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Quantitative trait loci associated with soybean seed weight and composition under different phosphorus levels

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Research Article

Abstract Seed size and composition are important traits in food crops and can be affected by nutrient availability in the soil. Phosphorus (P) is a non-renewable, essential macronutrient, and P deficiency limits soybean (*Glycine max*) yield and quality. To investigate the associations of seed traits in low- and high-P environments, soybean recombinant inbred lines (RILs) from a cross of cultivars Fiskeby III and Mandarin (Ottawa) were grown under contrasting P availability environments. Traits including individual seed weight, seed number, and intact mature pod weight were significantly affected by soil P levels and showed transgressive segregation among the RILs. Surprisingly, P treatments did not affect seed composition or weight, suggesting that soybean maintains sufficient P in seeds even in low-P soil. Quantitative trait loci (QTLs) were detected for seed weight, intact pods, seed volume, and seed protein, with five significant QTLs identified in low-P environments and one significant QTL found in the optimal-P environment. Broad-sense heritability estimates were 0.78 (individual seed weight), 0.90 (seed protein), 0.34 (seed oil), and 0.98 (seed number). The QTLs identified under low P point to genetic regions that may be useful to improve soybean performance under limiting P conditions.

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INTRODUCTION

Phosphorus (P) is an essential macronutrient required for plant growth and development. In terrestrial ecosystems, soil P availability constitutes a major limitation to plant productivity. It has been estimated that over 70% of terrestrial biomass occurs on soils with low P availability, which includes more than half of agricultural lands, worldwide (Lynch 2011). Furthermore, soil P undergoes significant organic complexation and inorganic fixation in the soil, which further limits its availability to plants (Kochian 2012). In particular, P is the most limiting essential nutrient for soybean (*Glycine max* (L.) Merr.), a legume food crop valued for its high seed protein and oil content. P deficiency in soybean first manifests as abnormal darker green color and shortened stems (Marschner 2011). However, P deficiency often results in stunted plants, aborted pod development, and reduced seed size (Bishnoi et al. 2007). As total soybean production in 2009 was 93 and 319 million metric tons for the United States and the world, respectively (USDA 2010; Hacisalihoglu and Settles 2017), P deficiency can severely impact food production, worldwide.

P fertilization is a major driver of crop productivity, resulting in increasing global consumption of phosphate

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fertilizers as P_2O_5 (FAO 2017). In general, most applied phosphate is rendered inaccessible to plants through adsorption to soil constituents, precipitation, or assimilation by soil microorganisms (Holford 1997). Consequently, phosphate is often applied at rates that exceed plant demand. Many studies predict that high-grade phosphate reserves around the globe will diminish within the current century (Runge-Metzger 1995; Cordell et al. 2009), which exacerbates the increasing pressure to improve agricultural production due to the increasing human population.

One approach to minimize low-P stress is to identify genotypic variation in crop plants that can be used to improve P-use efficiency (Ozturk et al. 2005). In the past few decades, considerable effort has been invested in the selection and development of P-efficient crops with superior growth and yield under low-P availability. Higher P-use efficiency has been associated with a number of plant physiological processes, such as root P uptake, shoot translocation, internal utilization, and mycorrhizal association (Ozturk et al. 2005; Marschner 2011).

Phosphorus is an immobile nutrient in soil and is largely concentrated in the upper soil layers (Marschner 2011). For these reasons, phosphorus capture depends on root system phenotypes that allow for increased soil exploration. For example, P-efficient common bean genotypes develop longer lateral roots (Lynch 2007), shallower basal roots, and higher density root hair distribution (Yan et al. 2004). Strategies for increasing soybean P acquisition efficiency have focused on improving root system architecture to maximize root exploration of P-dense soil space (Wang et al. 2010). Likewise, the molecular mechanisms that govern the P stress tolerance appear to modulate P uptake (Liang et al. 2010).

Quantitative trait locus (QTL) analysis, genome-wide association studies, and differential gene expression studies have been used to identify genetic regions associated with low-P tolerance in soybean (Li et al. 2005; Liang et al. 2010; Zhang et al. 2014; Wang et al. 2016; Zhang et al. 2016). Many of these studies focus on seedling and root traits because symptoms of P deficiency can be observed during early seedling growth. Further, many of the mechanisms of low-P tolerance are thought to be determined in the root system (Wang et al. 2010). Indeed, the only cloned QTL for soybean low-P tolerance encodes an acid phosphatase that is thought to improve P uptake from the soil (Zhang et al. 2014).

While studies have demonstrated that P deficiency in soybean reduces yield, due to aborted seed pods and reduced seed size (Zhang et al. 2010), the effect of P deficiency on seed quality is not known. Based on correlations between soybean seed size and composition traits, reduced seed size is expected to alter protein and oil composition under low-P growth conditions (Hacisalihoglu et al. 2016). Similar to low-P tolerance, a number of QTL studies have examined seed weight, seed protein content, and yield traits in various recombinant inbred line (RIL) populations (Pathan et al. 2013; Yesudas et al. 2013; Ramamurthy et al. 2014; Xie et al. 2014). However, no studies have directly compared seed yield and quality in contrasting P-availability soils.

Here, we aimed to investigate the relationship between soybean seed set, weight, and composition in response to low- and high-P soils. A RIL population generated by crossing the stress-tolerant cultivar Fiskeby III with the stress-sensitive cultivar Mandarin (Ottawa) was evaluated to obtain genetic variation in P tolerance and to identify seed trait-associated QTLs.

RESULTS

Characterization of phenotypic variations

Table 1 and Figure 1 present the mean values for several seed composition and morphological traits of the RILs and two parents (Fiskeby III and Mandarin (Ottawa)) under low-P, high-P, and optimal-P environments. In all environments tested, the stress-tolerant cultivar, Fiskeby III, produced a larger seed with greater weight and volume than when compared to the stress-sensitive cultivar, Mandarin (Ottawa). Mandarin (Ottawa) had lower protein content but higher seed number than Fiskeby III (Table 1). Overall, transgressive segregation that falls outside of the range of the parents was observed in most traits (Figure 1). Furthermore, seed traits, such as individual seed weight, protein content, intact pod weight (pod plus seed weight), and seed volume, exhibited transgressive segregation where individual RIL averages were lower or higher than both parental line averages (Figure 1).

Table 1. Soybean seed traits under three phosphorus (P) environments for recombinant inbred lines (RILs) derived from parents Fiskeby III and Mandarin (Ottawa)

Environment	Seed protein (%)	Seed oil (%)	Seed volume (mm^3)	Individual seed weight (mg $seed^{-1}$)	Seed number per plant	Single plant seed weight (mg $plant^{-1}$)	Intact pod weight (g)
Optimal P (2012; Minnesota, USA)							
RILs avg.	40.1	18.9	183	227			
RILs min.	34.6	15.7	128	171			
RILs max.	45.7	21.6	275	357			
RILs SD	2.24	1.20	22.9	28.8			
FiskebyIII avg.	41.7	18.1	188	234			
Mandarin (Ottawa) avg.	41.9	17.6	149	176			
Low P (2014; Turkey)							
RILs avg.	32.1	20.8	225	191	12	2311	4.34
RILs min.	21.3	12.9	140	117	3	736	1.45
RILs max.	40.9	26.7	305	323	23	4165	9.50
RILs SD	2.70	2.11	31.2	34.9	4.05	709	1.85
Fiskeby III avg.	34.2	20.9	237	225	13	2928	4.99
Mandarin (Ottawa) avg.	27.9	17.9	197	146	20	2925	5.06
High P (2014; Turkey)							
RILs avg.	30.9	20.5	218	180	18	3099	5.99
RILs min.	25.7	16.3	148	96.5	5	772	0.90
RILs max.	37.6	24.9	292	269	32	4754	11.5
RILs SD	2.35	1.90	28.2	31.6	5.16	855	2.29
Fiskeby III avg.	32.7	21.8	246	232	9	2092	3.53
Mandarin (Ottawa) avg.	29.2	18.5	198	153	15	2294	5.89
P-value (phosphorus)	$<0.001^a$	<0.278	<0.05	$<0.007^a$	$<0.0001^a$	$<0.0001^a$	$<0.0001^a$
P-value (genotype)	$<0.089^a$	$<0.0001^a$	$<0.0001^a$	$<0.001^a$	$<0.001^a$	$<0.001^a$	$<0.0001^a$
Broad-sense heritability (H^2)	0.90	0.34	0.65	0.78	0.98	0.97	0.95

[a]Significant at $P < 0.01$. SD, standard deviation. Descriptive statistics (mean, minimum, maximum, standard deviation), analysis of variance *P*-values, and broad-sense heritability estimates (H^2) are shown for each location and year.

The mean values of seed and plant traits showed significant variance among both RILs and environments (Table 1). Analysis of variance results showed significant P effects ($P < 0.05$) for all traits except seed oil concentration. Individual seed weights were higher in the field-grown Minnesota environment than either pot-grown environment in Turkey. Broad-sense heritability (H^2) was very high for protein, seed number per plant, total seed weight per plant, and intact pod weight with H^2 at least 0.90 for these traits (Table 1). There was substantial heritability for all other traits measured.

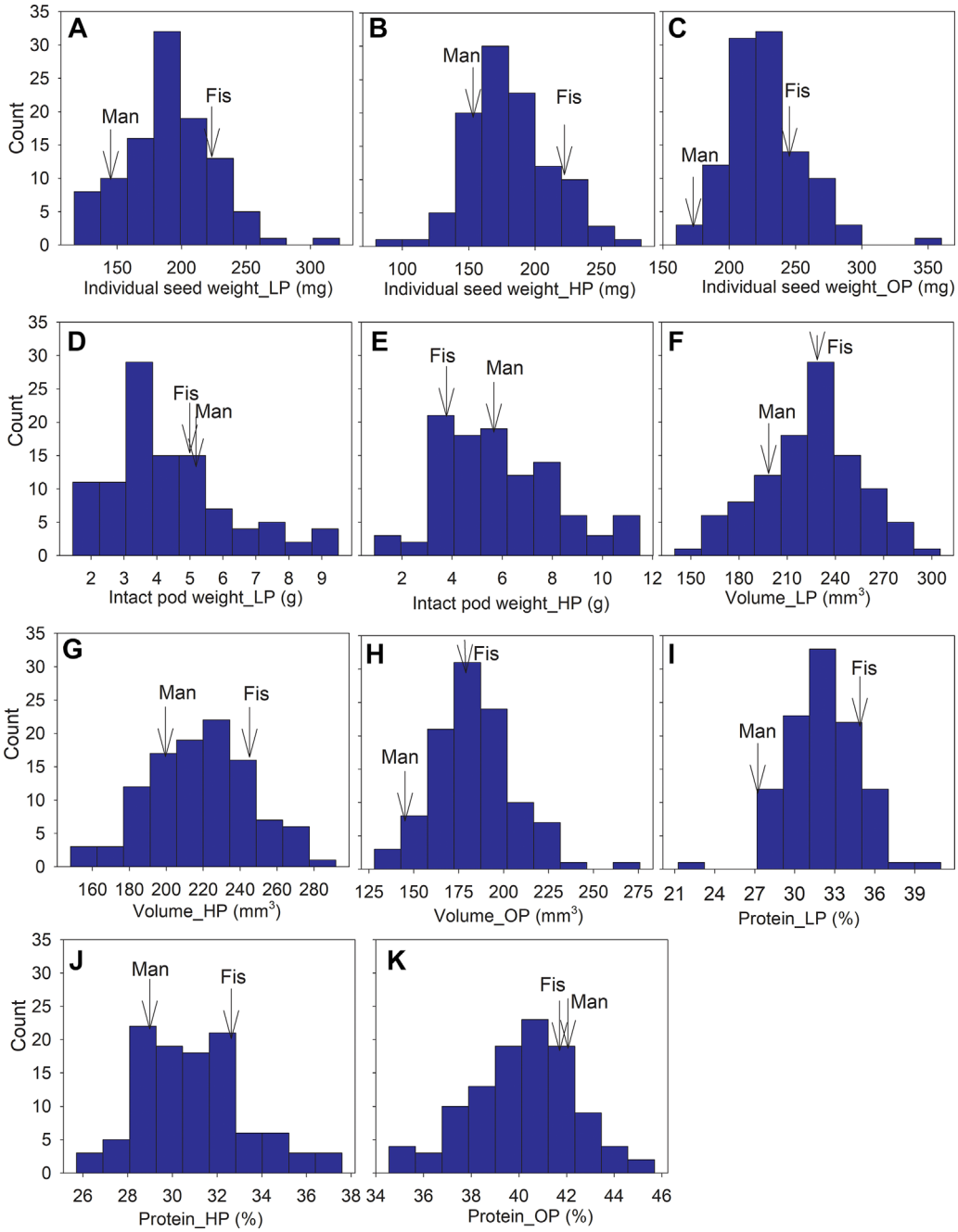


Figure 1. Frequency distribution of soybean seed traits in three phosphorus (P) environments

(**A**) Individual seed weight at low P. (**B**) Individual seed weight at high P. (**C**) Individual seed weight at optimal P. (**D**) Intact pod weight at low P. (**E**) Intact pod weight at high P. (**F**) Individual seed volume at low P. (**G**) Individual seed volume at high P. (**H**) Individual seed volume at optimal P. (**I**) Seed protein at low P. (**J**) Seed protein at high P. (**K**) Seed protein at optimal P. The soybean recombinant inbred line (RIL) population was developed using cultivars Fiskeby III and Mandarin (Ottawa). Arrows indicate the two parental lines of Fiskeby III ('Fis') and Mandarin (Ottawa; 'Man').

Physiological stress response

Low P caused physiological stress that impacted yield traits for the RIL population as a whole (Figure 2). Low P decreased intact pod weight (−37%), single plant seed weight (−33%), and seed number per plant (−31%) in comparison to high P. However, low P did not significantly alter individual seed weight. Seed weight distribution between high and low P is shown in Figure 2.

QTL analysis

No significant QTLs were detected for the traits measured in high-P conditions. However, four highly significant QTLs were found in the low-P condition (Figure 3, Table 2). On chromosome four, a QTL for intact pod weight (PodwtQTL4.2) explained 21% of the variance for this trait under low-P availability. Overlapping QTLs for individual seed weight and seed volume were identified on chromosome six (SeedwtQTL6.1 and VolQTL6.2, respectively) and explained more than 17% of the variance for these traits. On chromosome 20, ProtQTL20.1 explained about 4% of variance in seed protein content.

A second individual seed weight QTL was detected on chromosome four under low-P conditions (SeedwtQTL4.1). This locus had borderline significance that explained 7% of the trait variance. Finally, analysis of the Minnesota seeds used as the parental source for the P comparisons identified a lower-significance seed weight QTL on chromosome four (SeedwtQTL4.3) that overlaps with PodwtQTL4.2.

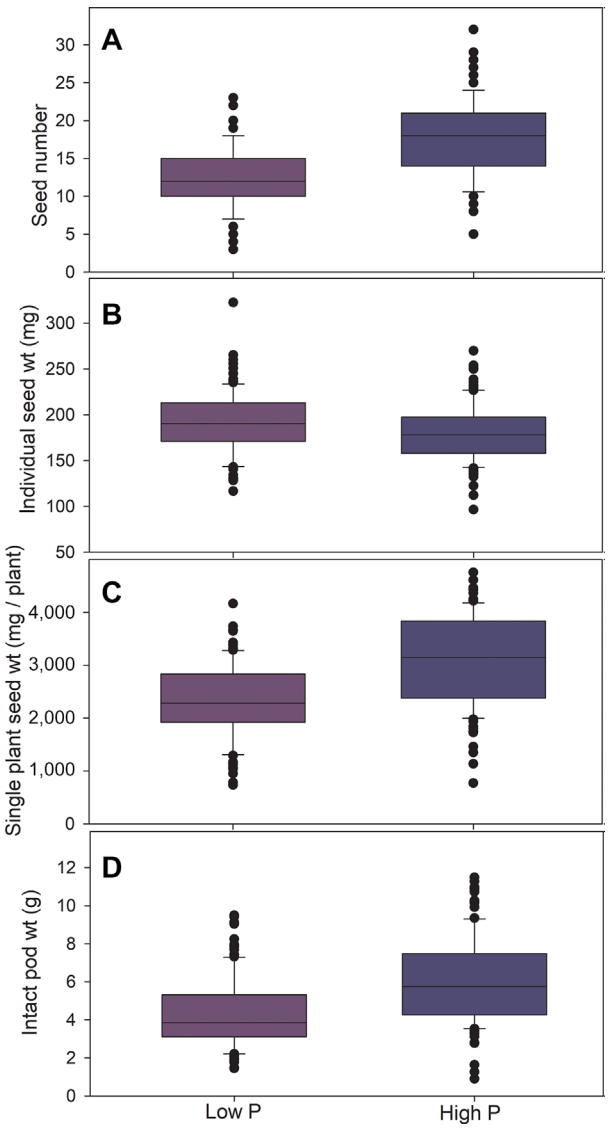


Figure 2. Comparison of seed traits in two phosphorus (P) environments

(**A**) Single plant seed number, (**B**) individual seed weight, (**C**) single plant seed weight, and (**D**) intact pod weight are shown in low-P and high-P environments. Treatment means were significantly different (Student's *t*-test, $P < 0.05$) for seed number, single plant seed weight, and intact pod weight. Treatment means were not significant for individual seed weight.

Correlations between phenotypic traits

The overlap of SeedwtQTL6.1 and VolQTL6.2 suggested that these QTLs may be a single locus detected through correlated traits. Correlations among eight plant and seed characteristics are shown in Table 3. Seed weight and total volume were highly correlated under both low-P ($r = 0.91$) and high-P ($r = 0.94$) conditions. A similarly strong correlation existed for single plant seed weight and seed number. Individual seed weight was also correlated with protein to a lesser extent, but this correlation was not sufficient to detect overlapping QTL for these two traits.

DISCUSSION

Soybean is one of the most important legume crop species worldwide because of its high seed protein and oil content. This study provides insights into the relationship between P deficiency, seed composition and morphological traits in soybean. Our data showed highly significant differences between low- and high-P treatments for seed number, single plant seed weight, and intact pod weight (Figure 2). These findings are consistent with previous findings in which soybean genotypes were grown in varying P conditions using hydroponic growth (Win et al. 2010).

We did not observe large changes in individual seed weight or relative protein or oil content in the RIL population grown in low-P conditions as compared to high-P conditions. Instead, low P primarily decreased seed number and other yield-related traits (Table 1). These observations showed that the P treatments were

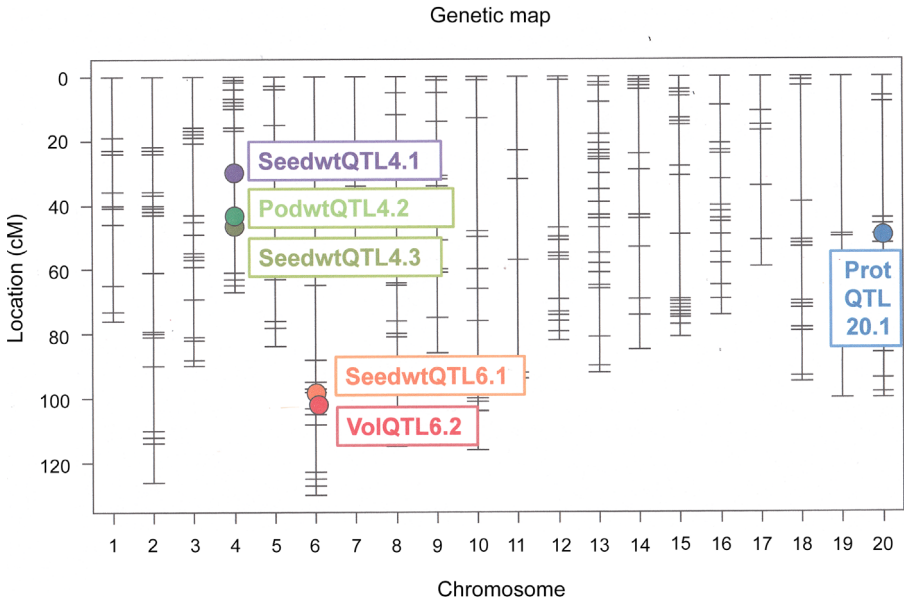


Figure 3. Quantitative trait loci (QTL) identified within the soybean genome for seed traits associated with phosphorus (P) availability

The chromosome map shows marker locations (dashes) and significant QTL (color-coded circles) for seed composition and morphological traits in the soybean recombinant inbred population derived from Fiskeby III x Mandarin (Ottawa).

sufficient to induce a physiological stress and confirm the known effect of low P on yield. The results also suggest that seed composition may not be affected by a decrease in P availability. Other studies have similarly found soybean to display phenotypic plasticity for single-seed traits. For instance, large variation in mineral nitrogen fertilizer application does not impact seed oil, seed protein, or seed weight (Boroomandam et al.

Table 2. Summary of quantitative trait loci (QTL) identified in the soybean recombinant inbred population Fiskeby III x Mandarin (Ottawa) for seed traits related to phosphorus (P) availability

Trait	QTL name	Marker	Chr	Position (cM)	LOD	LOD threshold	*P*-value	LOD alpha	% variance (R^2)
Individual seed weight (under low P)	SeedwtQTL4.1	044331-08673	4	37	4.50	4.47	0.09	$P \leq 0.10$	7.03
Intact pod weight (under low P)	PodwtQTL4.2	061273-17145	4	52	7.66	4.96	0.00	$P \leq 0.05$	21.0
Individual seed weight (under optimal P)	SeedwtQTL4.3	061273-17145	4	52	5.06	4.58	0.06	$P \leq 0.10$	1.91
Individual seed weight (under low P)	SeedwtQTL6.1	031099-06997	6	102	6.00	5.06	0.02	$P \leq 0.05$	17.7
Seed volume (under low P)	VolQTL6.2	031099-06997	6	102	6.56	5.10	0.00	$P \leq 0.05$	17.3
Seed protein (under low P)	ProtQTL20.1	025987-05207	20	53	5.09	4.85	0.03	$P \leq 0.05$	4.06

The trait, its abbreviation, marker, chromosome, position, LOD score, LOD threshold, *P*-value for the given locus, alpha value, and the percent variation explained by the locus are shown.

Table 3. Pearson correlation coefficients (*r*) of seven seed compositional and morphological traits in the soybean Fiskeby III x Mandarin (Ottawa) RIL population in low- and high-phosphorus conditions

Traits	Individual seed weight	Seed number per plant	Single plant seed weight	Intact pod weight	Seed volume	Seed protein
Low phosphorus						
Individual seed weight						
Seed number per plant	−0.40*					
Single plant seed weight	0.11	0.84**				
Intact pod weight	0.12	0.68**	0.85**			
Seed volume	0.91**	−0.38**	0.10	0.11		
Seed protein	0.78**	−0.32**	0.09	0.05	0.75**	
Seed oil	0.54**	0.01	0.28**	0.21*	0.51**	0.34**
High phosphorus						
Individual seed weight						
Seed number per plant	−0.34**					
Single plant seed weight	0.21	0.83**				
Intact pod weight	0.29**	0.67**	0.88**			
Seed volume	0.94**	−0.33**	0.20*	0.27*		
Seed protein	0.78**	−0.46**	−0.06	0.03	0.75**	
Seed oil	0.53**	−0.04	0.30**	0.27**	0.53**	0.29**

*, ** Significant at the $P < 0.05$ and $P < 0.01$ levels, respectively.

2009; Ferreira et al. 2016). Wilson et al. (2014) found a modest increase in relative protein content and seed weight after a large application of nitrogen in maturity group III soybean varieties with no change in seed oil content. In another study, high planting density reduced total yield but did not affect seed composition in soybean (Boroomandam et al. 2009). A common response for soybean under resource limitations is to reduce pod and seed number rather than sacrifice seed size and quality (Marschner 2011). Our findings suggest that P deficiency elicits a similar response by reducing yield components without influencing seed traits.

We primarily detected significant QTLs associated with seed characteristics in the low-P condition. The QTL with the largest effect was a region on chromosome four that explained 21% of intact pod weight variation in low-P conditions. Increased pod weight could be caused by increased seed size and/or increased seed number in the pod. Under P-deficient conditions, we found no evidence that seed weight changes. Therefore, the QTL controlling pod weight most likely affects seed number per pod. However, this chromosome four QTL was also identified as having a modest effect $P = 0.09$) on single seed weight in the Minnesota environment (Table 2). Independent linked genes may be causing the colocation of these two QTLs. The correlation between yield-related traits in low- and high-P environments was low. Consequently, one QTL for yield-related traits would not be expected to function in both environments. Environmental effects on plant phenotypes are known to influence QTL detection, and it is not surprising to find QTLs specific to the low-P environment. In maize, seed yield and yield components are strongly influenced by environment, and QTLs for yield-related traits are not consistently detected across multiple environments (Veldboom and Lee 1996). The number of RILs analyzed was potentially too few for sufficient power to detect lower-effect QTLs. Indeed, half of the QTLs detected individually explained more the 15% of trait variance. Five of the six QTLs explained yield-related traits of seed weight and seed volume on chromosomes six and four as well as intact pod weight on chromosome four. We also detected QTLs for soybean seed weight and volume on chromosomes four and six. Previous studies have identified soybean seed weight QTLs on chromosomes one, two, six, and ten (Panthee et al. 2005; Hu et al. 2013;

Ramamurthy et al. 2014; Xie et al. 2014). However, none of previously identified QTLs overlapped with the QTLs found in this study. Therefore, the low-P conditions used in this study revealed novel QTLs that may be useful for increasing seed weight in low-P soils.

These results indicate that the low P soils used in this study reduced yield traits but had minimal influence on seed size or composition. Therefore, it may be more useful to focus selection efforts on seed and pod number under low-P conditions as a metric for P-use efficiency. To aid in selecting P-use efficient lines, we identified a QTL on chromosome four for increased pod weight. Additional efforts to identify a tightly linked marker for this QTL would be useful in marker-assisted breeding efforts to increase yield in low-P soils. We also identified seed weight and protein content QTLs in low-P conditions that were not observed in other soil conditions. Genes that underlie these QTLs may modulate seed size and composition specifically in P-deficient conditions.

The controlled greenhouse conditions reported here allowed the relationship of plant nutrition with agronomic traits to be investigated under a less complex study design, but this means that the results obtained may not be consistent in diverse field conditions. The soil that was used in this study was taken from low-P sites, which should help in translating the QTLs to the field. However, many other field factors were not tested and are also likely to impact the measured traits. Nonetheless, we have identified new genetic loci that could be used to improve the performance of soybean under low P-conditions and thereby support sustainable soybean production.

MATERIALS AND METHODS

Mapping population and growing environments

The RIL population used in this study was derived from a cross between cultivars Fiskeby III and Mandarin (Ottawa) of soybean (*Glycine max*) as described previously (Burkey and Carter 2009; Burton et al. 2016). Fiskeby III is the stress-tolerant parental line, while Mandarin (Ottawa) is the stress-sensitive parental line. Original seed material for this study was kindly provided by Professor J. Orf at the University of Minnesota.

Minnesota cultivation: One set of 244 RILs and the two parents were planted at the University of Minnesota Research and Outreach Center, Rosemount, MN (44° 42'N 93°06'W) at the optimal P treatment conditions. The F_5-derived bulk seed was planted in May 2012 and harvested in October 2012. The resulting $F_{5:7}$ seeds were used as parent seed for the low- and high-P cultivation experiments (see below).

Low- and high-P cultivation: A subset of 103 $F_{5:7}$ RILs and the two parents were planted in greenhouses at Cukurova University, Adana, Turkey (37°00′N 35°16′E). Seeds were sown in 2 L plastic pots filled with 2.2 kg of P-deficient soil from the southern Anatolian region of Turkey. The soil was composed of 47% clay, 33% sand, and 20% silt. The soil contained 7.54 μg/g available Olsen P, which is considered low according to Ferguson et al. (2006). Therefore, the soil was supplemented with 30 μg/g P or 100 μg/g P to create low P and high P soils, respectively. The soil in the Rosemount, MN location was Waukegan silt loam with a P content of approximately 47 μg/g. Plants were grown from August 2014 and harvested in December 2014 under standard greenhouse growth practices, as described in Hacisalihoglu et al. (2004). Three biological replicates of three individual plants representing each RIL and parent were grown in both low-P and high-P soil. Agronomic traits were collected at harvest and included seed number per plant, individual seed weight, single plant seed weight, and intact pod weight (pod plus seed weight). Seeds were harvested and air dried prior to seed trait analysis.

Seed trait phenotyping

Seed composition traits were estimated with single-seed near-infrared spectroscopy (NIRS) (Hacisalihoglu et al. 2010, 2016). Average seed traits were estimated per plant by individually weighing and scanning 20 seeds three times each. If a plant produced fewer than 20 seeds, all seeds were assayed with NIRS. NIRS calibrations predicted seed oil, seed protein, and total seed volume, while average seed weight was determined using a microbalance. The oil and protein content of seeds were determined using a NIR-256-1-1,7T1 Near Infrared Spectrometer by triple scanning them in a 1 nm intervals (from 907 to 1,688 nm), with calibrations equations developed and described in Hacisalihoglu et al. (2016). The results were expressed as percentages.

Data analysis and heritability estimate

Two-way analysis of variance (ANOVA) and Pearson correlation coefficients (r) between measured seed composition and morphological traits were performed with IBM SPSS Statistics software package (IBM, Armonk, New York, USA) using a model with P and genotypes as factors. The data were plotted using SigmaPlot (Systat Software, San Jose, CA, USA).

Broad-sense heritability (H^2) was estimated for each trait using the procedure of Punnuri et al. (2016). H^2 was defined as:

$$H^2 = MSgen(MSgen + MSrep + MSer)^{-1}$$

where *MSgen* is the genotype mean square, *MSrep* is the replication mean square, and *MSer* is the error mean square from the two-way *ANOVA*.

Genetic linkage map construction and QTL detection by composite interval mapping

The *G. max* linkage map used in this study was constructed by Burton et al. (2016). The linkage map consisted of a total of 501 polymorphic single-nucleotide polymorphism (SNP) markers from the USDA soybean consensus map (Hyten et al. 2010). The QTL analysis for the traits was performed using the composite interval mapping (CIM) method in the software package R/qtl (Broman et al. 2003). CIM was carried out using three marker covariates with a 10 cM window size. LOD thresholds were determined for each trait from 1,000 permutation tests using significance thresholds of 0.05 and 0.10. Haley-Knott regression was used to determine position and marker effects. Mapping was carried out separately within each environmental location.

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AUTHOR CONTRIBUTIONS

G.H. designed and performed the research plan, collected and analyzed the data, and co-wrote the paper. A.L.B. contributed by constructing the soybean linkage map, conducting the essential QTL analysis, and co-writing the paper. S.E., S.A., E.H.H. contributed soil and carried out data collection and processing from Turkey. L.O., I.C., and A.Y. helped with soil and data collection from Turkey and provided technical guidance on P treatments. K.O.B. helped with evaluation of RIL seeds as well as interpretation of data. J.O. developed and grew the RILs in Minnesota and contributed seed. J.L.G. carried out trait predictions from NIRS data. A.M.S., J.L.G. interpreted data analyses and co-wrote the paper.

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