
111-120
W,N
D,V
Zinc (µg/g)
0-30
30.1-32.5
32.6-35
35.1-37.5
37.6-40
40.1-42.5
42.6-45
45.1-47.5
47.6-50
D,W
N
V

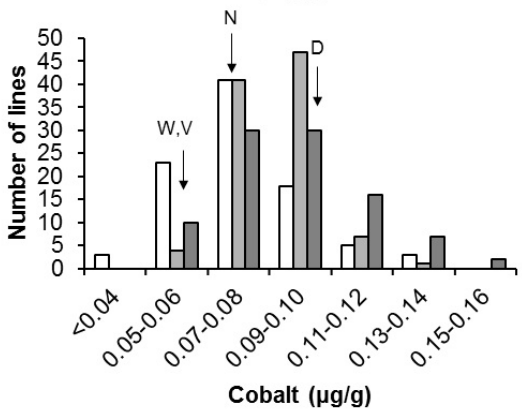

Number of lines
W
V
D
N
<2.0
2.01-2.20
2.21-2.40
2.41-2.60
2.61-2.80
2.81-3.00
3.01-3.20
Magnesium (mg/g)

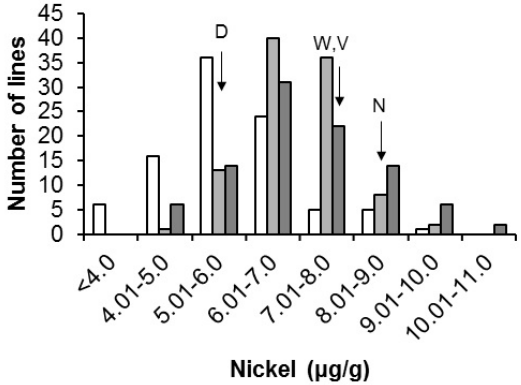

Number of lines
W
D,V
N
<5.0
5.01-5.5
5.51-6.0
6.01-6.5
6.51-7.0
7.01-7.5
7.51-8.0
8.01-8.5
8.51-9.0
Phosphorus (mg/g)


Number of lines
W,D,V
N
DSR population
NKS population
Vinton population
<17.0
17.1-18.5
18.6-20.0
20.1-21.5
21.6-23.0
23.1-24.5
24.6-26.0
26.1-27.5
27.6-29.0
Potassium (mg/g)


Number of lines
W
V,N
D
<1.6
1.61-2.0
2.01-2.4
2.41-2.8
2.81-3.2
3.21-3.6
3.61-4.0
4.01-4.4
4.41-4.8
Calcium (mg/g)


Number of lines
W,N,V
D
<25.0
25.1-28.5
28.6-32.0
32.1-35.5
35.6-39.0
39.1-42.5
42.6-46.0
Manganese (µg/g)


Number of lines
N
D
W,V
<0.04
0.05-0.06
0.07-0.08
0.09-0.10
0.11-0.12
0.13-0.14
0.15-0.16
Cobalt (µg/g)


Number of lines
D
W,V
N
<4.0
4.01-5.0
5.01-6.0
6.01-7.0
7.01-8.0
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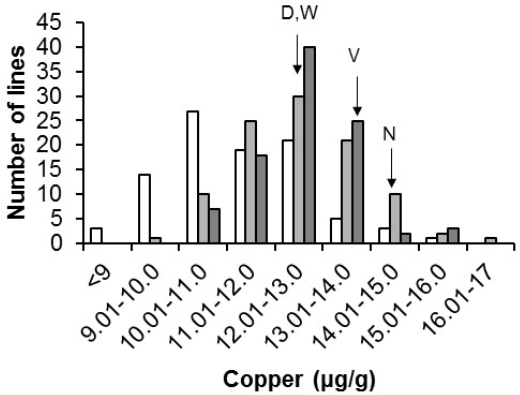
9.01-10.0
10.01-11.0
Nickel (µg/g)


Number of lines
D,W
V
N
<9
9.01-10.0
10.01-11.0
11.01-12.0
12.01-13.0
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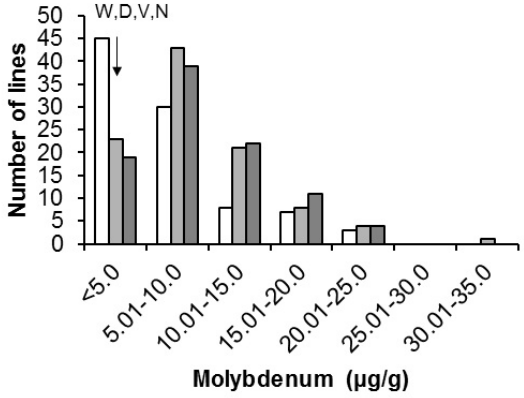
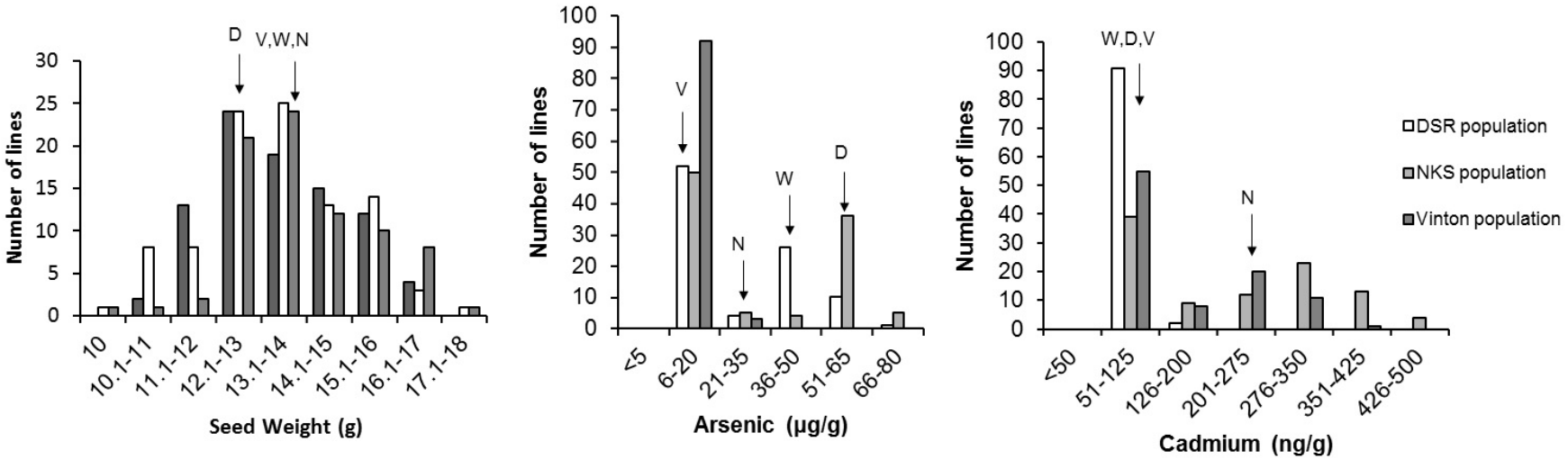
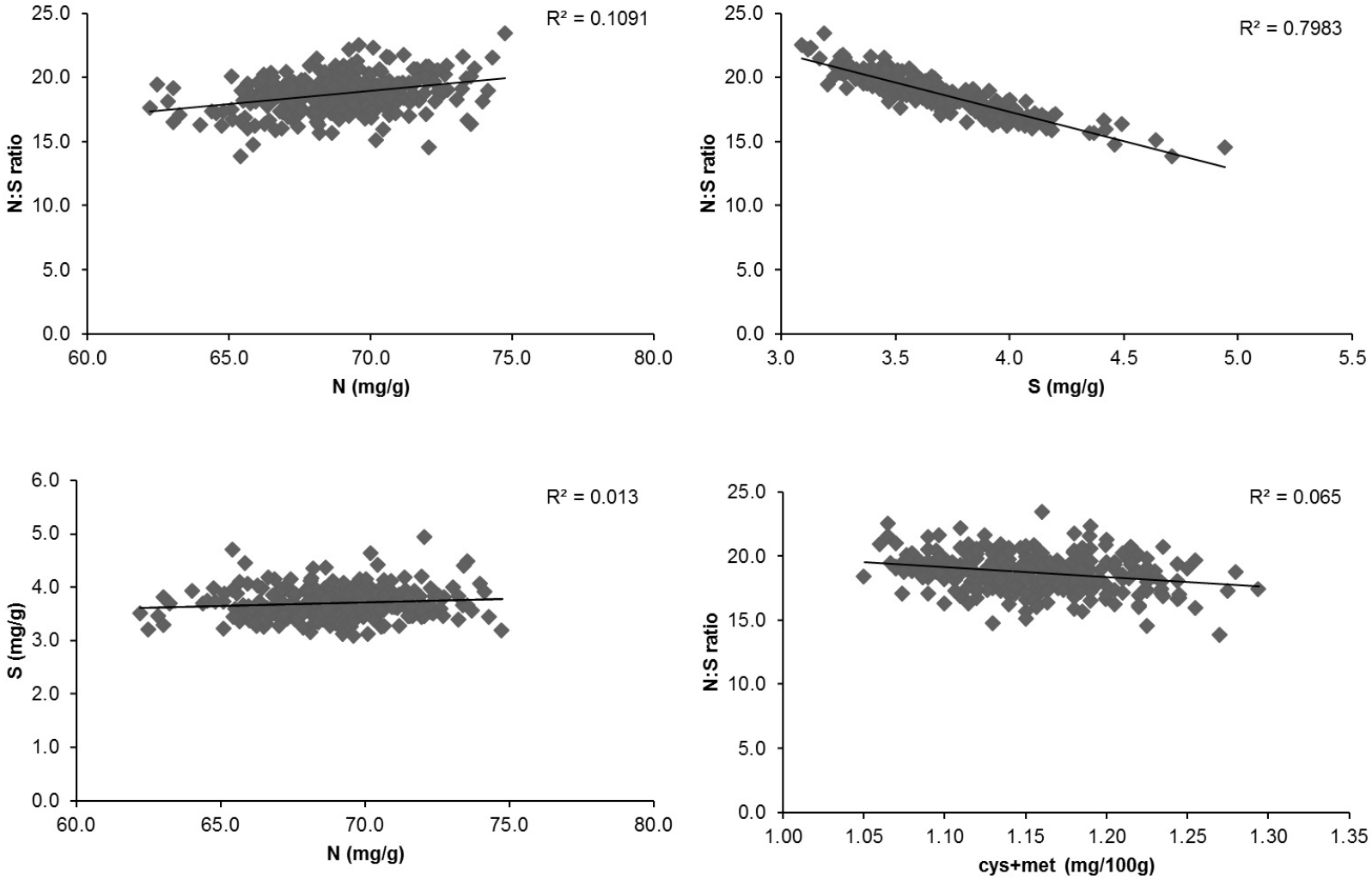
14.01-15.0
15.01-16.0
16.01-17
Copper (µg/g)


Number of lines
W,D,V,N
<5.0
5.01-10.0
10.01-15.0
15.01-20.0
20.01-25.0
25.01-30.0
30.01-35.0
Molybdenum (µg/g)



Supplemental Resource 1 Phenotypic distribution of seed concentrations of minerals, Cys, Met, Cys+Met N:S ratio and seed weight of RILs from DSR, NKS and Vinton populations. Bins are based on one standard deviation. Arrows indicate the trait value of the parental lines; Williams 82 (W), NKS19-90 (N), Vinton 81 (V), and DSR-173 (D).



Supplemental Resource 2 Scatterplots of association between N and N:S ratio, S and N:S ratio,S and N, and N:S ratio and Cys+Met concentrations. Data from 288 RILs of three soybean mapping populations. Regression co-efficient is shown on each plot.