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Meta-QTL analysis of seed iron and zinc concentration and content in common bean (*Phaseolus vulgaris* L.)

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Key Message

Twelve meta-QTL for seed Fe and Zn concentration and/or content were identified from 87 QTL originating from seven population grown in sixteen field trials. These meta-QTL include 2 specific to iron, 2 specific to zinc and 8 that co-localize for iron and zinc concentrations and/or content.

Common bean (*Phaseolus vulgaris* L.) is the most important legume for human consumption worldwide and it is an important source of microelements, especially iron and zinc. Bean biofortification breeding programs develop new varieties with high levels of Fe and Zn targeted for countries with human micronutrient deficiencies. Biofortification efforts thus far have relied on phenotypic selection of raw seed mineral concentrations in advanced generations. While numerous quantitative trait loci (QTL) studies have been conducted to identify genomic regions associated with increased Fe and Zn concentration in seeds, these results have yet to be employed for marker assisted breeding. The objective of this study was to conduct a meta-analysis from seven QTL studies in Andean and Middle American intra- and inter genepool populations to identify the regions in the genome that control the Fe and Zn levels in seeds. ~~total~~ Two meta-QTL specific to Fe and two meta-QTL specific to Zn were identified. Additionally, eight Meta QTL that co-localized for Fe and Zn concentration and/or content were identified across seven chromosomes. The Fe and Zn shared meta-QTL could be useful candidates for marker assisted breeding to simultaneously increase seed Fe and Zn. The physical positions for 12 individual meta-QTL were identified and within five of the meta-QTL, candidate genes were identified from six gene families that have been associated with transport of iron and zinc in plants.

Keywords

Common bean, QTL, Meta-analysis, biofortification, Iron, Zinc.

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume among the twenty that are commonly consumed in human diets (Joshi and Rao, 2017; Wilson et al., 2004). Common bean is a diverse crop in terms of morphological and seed variability, cultivation methods, environmental adaptation and consumer preferences and these factors have made it suitable for many different niches (Broughton et al., 2003). *P. vulgaris* was reported to have been domesticated independently twice - in Mexico and the Andes (Peru, Colombia) (Bitocchi et al., 2013). Members of the two genepools, Andean and Middle American, vary in seed size, seed nutritional quality, resistance to pathogens, yield, days to maturity, and other morpho-agronomic traits (Pérez-Vega et al., 2010; Blair et al, 2010a).

Dry bean is an important dietary source of iron and zinc (Bouis & Welch, 2010; Carrasco-Castilla et al., 2012). It has been included as a target crop in biofortification programs for countries with widespread human nutritional deficiencies. Microelement deficiencies are among the most common and devastating global nutritional problems (Hirschi, 2009). In terms of seed micronutrient concentrations, bean germplasm from the Andean and Middle American gene pools show some variability, Blair et al. 2010a reported that Andean gene pool and inter-gene pool crosses tend to have higher concentrations of minerals than Middle American beans. The natural variability for seed Fe and Zn has been utilized in breeding programs based on phenotypic selection for seed mineral concentrations (Blair et al., 2010b). However, phenotypic selections alone are not leading to the genetic gains needed to make biofortification successful (Vasconcelos et al., 2017). Genomic tools are needed to help breeders to reach target concentrations in dry beans.

One of the most common strategies to unravel quantitative traits are Quantitative Trait Loci (QTL) studies. Several QTL analyses have been conducted in common bean to identify regions associated with seed Fe and Zn levels (Guzma-Maldonado, et al., 2003; Cichy, et al 2009; Blair, et al., 2010d; Blair, et al., 2011; Blair & Izquierdo, 2012; Blair, et al., 2013). The use of common markers across different maps make it possible to integrate such QTL in order to improve the accuracy of positioning and decrease the confidence interval using meta-QTL analysis.

Meta-QTL analysis compiles information from multiple studies and improves QTL location by comparing individual experiments and narrowing down confidence intervals obtained from individual analyses (Goffinet and Gerber, 2000). Meta-QTL analysis has been conducted in several crops for various traits, including grain size and resistance to African gall midge in rice (Daware et al, 2017; Wu et al., 2016; Yao et al., 2016), grain traits in wheat (Tyagi, 2015), oil and protein in soybean (Van and McHale, 2017) and resistance to white mold in common bean (Vasconcellos et al., 2017). Various statistical methods have been developed for meta-QTL analysis. The software program Biomercator uses the transformed Akaike classification criterion (AIC) to determine the real number of QTL in a specific region (Arcade et al., 2004). To date, only one meta-QTL study for seed Fe and Zn has been published. That study was conducted

with five maize populations and it resulted in the discovery of ten **meta**-QTL involved in Fe and/or Zn accumulation. The phenotypic variation contributed to the 10 MQTL ranged from 9% to 28 % (Jin et al., 2013). Genomic advances have led to the identification of several key nutrient-regulation related genes relevant to biofortification (Carvalho & Vasconcellos, 2013). The accumulation of Fe and Zn in seeds is determined through several mechanisms. Gene families involved in root mineral uptake include ZIP (Zinc/Iron-regulated transporter-related protein), ZIF (zinc induced facilitator), HMA (heavy metal associated), FRO (ferric reductase oxidase), and NA (nicotianamine) (Haydon & Cobbett, 2007; Curie et al., 2009; Haydon et al., 2012). Shoot transport gene families include ZIP, FRO, NA and MATE (multidrug and toxic compound extrusion) (Vert et al., 2002; Wintz et al., 2003, Bashir et al., 2006; Ishimaru et al., 2005, 2011, 2012) and seed filling genes include HMA and NRAMP (Natural Resistance Associated Macrophage Protein) (Connorton et al., 2017a; Mary et al., 2015; Carvalho & Vasconcelos, 2013; Lopez-Millan et al., 2016). Genes in the NA, IRT (iron regulated transporter), VIT (vacuolar iron transporter) and ferritin families have been used to increase the concentration of Fe and/or Zn in grain of wheat and rice (Borg et al., 2012; Moreno-Moyano, et al, 2016; Connorton., et al., 2017b; Singh et al., 2017).

In this study, individual genetic maps and QTL for seed Fe and Zn concentration and content from seven populations were used to develop a consensus map and to identify Fe and Zn **meta**-QTL. These **meta**-QTL narrowed down confidence intervals of initial individual analysis' and the physical regions of the **meta**-QTL were identified. Furthermore, candidate genes within the **meta**-QTL intervals that belong to families of genes that have been reported in the literature in the process of uptake, transport and/or remobilization of Fe and Zn were selected.

Materials and Methods

Populations

QTL information related to Fe and Zn in common bean seed was collected for seven populations, five of which have been previously published. We have summarized the details in Table 1. The seven populations include two Andean (AND 696 x G19833 (AG), G21242 x G21078 (GG2)), two Middle American (G14519 x G4825 (GG1), Black Magic x Shiny Crow (BS)), and three

inter-gene pool (DOR 364 x G19833 (DG), BAT 93 x Jalo EEP (BJ), Cerinza x G10022 (CG)) populations of common bean. Additional description of field trials, statistical analysis, and molecular markers for the published studies have been previously reported in detail (Blair et al., 2009; Cichy et al., 2009, Blair et al., 2010c, Blair et al., 2011, Blair & Izquierdo, 2012, and Blair et al., 2013). The description of field trials and the map development for BS has been previously reported (Cichy et al., 2014).

Phenotypic data

The seven populations were planted in multiple years, environments, and locations. The GG1 and GG2 populations were planted in three locations in Colombia: Darien, Palmira and Popayan. DG was planted in Popayan and Darien and CG in Palmira and Darien. The AG population was planted in Darien under high and low soil P treatments. The BS population was planted in Michigan, USA and the BJ population in Darien, Colombia (Table 2). Two methods of mineral analysis were implemented in the studies, Inductively Coupled Plasma–Optical Emission Spectrometry (ICP) and Atomic Absorption Spectroscopy (AAS) as described in (Blair et al, 2009). AAS was used to quantify minerals in the AG population and ICP was used in the BS. The other studies used both methodologies to quantify Fe and Zn. All seven populations have data for seed Fe and Zn concentration. The CG and GG1 populations have data for seed Fe and Zn content, DG and CG have data for seed Fe and Zn concentration in cotyledon tissue, and CG also has seed coat Fe and Zn concentration data. The DG data on Fe and Zn concentration in cotyledon has not been published previously. For the analysis of cotyledon and coat were used the methodology described by Blair et al 2013 where 12g of seed were washed with sterile water and peeled by hand using a sterile scalpel to remove the seed coat from the cotyledons. The separate seed coat and cotyledon samples were dried before grinding. Seed coat and cotyledonary tissues were weighed out into two replicates of ~0.25g dry weight each which were analyzed separately before averaging. Phenotypic data were analyzed using Pearson’s correlations between traits and years using the software SAS, v 9.3 (SAS-Institute 2011).

QTL analysis

The seven populations range in size from 77 lines in AG to 138 lines in CG. The number of markers range from 114 in GG1 to 681 in BS. Eighty eight QTL for seed Fe and Zn concentration and/or content have been reported for the populations AG, CG, DG, GG1, and GG2 (Blair et al., 2009; Cichy et al., 2009, Blair et al., 2010c, Blair et al., 2011, Blair & Izquierdo, 2012, and Blair et al., 2013). While the DG QTL study reported previously found 26 QTL for seed Fe and Zn concentration (Blair et al., 2009), the QTL data we present here is based on a **reanalysis** of the phenotypic data with a saturated genetic map of this population that became available following the publication of the original DG QTL study (Galeano et al., 2011). This new map provided high resolution for QTL detection, and for that reason we **repeated** the QTL analysis with the new genetic map. We conducted a new QTL analysis for the BS and BJ populations via composite interval mapping (CIM). **This** is a new analysis that has not been previously published. The QTL analysis was carried out using the program Windows QTL cartographer version 2.5 (Wang et al., 2012). To identify an accurate significance threshold for each trait, an empirical threshold was determined using 1000 permutations (Churchill and Doerge, 1994).

Map projection and Meta-analysis

In total data from 87 QTL were used for analysis (Table 1, Tables S1). In the population AG, all published QTL were used for meta-analysis except the QTL reported on chromosome 11. In AG chromosome 11 is comprised of 15 random amplified polymorphic DNA (**RAPD**), 1 amplified fragment length polymorphism (**AFLP**), and 1 simple sequence repeat (**SSR**). Due to the lack of sequence information for these markers it was not possible to use these QTL in the meta-analysis.

The program Biomercator v3.0 (Arcade et al., 2004) was used to develop a consensus map and for subsequent **meta-QTL** analysis. The DG map was chosen as the anchor for the consensus map since it has a high marker density, including 499 molecular markers of which 462 are SSR or SNPs that have information about their physical position (Galeano et al., 2011). The other six populations were projected onto the DG map to integrate the seven populations into the consensus map. Six of the seven maps share common markers, the exception is **that** of the BS population **which** is composed of 681 SNPs (Cichy et al., 2014). The BS population was integrated in the analysis using the physical position of SSR and SNPs of the DG map in the *P. vulgaris* v.2.1

reference genome available in Phytozome v12 (Goodstein et al., 2012). The DG SSR sequences were obtained from Phaseolus genes website at <http://phaseolusgenes.bioinformatics.ucdavis.edu>. Using the DG SSRs and SNPs and BS SNPs physical positions, we made a bridge between both genetic maps, taking into account the distance in cM estimated by Schmutz et al., 2014. Positions of Fe and Zn concentration and content QTL were extrapolated onto the consensus map on the basis of common genetic marker positions. Co-localization of meta-QTL was determined by the Akaike's information criterion (AIC) (Akaike, 1974), and the lowest value was considered the best fit model for meta-QTL prediction.

Candidate genes

The search for candidate genes was performed based on the physical positions of the meta-QTL regions. The most recent annotated version of the *P. vulgaris* reference genome v.2.1 in Phytozome was used to identify the physical position of the meta-QTL and genes contained in these regions (Goodstein et al., 2012). The candidate genes were selected on the basis of a literature review of genes that have been reported as having a role in root uptake, transport and accumulation of Fe and Zn in plants (Vert et al., 2002; Wintz et al., 2003; Ishimaru et al., 2005, 2011, 2012; Bashir et al., 2006; Haydon & Cobbett, 2007; Curie et al., 2009; Borg et al., 2012; Haydon et al., 2012; Carvalho & Vasconcelos, 2013; Mary et al., 2015; Lopez-Millan et al., 2016; Moreno-Moyano, et al, 2016; Connorton et al., 2017a, b; Singh et al., 2017). In the case of bZIP family where has been reported that only two genes of that family have a relationship with the uptake of minerals on plants (Assuncao et al., 2010), the protein sequences of those two genes were aligned to the *P. vulgaris* reference genome v.2.1 using BLASTP in NCBI (<https://blast.ncbi.nlm.nih.gov/>).

Results

Single Population QTL Analysis

This study combines 87 QTL for seed Fe and Zn concentration and content, including 39 for Fe and 48 for Zn across seven common bean populations. It includes 56 QTL collated from previous studies within the AG, CG, GG1, and GG2 populations (Table S2). Additionally, 31

previously unreported QTL were identified with CIM analysis in the BJ, BS and DG populations (Table 1). Among the QTL identified with CIM, three were identified for Fe seed cotyledon and one for Zn seed cotyledon concentration were found in DG population. The QTLs for Fe seed cotyledon are on chromosomes Pv02, Pv08 and Pv11, while the QTL for Zn seed cotyledon is on chromosome Pv11. The other 27 QTLs of populations BJ, BS and DG include 11 QTL for seed Fe and 16 QTL for seed Zn concentration. The seed Fe/Zn concentration QTL are distributed on chromosomes Pv01, Pv02, Pv03, Pv04, Pv05, Pv06, Pv08, Pv09 and Pv11. R^2 values for Fe concentration QTLs ranged from 0.08 on chromosome Pv03 to 0.27 on chromosome Pv11, and R^2 values for Zn concentration QTLs ranged from 0.09 on chromosome Pv02 to 0.27 on chromosome Pv04 (Table S1).

Phenotypic values for seed Fe concentration ranged from 28 to 114 ppm and for seed zinc concentration from 16 to 57 ppm (Table 2). Seed Fe and Zn concentration were positively correlated across the seven populations, and the average of those correlations was 0.66, 0.67, and 0.50 for the Andean (AG, GG2), Middle American (GG1, BS) and inter-gene-pool crosses (DG, BJ, CG) respectively. The average correlation between Fe and Zn concentration on the seven populations was 0.59.

Chromosomes Pv01 and Pv06 contained the highest number of QTL while Pv10 was the only chromosome without a single QTL identified (Table S1). Individual QTL explained between 4 and 55% of the phenotypic variance. In total, 25 QTL had R^2 values greater than 20%, and therefore are considered major QTL. All QTL detected on Pv06 and Pv09 were detected only in intra-gene-pool populations, whereas the remaining chromosomes (Pv01 through Pv05, Pv08, and Pv11) contained QTL from both intra- and inter-gene-pool populations. Fifty five percent of QTL had an Andean source while the other forty five percent had Middle American sources. Pv02 had the most consistently detected QTL, such that QTL were detected in the same region in five out of the seven populations.

Consensus Mapping

The DG genetic map was used as a reference to develop the consensus map. The DG map is highly saturated and, with the exception of the BS population, it had markers in common with

all maps that were integrated into this study. The consensus map consists of 1,038 markers with a total length of 2,012 cM and an average distance between markers of 3.6 cM. Of the 87 QTL that were identified in the 7 populations, 72 were projected on the consensus genetic map (Table S3). The remaining 15 QTL that were not projected were in regions that did not have sufficient common markers to make a reliable projection on the consensus map. For consensus QTL projection, the chromosomal position, LOD score, and R^2 of the individual QTL was taken into consideration. Chromosome Pv06 had the highest number of consensus QTL (18 QTL) and Pv02 contained the highest number of QTL that came from different populations (5 populations) (Fig 1). The order of each chromosome was estimated with the physical position of SSR and SNP markers in the *P. vulgaris* reference genome v.2.1.

Meta-QTL analysis

The meta-analysis of the 72 QTL projected in the consensus map was performed in Biomecator 3.0, the AIC was used to select the best QTL model on each chromosome (Table 3). The meta-analysis resulted in a genetic model with 12 meta-QTL that covered 47 of the 72 individual QTL from the seven populations (Fig 2 and Table S4). The number of meta-QTL identified on each chromosome varied from one on chromosomes Pv01, Pv04, Pv09 and Pv11, and two on chromosomes Pv02, Pv06, Pv07 and Pv08. The mean R^2 of the MQTL ranged from 10.3 to 27.0%, while the 95% confidence intervals for the MQTL varied between 3.1 and 18.1 cM, with an average of 7.6 cM. The CI was narrower in all MQTL than the mean CI identified for the original QTL. The physical length of MQTL varied from 0.36 to 11.93 Mb. MQTL_Fe&Zn_6.1 and MQTL_Fe&Zn_6.2 contained the highest number of individual QTL, each one with 8 QTL. MQTL_Fe&Zn_11.1 contained QTL from three different inter gene pool populations.

Three types of MQTL were identified: 1) Fe-MQTL, 2) Zn-MQTL, and 3) Fe & Zn-MQTL. Fe-MQTL included MQTL_Fe_7.2 and MQTL_Fe_8.1. MQTL_Fe_7.2 and MQTL_Fe_8.1 have two individual QTL each that come from two different populations, in the case of MQTL_Fe_7.2 the source in GG2 was the Andean line G21078 and for GG1 was the Middle America genotype G4825. Similarly the sources of MQTL_Fe_8.1 came from both the Andean (Cerinza) and Middle American (Black Magic) gene pools. Zn-MQTL are distributed on chromosomes Pv02 and Pv07.

MQTL_Zn_2.2 includes QTL from two inter genepool populations (BJ and DG). The source of the BJ-QTL the source is the Middle American line BAT93 and in the DG-QTL the source is the Middle American DOR364. MQTL_Zn_7.1 is specific to CG and included two QTL that were identified in the CG cross between an Andean line and a Mexican wild genotype. One of these QTL is for seed Zn content with as source the wild genotype G10022, while the other QTL is for seed coat Zn content, with as source the Andean line Cerinza. The last group is the Fe & Zn-MQTL, this is the most abundant group with eight MQTL across seven chromosomes. MQTL_Fe&Zn_1.1 and MQTL_Fe&Zn_8.2 have Andean sources, MQTL_Fe&Zn_4.1 has Middle American sources and MQTL_Fe&Zn_2.1, MQTL_Fe&Zn_6.1, MQTL_Fe&Zn_6.2, MQTL_Fe&Zn_9.1 and MQTL_Fe&Zn_11.1 have both gene pools as sources of favorable alleles for Fe and Zn concentration/content in seed. The individual Fe and Zn contribution to the shared meta-QTL is available in Table S5.

Candidate genes

In total, 12 candidate genes were identified related to mineral transport or storage. These were found within 5 of the 12 MQTL regions (Table 4). The gene families identified in the MQTL have been previously reported to function in various points in Fe and Zn acquisition, including 1) Root uptake (ZIP, FRO and NA), 2) Translocation within the plant (ZIP, FRO, NA and MATE), and 3) Storage in seed (NRAMP). The name, position and family of each of the 15 candidate genes is reported in Table 4.

ZIP family: In both MQTL_Fe&Zn_9.1 and MQTL_Fe&Zn_11.1 there is a single ZIP family gene. Members of this family participate in mineral uptake, transport to leaves and translocation to seeds, embryo, endosperm, and seed coat (Vert et al., 2002; Ishimaru et al., 2005). The ZIP genes in MQTL_Fe&Zn_9.1 and MQTL_Fe&Zn_11.1 have both been annotated in the reference genome as zinc/iron transporters (Goodstein et al., 2012). In addition, a bZIP transcription factor was found in MQTL_Fe&Zn_11.1. Although there are other bZIP elements in the MQTL, bZIP Phvul.011G035700 is the only one that aligned at the protein level with the genes bZIP19 and bZIP23 (e-value of 4×10^{-120} and 5×10^{-116} respectively) that have an important function in Zn uptake capacity in *Arabidopsis* (Assuncao et al., 2010).

FRO family: Three FRO genes were identified within MQTL_Fe&Zn_6.1. FRO genes have important roles in iron uptake and in its transport in the vascular system (Wu et al., 2005; Kim & Guerinot, 2007). Furthermore other members of this family play an important role in chloroplast iron acquisition (Jeong et al., 2008).

NA family: MQTL_Fe&Zn_1.1 contains an NA family gene. NA genes have been related with mechanisms to acquire Fe and other minerals from the soil (Waters et al., 2006). NA chelates metal cations (Masuda et al 2009), and there is evidence that suggests that NA family play a role in the internal transport of Fe, Zn and other metals in plants (Takahashi et al., 2003; Schuler et al., 2012; Singh et al, 2017)

MATE family: Members of the MATE family are involved in the efflux of molecules from the cytoplasm to the outside of the cell or into the vacuole, and it is likely that these genes products export an Fe chelator that allows the movement of Fe in the plant (Grotz & Guerinot, 2006, Rogers et al., 2009). One MATE gene was found in MQTL_Fe&Zn_6.1, and two genes were located in MQTL_Fe&Zn_4.1.

NRAMP family: NRAMP Genes are involved in transport of metals out of vacuoles. In *Arabidopsis*, members of this family are required for iron mobilization in germinating seeds (Thomine et al., 2003; Lanquar et al., 2010; Gollhofer et al., 2014; Mary et al., 2015). NRAMP genes were located in MQTL_Fe&Zn_1.1 and MQTL_Fe&Zn_9.1.

Discussion

Micronutrient deficiencies are widespread nutritional disorders affecting billions of people around the world (Nestel et al., 2006; Zhao et al., 2009; Vasconcellos et al., 2017). To date biofortification programs have increased Fe and Zn content in several crops, however more efforts are still needed for **at risk human populations** to reach the recommended dietary requirements (Vasconcelos et al. 2017). Additional progress can be made through molecular breeding. Next generation sequencing information has allowed genome sequencing of the most important crops to human consumption. There has also been progress in identifying genes that are involved in the movement of Fe and Zn in plants and using these genes for biofortification of

rice (Goto et al., 1999), cassava (Ihemere, 2012), wheat (Borg et al., 2012), maize (Kanobe et al., 2013), lettuce (Goto et al., 2000) and soybean (Vasconcelos et al., 2014).

Seed Fe and Zn concentrations are quantitative traits with wide genotypic variability. As was reported by Beebe et al., 2001 and Blair et al., 2008, there is a difference in Fe and Zn concentration between the gene pools. Andean genotypes tend to have higher Fe but lower Zn than genotypes from the Middle American gene pool. Another important factor in the analysis of seed Fe and Zn concentration in common bean is the genotype–environmental interaction (GxE) (Araújo et al., 2003; Pereira et al., 2014). Beebe 2012 and Hossain et al 2013 reported that environmental factors such as soil characteristics and precipitation have an important influence in mineral accumulation in common bean.

To unravel the genetic complexity of seed Fe and Zn concentration and content we collected data from seven populations over four locations, nine years, and with multiple methodologies being used for mineral quantification, including whole seed, cotyledon, and seed coat measurements. The seed mineral data from the seven populations was positively correlated among locations, years and traits (Fe-Zn). The average correlation between Fe and Zn **concentration** in the seven populations was 59%. The correlation between Fe and Zn support the well-reported observation that these traits are linked and if we increase the concentration of one of them, we will increase the other as well (Blair et al., 2010b; Blair and Izquierdo, 2012). The correlation may be related to the similar movement of Fe and Zn through the plant, ultimately to the seed. Many of the same genes are involved in both Fe and Zn transport (Kim and Guerinot, 2007; Bashir et al., 2013). The positive correlation between seed Fe and Zn concentrations has been reported in other crops as well, including chickpea (Diapari et al., 2014; Upadhyaya et al., 2016).

In this study we used seven populations that involve the two major gene pools of common bean. We included four intra- and three inter genepool crosses. In total we tested 87 individual QTL detected in seven populations and were able to project 72 in the consensus map (41 have an Andean source, 25 have a Middle American source, and 6 **have** a wild Middle American source). The 72 QTL projected were distributed across all chromosomes except Pv10. The

numerous QTL reflect the genetic complexity of the accumulation of Fe and Zn in common bean seeds.

The consensus map generated from the seven maps has a size of 2,012 cM with 12 MQTL including two MQTL for Fe, two for Zn and 8 MQTL co-localized for Fe and Zn. It is interesting that of the nine QTL projected in the consensus map from the BS population, all but two clustered with QTL of other populations. All populations used in this study were planted in Colombia except the BS that was planted in Richville, MI – US, and although the environmental conditions are different (e.g. soil type, PH, average yearly rainfall), the BS-QTL are mainly close to the QTL of the other populations that have a Middle American genotype as a source. The proximity of BS-QTL with the Middle America QTL suggests that although there is a GxE interaction (Araújo et al., 2003; Beebe 2012; Hossain et al 2013 and Pereira et al., 2014;) in the accumulation of Fe and Zn, the gene-pool origin has an important effect in the accumulation of these minerals in the seed of common bean.

In this study we narrowed down the CI in all 12 MQTL regions that allowed us to identify 12 candidate genes that could be responsible for some of the differences in the seed Fe and Zn concentration/content in the populations included in this study. Out of the 12 MQTL, the eight Fe-Zn shared MQTL distributed over chromosomes 1, 2, 4, 6, 8, 9 and 11 have major potential for molecular breeding because they are associated with both Fe and Zn concentration and/or content and could potentially be used to increase the content of both elements in common bean seed. In five of these eight meta-QTL there are 12 candidates for validation and subsequent application of allelic variation in breeding, e.g. by use of genetic transformation or allele screening in germplasm collections (ecoTilling). The genes Phvul.006G030500, Phvul.006G030550 and Phvul.006G030600 of the FRO family in MQTL_Fe&Zn_6.1 are of special interest, because genes of this family have been used successfully to increase mineral concentration in rice, wheat, and soybean (Goto et al. 1999; Borg et al. 2012; Vasconcelos et al. 2014). FRO genes are responsible for reducing iron at the root surface (Wu et al., 2005; Mukherjee et al., 2006). Dicots acidify the rhizosphere to acquire Fe from the soil. The roots release organic acids and phenolic compounds to increase Fe³⁺ concentrations in the soil solution. These compounds chelate Fe³⁺ which subsequently is reduced to Fe²⁺ in the plasma

membrane of root epidermal cells by ferric reductases which are encoded by members of the FRO gene family (Wu et al., 2005; Mukherjee et al., 2006; Connolly and Guerinot, 2002; Kobayashi and Nishizawa, 2012). We identified twelve candidate genes with the information available in the literature of the better-known gene families that have a relationship in the process to uptake, transport, and accumulation of Fe and Zn in plants. For the above, it is possible that we missed reporting some genes that belong to families that do not have a well-reported role in the movement of minerals in plants or genes that belong to families with unknown function.

Quantitative traits are a challenge in plant breeding due to the genetic complexity that governs these traits, and the difficulty of stacking numerous alleles that control them. Meta-QTL analysis made possible the consolidation of 47 single QTL into 12 meta-QTL. These results showed a greater consolidation than a maize meta-QTL analysis for grain Fe and Zn, where 28 single QTL were consolidated into 10 meta-QTL (Jin et al., 2013). While we show that there are at least 12 regions that control the seed concentration/content of Fe and Zn in the common bean genome, the eight regions that associate with both Fe and Zn are most promising for focus in future studies. The stacking of eight independent regions in a single breeding line is challenging as there is a probability of one in 256 to stack the eight regions with the favorable alleles. The MQTL identified in this study have three potential uses, the first one is to generate markers for Marker assisted selection (MAS) for gene stacking breeding lines, the second way is the validation and subsequent use of the genes identified in this study in bean genetic transformation or eco-Tilling programs, and the last and perhaps most promising is the use of the MQTL regions in Genomic Selection (GS) models to increase the models accuracy in their use in bean breeding programs. Although this is a new study field, Spindel et al., 2016 reported promising results in rice using the regions identified by Genome-wide association studies (GWAS) as fixed effects in GS models.

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List of Supplementary Tables

Table S1. List of Fe and Zn QTL identified in the seven common bean mapping populations.

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Table S4. List of the QTL that comprise the MQTL regions.

Table S5. Shared Fe-Zn **meta**-QTL disaggregated data.

Table 1. A summary of the common bean mapping populations considered for meta-QTL analysis.

ID	Parents	Genepool	Donor parent	Type of population	Population Size	Markers Used	No. of markers	QTL reported	QTL for Meta analysis	QTL for Meta Analysis by type			
										Fe conc.	Zn conc.	Fe content	Zn content
AG	AND696 x G19833	A x A	G19833	RIL	77	SSR - RAPD - AFLP	167	23	17	9	8		
GG2	G21242 x G21078	A x A	G21242	RIL	100	SSR - RAPD	118	9	9	6	3		
GG1	G14519 x G4825	M x M	G14519	RIL	110	SSR - RAPD	114	17	17	4	9	3	1
BS	Black Magic x Shiny Crow	M x M	Shiny Crow : Fe Black Magic: Zn	RIL	92	SNP-PAV-DArT	681	NR	8	3	5		
DG	DOR364 x G19833	M x A	G19833	RIL	87	RFLP - SSR - SNPs STS	499	26	14	8	6		
BJ	BAT93 x Jalo EEP	M x A	BAT93 : Zn Jalo EEP : Fe	RIL	78	SSR - RAPD - AFLP RFLP	458	NR	9	3	6		
CG	Cerinza x G10022	A x W	G10022	AB	138	SSR	143	13	13	2	6	1	4

Genepool: A – Andean; M – Middle American; W – Wild.

NR: Not previously reported.

Conc: concentration.

AG: Cichy et al, 2009, GG2: Blair et al, 2011, GG1: Blair et al., 2010c, BS: Cichy et al., 2014, DG: Blair et al. 2009; Galeano et al., 2011, BJ: Freyre et al., 1998, CG: Blair & Izquierdo, 2012, Blair et al., 2013.

Table 2. Summary of the location, years, range of Fe and Zn, and environmental conditions of QTL studies included for meta-QTL analysis.

Population	Location	Year	Range ppm		MASL	AT (°C)	AR (mm)	PH	Soil type
			Fe	Zn					
AG	Darien - Colombia	2000	53-77	16-29	1485	20	1,288	5.6	Udand (LP - HP)**
	Darien - Colombia	2005	39-79	20-23					
GG2	Darien - Colombia	2011	28-95	17-37	1450	20	1,650	5.6-6.1	Udand
	Palmira - Colombia	2011	48-93	17-49	1000	24	905	7.8	Haplustoll
	Popayan - Colombia	2011	30-88	22-57	1730	18	2,124	5.6-6.1	Dystrudepts
GG1	Darien - Colombia	2010	35-81	17-39	1400	20	1,500	5.6	Udand
	Palmira - Colombia	2010	43-97	17-32	1000	24	905	7.8	Haplustoll
	Popayan - Colombia	2010	44-77	30-49	1730	18	2,124	6.1	Dystrudepts
BS	Richville, MI - US	2010	48-102	24-37	190	22*	871*	7.9	Tappan-Londo loam
		2011	48-99	23-38					
		2013	55-108	28-43					
DG	Popayan - Colombia	1998	40-79	19-37	1730	18	2,124	5.6	Dystrudepts
	Darien - Colombia	2003	42-84	17-42	1400	20	1,650	5.6	Udand
BJ	Darien - Colombia	2007	46-114	20 - 57	1450	20	1,650	5.6	Udand
CG	Palmira - Colombia	2012	54-100	27-39	996	24	950	7.2	Vertisol
	Darien - Colombia	2012	58-92	23-38	1485	20	1,288	5.6	Vertisol

MASL: Meter above sea level.

AT: Average yearly temperature in °C.

AR: average yearly rainfall.

*average between June-September.

**LP and HP is low and high P respectively.

Table 3. Summary of the meta-QTL associated with seed iron and zinc concentration.

MQTL ^a	Chr	Trait	Initial number of QTL	Mean R2 (S. D.) ^b	Mean initial CI ^c (cM)	MQTL CI (cM)	CRL ^d	Physical position (Mb) ^e		MQTL Size (Mb)	No. genes in MQTL
								Start	End		
MQTL_Fe&Zn_1.1	1	Fe - Zn	6	24.3 (9.3)	9.7	4.2	2.3	43.3	48.5	5.24	553
MQTL_Fe&Zn_2.1	2	Fe - Zn	3	10.3 (5.69)	10.7	3.1	3.4	34.5	35.0	0.44	24
MQTL_Zn_2.2	2	Zn	2	11.0 (2.83)	14.7	7.1	2.1	40.5	42.6	2.15	216
MQTL_Fe&Zn_4.1	4	Fe - Zn	2	18.0 (12.73)	30.5	18.1	1.7	44.8	46.0	1.21	108
MQTL_Fe&Zn_6.1	6	Fe - Zn	8	20.4 (5.07)	21.4	8.7	2.5	10.2	12.4	2.17	69
MQTL_Fe&Zn_6.2	6	Fe - Zn	8	27.0 (11.3)	26.1	5.4	4.9	28.2	29.5	1.28	172
MQTL_Zn_7.1	7	Zn	2	11.5 (2.12)	8.8	6.6	1.3	0.1	0.5	0.36	42
MQTL_Fe_7.2	7	Fe	2	12.0 (1.41)	17	8.6	2.0	29.5	36.9	7.44	698
MQTL_Fe_8.1	8	Fe	2	11.5 (0.71)	9.1	5.2	1.7	0.8	3.5	2.63	331
MQTL_Fe&Zn_8.2	8	Fe - Zn	4	12.3 (3.77)	10.7	4.8	2.2	12.5	24.4	11.93	300
MQTL_Fe&Zn_9.1	9	Fe - Zn	2	15.1 (5.66)	12	3.5	3.4	11.7	13.5	1.79	160
MQTL_Fe&Zn_11.1	11	Fe - Zn	6	15.0 (7.27)	16.1	10.1	1.6	2.3	5.3	2.98	337

^a The Akaike's information criterion (AIC) was used to select the best QTL model for Fe and Zn, the one with the lowest AIC value was chosen as a significant model to indicate the number of MQTL on each chromosome.

^b S.D. = Standard deviation of R2 values of individuals QTL involved in MQTL

^c CI = confidence interval of 95% of QTL location

^d Coefficient of reduction in length from mean initial QTL to MQTL

^e Physical size and position of MQTL were estimated with the location of SNPs and the alignment of the SSR sequences in the reference genome *P. vulgaris* v.2.1

Table 4. Candidate genes found within meta-QTL for seed Fe and Zn concentration and content.

MQTL	Candidate Gene Name	Chr	*Start (bp)	End (bp)	Gene family
MQTL_Fe&Zn_1.1	Phvul.001G177500	Chr01	43,453,927	43,463,444	NRAMP
MQTL_Fe&Zn_1.1	Phvul.001G225000	Chr01	47,980,872	47,982,640	NA
MQTL_Fe&Zn_4.1	Phvul.004G152100	Chr04	45,482,068	45,486,874	MATE
MQTL_Fe&Zn_4.1	Phvul.004G152400	Chr04	45,504,102	45,509,691	MATE
MQTL_Fe&Zn_6.1	Phvul.006G028701	Chr06	10,873,033	10,876,474	MATE
MQTL_Fe&Zn_6.1	Phvul.006G030500	Chr06	11,701,246	11,702,422	FRO
MQTL_Fe&Zn_6.1	Phvul.006G030550	Chr06	11,747,123	11,748,871	FRO
MQTL_Fe&Zn_6.1	Phvul.006G030600	Chr06	11,804,817	11,806,566	FRO
MQTL_Fe&Zn_9.1	Phvul.009G069700	Chr09	12,141,306	12,145,376	NRAMP
MQTL_Fe&Zn_9.1	Phvul.009G077700	Chr09	13,064,171	13,066,165	ZIP
MQTL_Fe&Zn_11.1	Phvul.011G035700	Chr11	3,293,008	3,295,446	bZIP
MQTL_Fe&Zn_11.1	Phvul.011G058500	Chr11	5,228,604	5,232,591	ZIP

*Physical position in the reference genome *P. vulgaris* v.2.1.



