Full title:

Developmental Pleiotropy Shaped the Roots of the Domesticated Common Bean (*Phaseolus vulgaris* L.)

Short title:

Developmental pleiotropy shaped root domestication

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Author Contributions:

J.S. and C.E.V. conceived the original research plans; J.S. performed the experiments; J.S. and S.A.G. designed the experiments and analyzed the data; J.S. and C.E.V. conceived the project and wrote the article with contributions from S.A.G.; all authors have read and approved the final version of the manuscript.

One-sentence summary:

Genetic analysis revealed the role of pleiotropic gene action in shaping the root system architecture during domestication of common bean (*Phaseolus vulgaris* L.).

Abstract

Roots have been omitted from previous domestication analyses owing mostly to their subterranean nature. We hypothesized that domestication-associated changes in common bean (*Phaseolus vulgaris* L.) roots were due to direct selection for some aboveground traits that also affect roots and to indirect selection of root traits that improved aboveground plant performance. To test this hypothesis, we compared the root traits of wild and domesticated accessions and performed a multistep quantitative trait locus (QTL) analysis of an intra-Andean recombinant inbred family derived from a landrace and a wild accession. Multivariate analysis of root traits distinguished wild from domesticated accessions and showed that seed weight affects many root traits of young seedlings. Sequential and methodical scanning of the genome confirmed the significant effect of seed weight on root traits and identified QTL that control seed weight, root architecture, shoot and root traits, and shoot traits alone. The root domestication syndrome in the common bean was associated with genes that were directly selected to increase seed weight but had a significant effect on early root growth through a developmental pleiotropic effect. The syndrome was also associated with genes that control root system architecture and that were apparently the product of indirect selection.

Keywords: Wild, Domesticated, Genetic mapping, QTL analysis, Root growth, Root phenotyping, Linkage, Pleiotropic gene action.

Introduction

Domestication produces a biological group that displays clear phenotypic and genetic differences from its wild ancestor (Garcia et al., 1997; Ross-Ibarra et al., 2007). However, plant root traits have not yet been considered as part of the "domestication syndrome" as described by Harlan (1992). A change in edaphic conditions imposed by early farmers on the ancestral wild populations may have imposed selection pressure resulting in allele frequency changes of root-specific genes. Also, functional equilibrium between root and shoot might have indirectly altered the root system due to direct selection of shoot traits.

Quantitative trait locus (QTL) analysis has been used effectively in the identification of genes associated with domestication (Burger et al., 2008; Olsen and Wendel, 2013), and it has also led to their isolation and molecular characterization (Doebley et al., 1997; Wang et al., 2005). QTL analysis using soil-free root phenotyping platforms has also identified genetic determinants of quantitative variation in root system architecture (RSA; Clark et al., 2013; Ron et al., 2013; Topp et al., 2013; Burton et al., 2014; Liang et al., 2014; Zurek et al., 2015; Ye et al., 2018). This work has led to the identification of genes that control root traits, some of which were previously identified through mutant analysis (Mouchel et al., 2004; Sergeeva et al., 2006). Although some studies have included progenies with wild accessions (Prince et al., 2015), as far as we know, none have directly addressed genes controlling root traits associated with domestication.

Phaseolus vulgaris L., the common bean, was domesticated independently in Mesoamerica and the Andes about 8,000-10,000 years ago (Kwak and Gepts, 2009; Rossi et al., 2009). The Mesoamerican and Andean gene pools are separated by partial reproductive isolation and exhibit significant inter- and intra-gene pool variation. Comparative analysis of wild and domesticated accessions previously identified several aboveground domestication-related traits, and genetic analysis of these traits has suggested the presence of a few major QTL with large effects (Koinange et al., 1996).

The main objective of this study was to determine both the extent to which domestication had modified root traits of common bean and the genetic complexity of those traits through QTL analysis in an intra-Andean segregating progeny. We conducted a comprehensive phenotypic analysis using multivariate statistics and genetic characterization of 28 root and shoot traits to evaluate effects of domestication on root system architecture of common bean.

Results

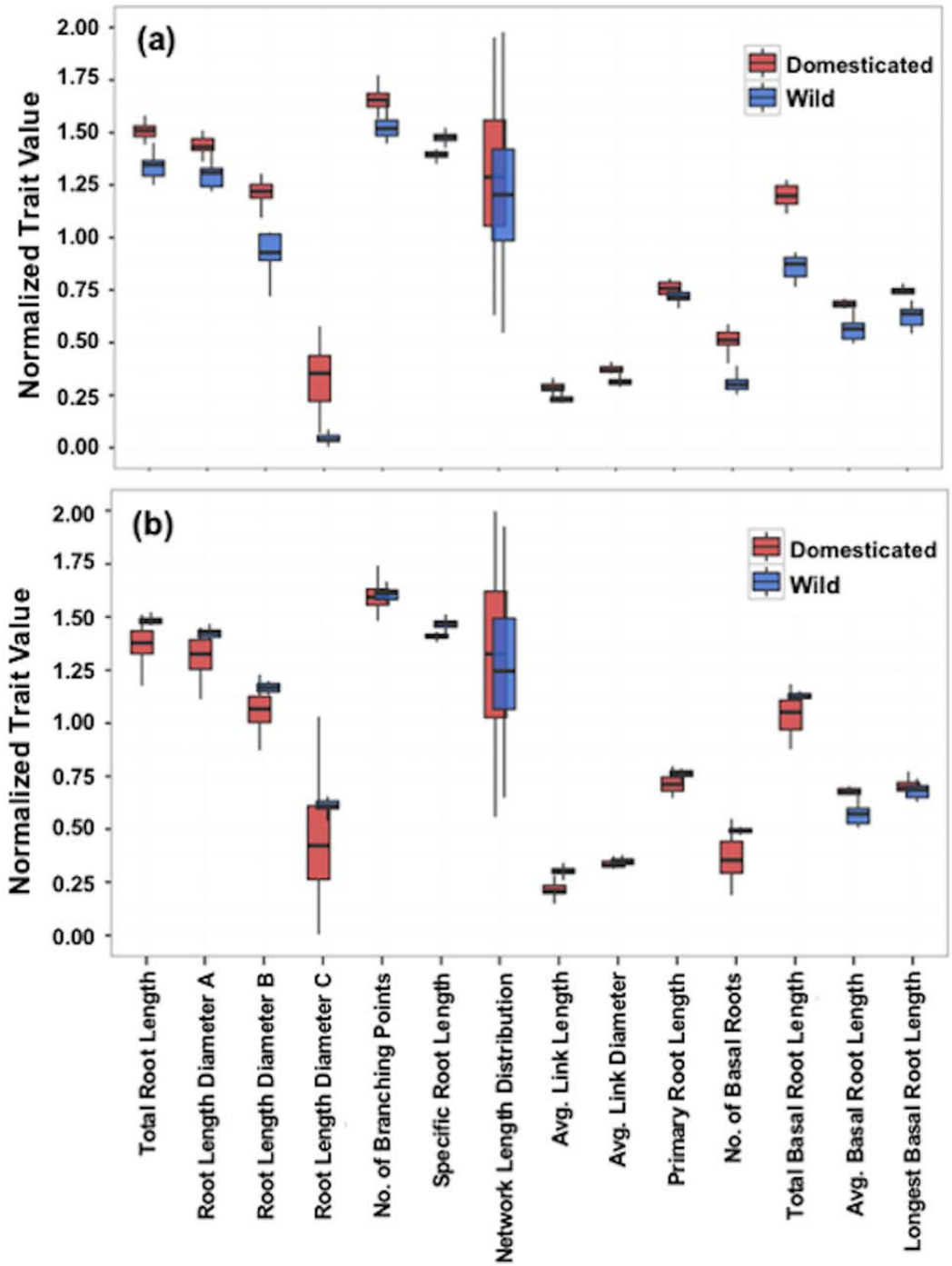
Comparative root system analysis of wild and domesticated accessions of the common bean.

Statistical comparisons of wild and cultivated accessions revealed highly significant differences ($p < 0.01$) in the RSA of young seedlings (Fig. 1a; Supplemental Table S1). Cultivated accessions have significantly larger root systems than their wild counterparts and display marked differences in the distribution of root sizes (Supplemental Table S1). Domesticated beans have primary root lengths that are 1.27 times longer than those of wild accessions, but the increased number and length of basal roots significantly lowers the root apical dominance of domesticated beans. The primary root length of wild accessions is on average 47 % of the total basal root length, while this relative proportion drops to 13% in domesticated accessions.

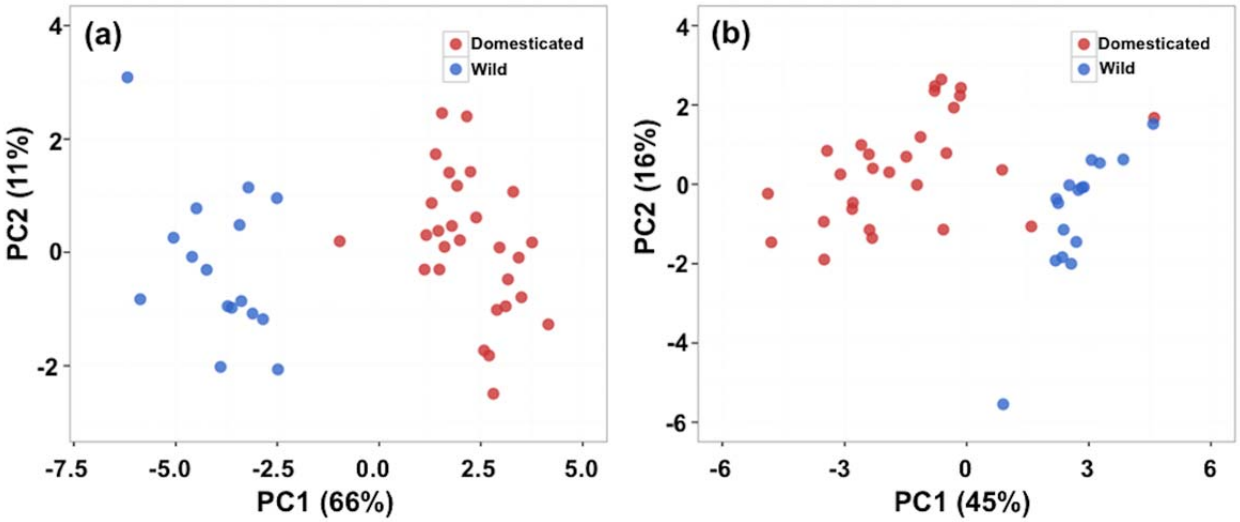
Principal component analysis (PCA) of root traits clearly shows that wild and domesticated genotypes form distinct clusters, suggesting selective forces applied during domestication significantly altered size and RSA (Fig. 2a; Supplemental Table S2). Principal component (PC) 1 explained 66% of the variation, PC2 11%, and 4 additional PCs explained most of the remaining (20%) variation (Supplemental Table S2). All root traits contributed to variation explained by PC1, but PC2 had significant contributions from fewer traits (Supplemental Fig. S1). In contrast to other traits, basal root growth had a negligible contribution towards PC2. A negative correlation between *Specific Root Length* and the other PC1 traits could be explained by the fact that as roots grow their spatial arrangement changes, resulting in a sparser distribution. This is also in agreement with the box plots pattern where the *Specific Root Length* of the wild accessions has a pattern opposite to the other traits (Fig. 1). Negative correlations for other traits were observed in the remaining principal components, and these likely explain the hidden genetic variation contributed by the wild genotypes.

PCA results could be biased because *Seed Weight* was the main target of selection during domestication, and we have shown that seed reserves can significantly influence early heterotrophic growth (Singh et al., 2017). Thus, *Seed Weight* differences between wild and domesticated accessions could explain some of the root growth differences between the accessions as suggested by the significant phenotypic correlations between seed weight and many root traits, and root size traits in particular (Supplemental Tables S3 and S4). To address this point, we re-analyzed the trait differences through covariate analysis to remove the dependency of root trait variation on seed weight.

Domesticated accessions had lower root trait values than those of wild accessions after they were adjusted for *Seed Weight* (Fig. 1b). For example, *Total Root Length* was reduced on average by 36% in the domesticated accessions. Overall, *Seed Weight* can significantly affect several root traits and should



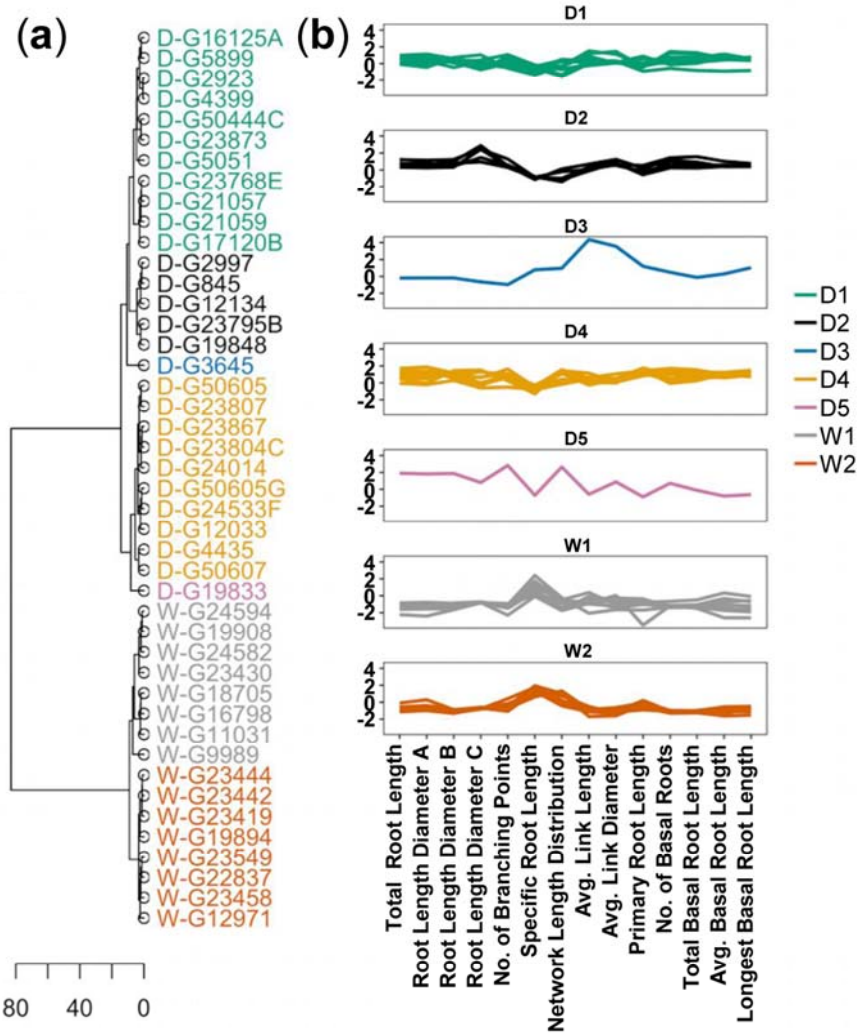
be considered as a covariate during further genetic analysis. Results from PCA using this covariate showed that the wild and domesticated clusters remained distinct from each other (Fig. 2b), although their positions were inverted along the PC1 axis. Nine PCs explained 99 % of the variation, and unlike the previous PCA, PC1 explained only 45 % of the variation. PC2 and PC3 explained about 16% and 13% of the variation, respectively, while variation was evenly distributed among the remaining components (Supplemental Table S2). These results showed that domestication significantly altered various aspects of



RSA because most root traits, without or with *Seed Weight* as covariate, show a relatively high degree of correlation as part of PC1, and that wild and cultivated accessions form distinct clusters along this axis. Moreover, genes controlling the targeted traits may be involved in adaptation to distinct edaphic conditions.

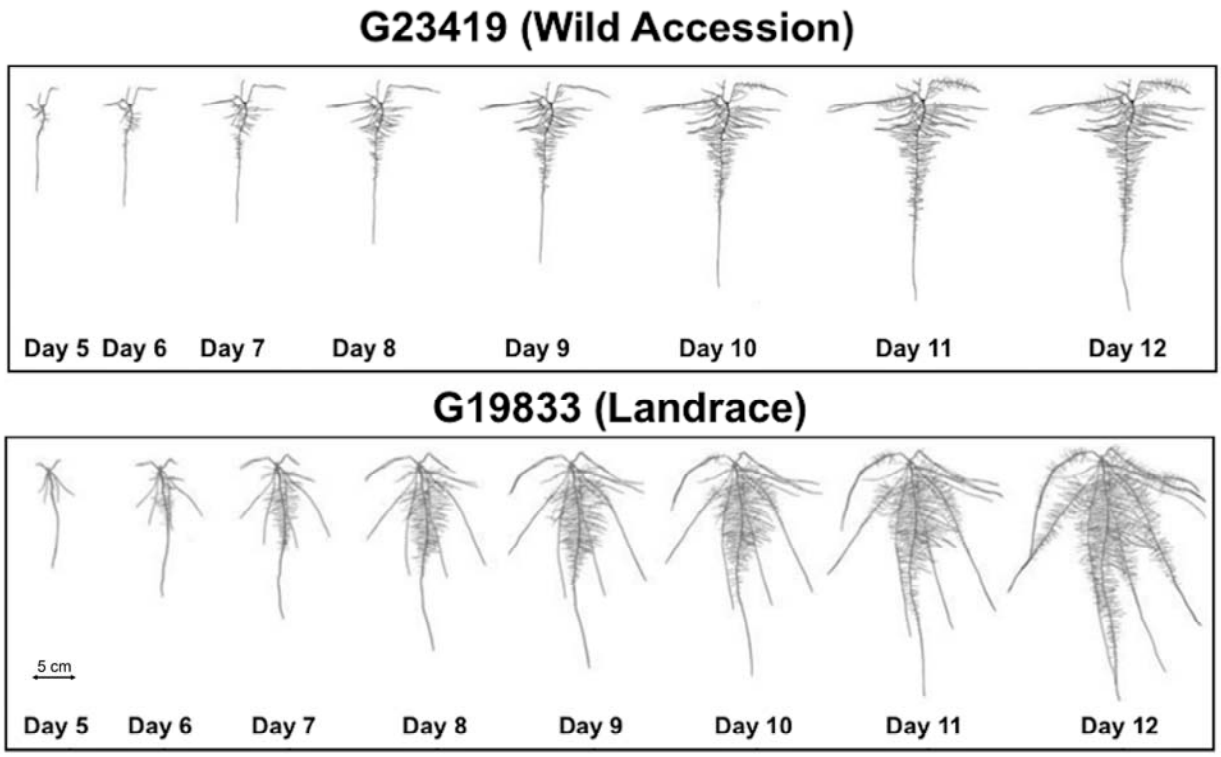
We further explored relationships between wild and domesticated accessions with hierarchical clustering, which, in contrast to PCA, analyzes the extent of similarities among entries. This analysis detected a total of seven sub-groups (threshold tree height = 8); five domesticated clusters (D1, D2, D3, D4, D5) and two wild clusters (W1, W2) (Fig. 3a). Clusters D1 and D2 contained each Andean and Mesoamerican accessions, while cluster D4 had 10 Andean domesticated genotypes (Supplemental Table S5). Clusters D3 and D5 represented only one accession each from the Mesoamerican and Andean gene pools, respectively. Wild clusters W1 and W2 had Andean and Mesoamerican accessions. This analysis distinguished wild from domesticated accession and, to a limited extent, discriminated between gene pools. A parallel coordinate plot of normalized trait means showed unique root trait patterns that distinguish each cluster (Fig. 3b). An additional clustering analysis using seed weight as covariate revealed seven distinct groups (Supplemental Fig. S2a, Supplemental Table S5); six of them contained domesticated accessions and one contained only wild accessions. The clustering pattern was highly similar to the previous one, with the exception of cluster D4, which included one domesticated accession from two different previous clusters and a wild accession. In addition, clusters D3, D5, and D6 displayed gene pool specificity (Supplemental Table S5). A large amount of significant trait variation was apparent within and across clusters (Supplemental Fig. S2b).

Phenotypic trait segregation and genetic correlations among root and shoot variables



Clustering of wild and cultivated beans by root traits suggested that genetic analysis of a segregating progeny may reveal the underlying genes. For this purpose, we conducted a QTL analysis of root traits using a recombinant inbred family (RIF) generated between a landrace and a wild accession from the Andean gene pool (Fig. 4; Supplemental Table S6). The frequency distributions of root and shoot traits (Supplemental Fig. S3 and S4) were continuous, indicating the quantitative nature of these traits. Transgressive behavior in all the traits, with the exception of seed and seed coat dry weights, indicated that each parent had genes that contributed to the traits in opposite directions. Broad-sense heritability estimates calculated for each of these traits (Supplemental Table S6) indicated the feasibility of identifying genes responsible for the phenotypic variation. In general, simple traits (weights and dimensions) had higher heritabilities than traits derived from a relationship of traits.

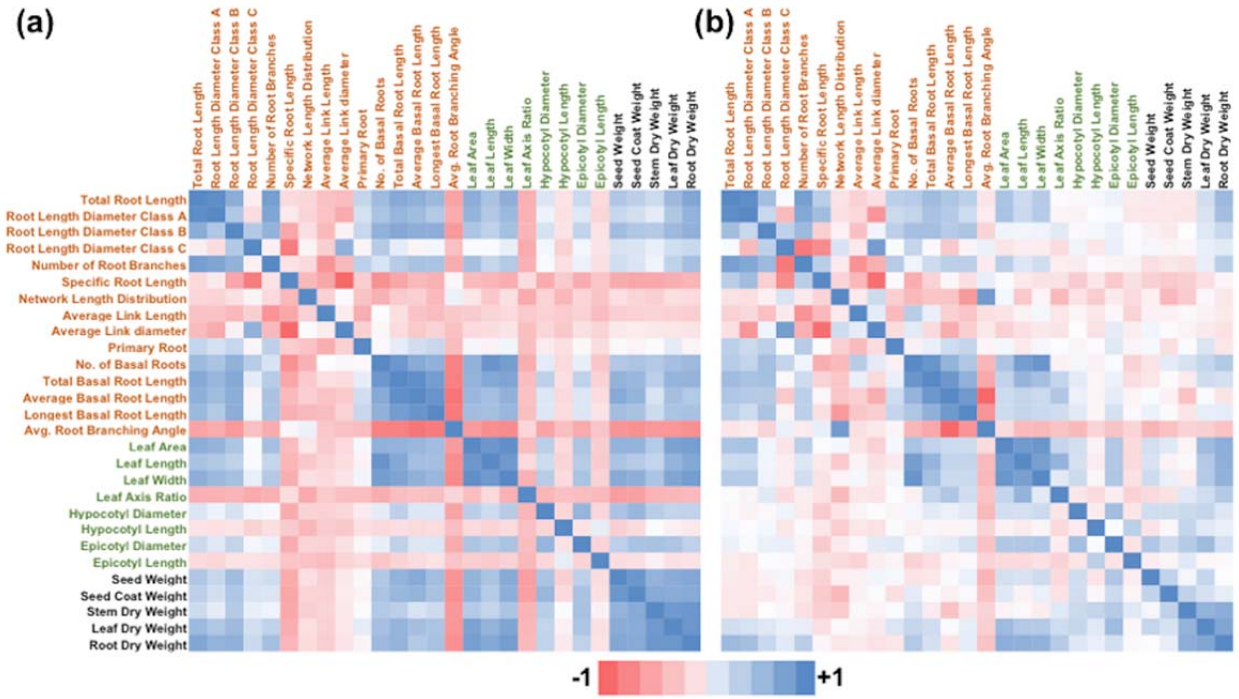
Genetic correlations obtained between trait pairs identified strong positive correlations among organ size traits, and negative genetic correlations between these and morphological traits (Fig. 5a).



Correcting for the *Seed Weight* covariate significantly altered trait correlations (Fig. 5b). The strength of correlations between organ size and morphological traits was decreased by the seed weight covariate. However, correlations between shoot and root size traits remained strong in general. Correlations involving *Hypocotyl* and *Epicotyl Length* were the least affected by the seed weight covariate. Overall, these results indicated that seed weight has variable effects on different traits.

Multivariate analysis of root and shoot growth traits revealed eleven PCs contributing to 90% of the total variation in the RIF (Supplemental Table S7). PC1 explained 44% of the variation and had representation from almost all the traits except leaf axis ratio (Fig. 6a; Supplemental Fig. S5). The remaining PCs explained the variation to a lesser extent and varied from 10% for PC2 to 2% for PC10. The relatively higher contribution of PC1 over the other PCs indicated a high level of correlation among different root and shoot variables and suggested common genetic determinants. When PCA was conducted after including seed weight as a covariate, PC1 explained only 26% of the total variation in the dataset (Fig. 6b; Supplemental Table S7) suggesting again that most young seedling traits are affected by seed weight. In fact, some traits that appeared to cluster along with seed weight in the first biplot analysis (Fig. 6a) showed a dispersed pattern when seed weight was used as covariate (Fig. 6b).

Construction and characterization of the linkage map of the RIF family



Genotyping-by-sequencing (GBS) produced an average of 225 million reads in each of two 96-genotype sequencing lanes, which yielded an average of 1.5 million reads per genotype after filtering. The alignment of the wild G23419 sequence reads against the G19833 reference genome (landrace parent) identified 1,984 single nucleotide polymorphisms (SNPs). Selection for high-quality SNPs present in 90% of the RIF yielded 905 informative markers. These were complemented by 75 locus-specific high-resolution melting PCR markers that were used to cover low-density marker sectors of the GBS map. We used 980 segregating markers for linkage analysis.

Linkage analysis identified 196 recombinationally unique markers distributed among the 11 linkage groups. The linkage map spans over a total distance of 827 cM, with an average of 75.2 cM per chromosome (Fig. 7, Supplemental Table S8). Chromosome lengths ranged from 49.6 cM (Chr04) to 149.8cM (Chr02). The average inter-marker distance ranged from 2.97 cM (Chr05) to 6.4 cM (Chr04), with an overall average of 4.2 cM. Chr02 contained 39 marker loci, the highest number of recombinationally unique loci, followed by Chr03 with 25 marker loci. Chr04 had the lowest number with only eight marker loci.

The GBS linkage map covered 94 % of the *P. vulgaris* sequenced genome (Supplemental Table S8), ranging in coverage from 98.8% for Chr08 to 81.8% for Chr06; low coverage of Chr06 might be due to its telocentric structure (Pedrosa et al., 2003; Bhakta et al., 2015).

Markers were not evenly distributed across the linkage map showing some gaps. To investigate the nature of these gaps, we plotted the first derivative function of cM over Mbp distances (Supplemental

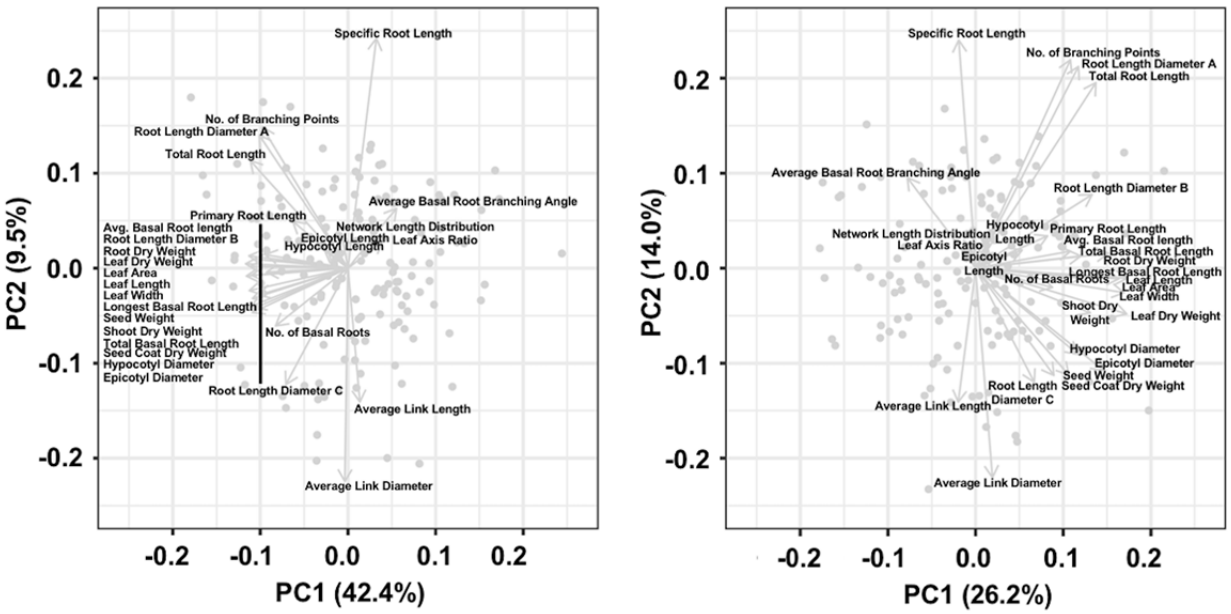
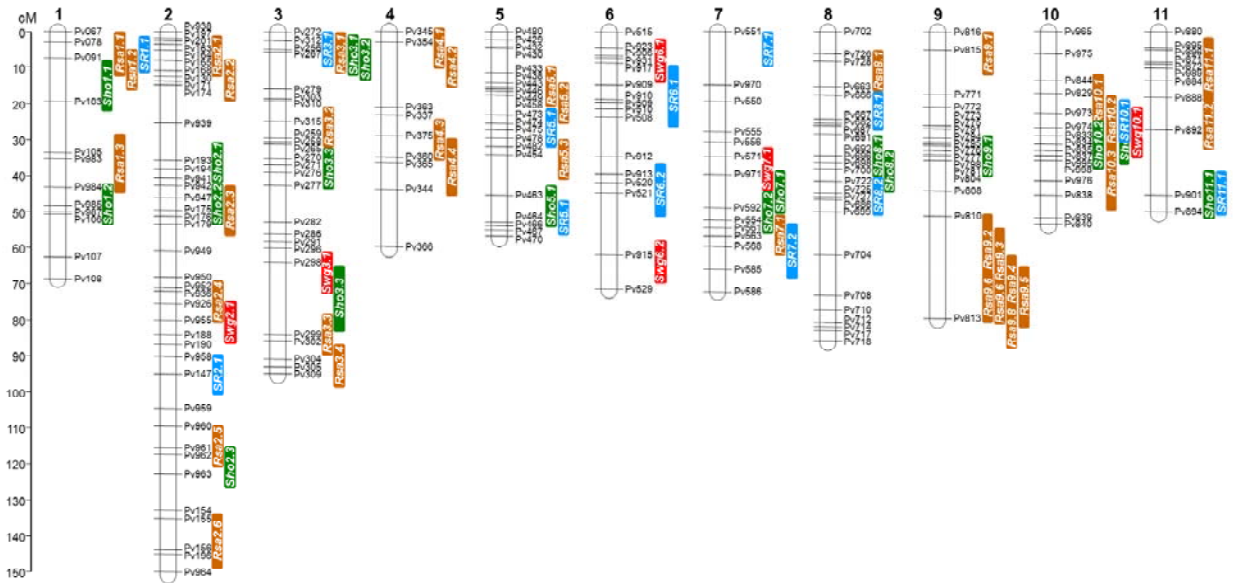


Fig. S6). These plots showed vast sectors of suppressed recombination corresponding to pericentromeric regions and also recombination hotspots distally located on the chromosomes, which were similar to the positions reported by Bhakta et al. (2015). The number of recombination hotspots as well as their recombination frequencies differed among chromosomes, with the highest cM/Mbp peak detected on Chr10 followed by Chr02 and Chr07. Interestingly, Chr01 exhibited suppressed recombination over a region covering approximately 38 Mbp where we found 51 markers with similar segregation patterns forming a single haplotype. This observation suggests that some structural differences may exist between the two parental genomes, which could be responsible for suppressed recombination in this wide genomic region.

Genetic architecture of domestication syndrome in early seedlings of common bean

We conducted QTL analyses in multiple and progressive steps to identify chromosome regions that might have been targeted during domestication – directly or indirectly. We first used composite interval mapping (CIM) to scan the linkage map for QTL associated with PCs. Out of 12 QTL (Supplemental Table S9), four were associated with PC1 ($\Sigma R^2 = 0.4$), five with PC2 ($\Sigma R^2 = 0.52$), and three with PC3 ($\Sigma R^2 = 0.28$). A second scan for the first three PCs, identified with *Seed Weight* covariate corrections, detected seven QTL. Only three of these QTL represented genomic regions that overlapped with those detected in the first scan, and the remaining four were new. These contrasting results highlight the strong effect that seed weight has on multiple traits of young seedlings.



Next, CIM analysis of 28 traits (Supplemental Table S10) identified 84 QTL with log of odds (LOD) thresholds ($p < 0.05$) that ranged from 2.66 to 2.97 and LOD maxima that varied between 2.85 and 11.34 (Supplemental Table S11). The map distances covered by these QTL varied between 3.7 and 34.1 cM; there was a relatively high correlation ($R^2 = 0.65$) between map distance range and LOD maximum. No QTL were detected for *Network Length Distribution*. All the other traits were associated with one to six QTL. The variation for each trait explained by the associated QTL ranged from 0.08 for root dry weight to 0.47 for root length. QTL were detected in all 11 chromosomes.

An alignment of all QTL-associated chromosome segments to the linkage map revealed 22 overlapping genomic sectors with 1 to 3 QTL sectors per chromosome (Supplemental Table S11). Several single QTL sectors had marginal LOD values and could be false positives, while others, like *Epicotyl Length*, had high LOD values (11.3). We detected segments on chromosomes 2, 5 and 7 with 9, 13, and 13 QTL, respectively. Each of these large clusters had a QTL for *Seed Weight*, and the associated QTL displayed a large range of LOD values. Interestingly, these three sectors overlapped with sectors detected with PC-linked QTL.

A new genome-wide scan using *Seed Weight* as covariate detected 80 QTL altering the previous QTL landscape. The variation explained by these QTL ranged from 14% for the *Average Basal Root Length* to 45% for *Specific Root Length* and *Seed Coat Dry Weight* (Supplemental Table S12). Removing the seed weight effect increased the number of QTL sectors. For instance, a single 19 cM multi-QTL sector was found on chromosome 1 before including the covariate, and a new QTL in that sector and two additional QTL sectors, outside the first one, were detected after including the covariate. A more pronounced effect was observed on chromosome 2 where three QTL sectors were detected without the *Seed Weight* covariate, and six sectors were detected after the inclusion of the covariate. Two of the six

sectors have marginally significant QTL, but the other four have LOD values that exceed the threshold by up to 7 units. A similar situation was observed for chromosome 5. Each one of these chromosomes carry one QTL for *Seed Weight*, which was exerting a significant pleiotropic influence on other traits before the seed covariate was applied. These findings indicated again that *Seed Weight* has a significant effect on the genetic analysis of several seedling traits.

We conducted a final genome scan with multiple interval mapping (MIM) using trait values after *Seed Weight* covariate correction. This analysis identified additional QTL, repositioned most CIM-based QTL, resolved some single CIM-QTL into two closely linked QTL with opposing effects, and identified a few QTL exerting additive-by-additive epistatic interactions (Cheverud and Routman, 1995). A total of 265 QTL were detected for 26 traits with *Seed Weight* as covariate and with LOD values that ranged from 0.62 to 12.9 units. The lack of a reliable test of significance for MIM results led us to adopt a conservative *ad-hoc* approach in which we selected a threshold that established the presence of a QTL if it was at least three times as likely as the threshold identified by CIM, that is, an LOD value of 0.5 above the CIM threshold of each trait.

The total number of QTL with an additive effect was reduced to 142 (Supplemental Table S13). Ten of the 80 QTL identified by CIM were not detected by MIM. MIM added 72 QTL, four of which corresponded to two QTL pairs that have been resolved each from single QTL detected by CIM; in each case the QTL were closely linked and had opposing effects. The selected MIM QTL had LODs that ranged from 3.25 to 13.82, and 122 exceeded the CIM LOD threshold by 1.0 and 20 of them by 0.5 to 1. The combined QTL effect for each trait varied from 11.1% for the *Number of Basal Roots* (1 QTL) to 84.7% for *Specific Root Length* (7 QTL). Other traits with a strong genetic component include *Leaf Length*, *Root Length Diameter B*, *Seed Weight*, *Average Link Length*, and *Stem Dry Weight*. MIM also detected five epistatic interactions; of these, four were between QTL that had additive effects, and one interaction was between QTL with and without an additive effect (Supplemental Table S13).

Correlations among traits and PC QTL suggested the presence of pleiotropic QTL and/or QTL-linked clusters. To sort out these possibilities, all QTL-associated sectors, established by being within 1 LOD unit from that of the MIM-estimated peak position, were aligned to the linkage map to determine the extent to which they overlapped. Multiple Trait – MIM was used to analyze QTL within overlapping intervals to determine the likelihood of being pleiotropic or closely linked (Fig. 7; Supplemental Table S14). These analyses reduced the number of QTL to 72, 32 of them were single trait QTL and 38 were found to be pleiotropic affecting between two and six traits. The QTL were split into 4 groups: 35 *Root System Architecture* (RSA) QTL, 21 of which controlled single traits and the others controlled two (6), three (6), and four (2) traits; 18 *Shoot* (SHO) QTL found on nine chromosomes, 11 controlled single

traits, and the others controlled two (5), three (1), and five (1) traits; 13 *Shoot and Root* (SR) pleiotropic QTL found on nine chromosomes; and 6 *Seed Weight* (SWG) QTLs.

Discussion

Univariate, bivariate and multivariate analyses of young seedling root traits clearly distinguished wild from domesticated accessions of the common bean. The high degree of correlation between most traits also suggested the presence of a root domestication syndrome. Differences in root-size traits between the groups raised the possibility that the 5-fold difference in seed weight between the groups could be responsible for the root trait differences. This is because bean cotyledons are the only source of nutrients during the early heterotrophic growth phase of seedlings. However, differences in root traits persisted even after correcting for the *Seed Weight* covariate. This observation indicated that other changes have taken place outside of the seed weight effect. The covariate correction also showed that most domesticated root traits have smaller values than those of the wild accessions. Overall, these comparisons indicated that domestication brought about genetic changes that directly affected root traits. Correlations between traits were reduced after correcting for seed weight, but were not eliminated, suggesting the presence of genetic correlations among some traits.

We conducted genetic analysis of young seedling traits that distinguishes wild from domesticated accessions with an intra-Andean RIF to avoid detection of genes responsible for inter-gene pool polymorphisms. This analysis was used to test our hypothesis that genes selected during domestication could be divided into three groups; one controlling shoot traits that were likely targets of direct selection, another controlling shoot and root traits, also mostly targets of direct selection, and a third group that exclusively controls root traits, which should be considered targets of indirect selection.

Root and shoot traits targeted for genetic analysis displayed normal distributions and relatively high heritability values, indicating the feasibility of detecting underlying QTL and important genetic gains to be achieved. With the exception of seed weight, all traits showed transgressive behavior revealing the presence of QTL alleles in each parent with opposing phenotypic effects. The linkage map constructed for QTL analysis had an average inter-marker distance of 4.2 cM, which provided sufficient QTL power of detection, although the precision for localizing the QTL may have been decreased somewhat by that marker density (Stange et al., 2013).

QTL analysis of PCs and of root and shoot traits before and after correcting for *Seed Weight* revealed the strong effect that seed weight has on those young seedling traits. Thus, segregation of seed weight genes can lead to the detection of QTL artifacts when mapping growth and developmental traits of young seedlings. For example, CIM detected 14 QTL on chromosome 5 before *Seed Weight* was used as covariate. However, only 11 of those QTL were detected after the covariate correction, resulting in a net loss of three QTL, but three new ones were gained with the covariate. Furthermore, 12 QTL were detected in one sector of overlapping QTL ranges on chromosome 7, including one seed weight QTL,

before covariate correction. This sector was resolved into five different QTL sectors after the covariate correction – the *Seed Weight* QTL range did not overlap with any of the other four QTL sectors. Introduction of the covariate also resulted in relevant QTL position changes. Similar changes were detected in other chromosomes, but changes were more dramatic for those with a *Seed Weight* QTL.

MIM analysis detected 70 additional QTL using our *ad-hoc* LOD threshold, bringing up the total to 142 QTL. Bivariate and multivariate analysis, and the overlapping genetic ranges of various QTL, suggested that some of these QTL may have pleiotropic effects, while others belonged to clusters of closely linked QTL. Multiple-trait MIM (MT)-MIM resolved these into 72 QTL, half of which are single trait QTL and the remaining appear to be pleiotropic. The MT-MIM QTL fell into one of four trait categories: *Seed Weight*, SWG; *Shoot*, SHO; *Shoot/Root*, SR; and *Root System Architecture*, RSA. The identification of these QTL provided evidence in support of our hypothesis. SWG and SHO are QTL that control aboveground traits, which were subjected to direct selection during domestication, while RSA QTL control traits that were selected indirectly during domestication as these traits are not visible and represent a product of "unconscious selection" – a term and concept first used by Darwin (1865) and considered by Heiser (1988), which refers to selection of traits without 'a predetermined purpose to improve the breed'. SR QTL are clearly pleiotropic as they appear to control both above and below ground traits. For instance, *Sr2.1* on chromosome 2 controls *Leaf Length* and *Basal Root Length*, potentially through the control of cell division in both organs; although selection was exerted only on the leaf, it appears to have had an unintended effect on the length of the basal roots. The shoot:root relationship has been framed under a functional equilibrium system with coordinating channels of chemical/hormonal communication (Wilson, 1988; Farrar and Jones, 2000; Bouteille et al., 2012; Puig et al., 2012; Dignat et al., 2013).

Six SWG QTL explained 70% of the variation of this trait and had LOD values that exceeded the threshold by 3 to 11 LOD units. These QTL were clearly among the principal targets of direct selection during domestication and had significant pleiotropic effects that fall within both developmental and selectional pleiotropies, according to the classification proposed by Paaby and Rockman (2013). In the former, the phenotype selected for one stage of development (large seeds) has significant effects on traits expressed in subsequent developmental stages – germination and early seedling growth. Large seeded genotypes produce larger organs during heterotrophic growth, which increase the starting material for the subsequent exponential growth.

Selectional pleiotropy impacts on adaptation could be appreciated by examining certain root traits. Wild accessions have smaller root systems, less root branching, fewer and shorter basal roots than the domesticated counterparts, and comparatively greater root apical dominance than domesticated

accessions. These root characteristics appear to condition adaptation to less favorable soil characteristics. For instance, the relatively long primary root allows the seedling to have access to water in lower soil horizons soon after germination, while the more horizontal basal roots provide the seedling with "top soil foraging" capability for phosphorous acquisition (Lynch and Brown, 2001). These root traits do not appear to be of great adaptive value in the fertile alluvial soils where water is not a limiting factor, and where agriculture and domestication were initiated. Thus, larger root systems promoted by large seeds would be at a disadvantage in soils with limited water availability. The selection for larger plant organs, as reported for beans (Koinange et al., 1996; Araujo et al., 1997), could only be viable when optimum resources are available.

We acknowledge that the QTL reported in this manuscript, even those with high LOD values, were identified by statistical inference and for this reason they need to be validated experimentally. This task could be accomplished through Mendelization using recurrent selection and/or through comparative sequence analysis of the associated genomic regions to determine whether they possess signatures of selection due to domestication. The same word of caution applies to inferred pleiotropy, which may be the result of suppressed recombination. Methods designed to increase the resolution of QTL (Heifetz and Soller, 2015; Xu and Vision, 2005) could be applied to address this issue. Finally, these results underscore the potential usefulness of wild germplasm as a potential source of adaptive root traits to stressful soil environments.

A number of studies have addressed the genetic control of the domestication syndrome (Koinange et al., 1996; Weeden NF, 2007; Wills and Burke, 2007; Kaga et al., 2008; Isemura et al., 2012), but none of them has directly focused on root growth and development. This study begins to fill the gap in knowledge about domestication-associated changes in various root traits. The work presented here documents a case of unconscious selection in which selection for increased seed weight had consequences in subsequent developmental stages in the common bean—an effect that could probably be extended to other domesticated species, in particular those that experienced a significant increase in seed weight. In contrast to other domestication studies that detected few genes with major effects on the shoot phenotype (Doebley et al., 1997; Wang et al., 2005; Olsen and Wendel, 2013), we have identified many more genes that appear to have smaller contributions to the domesticated root phenotype, perhaps suggesting that changes by indirect selection are more gradual and can accumulate more mutations over time.

MATERIALS AND METHODS

Plant Material

A set of wild and landrace accessions of the common bean from the Andean and Mesoamerican gene pools was phenotyped for various root growth traits (Supplemental Table S15). A recombinant inbred family with 186 genotypes (RIF; $F_{6:8}$) was generated from a cross between a landrace (G19833) from Northwestern Peru (6.27° S, 77.75° W) and a wild accession (G23419) from Central Peru (11.23° S, 75.53° W). The two genotypes exhibit significant phenotypic and gene expression differences at early seedling stage (Singh et al., 2017; Singh et al., 2018). The intra-Andean wild-landrace RIF excluded traits that may be found in modern cultivars and those controlling inter-genepool variation. The RIF was generated minimizing any type of selection to maintain its expected diversity.

Plant Growth and Root Phenotyping Platform

Seeds were weighed and surface sterilized with 50% commercial bleach for 5 minutes, rinsed in sterile deionized water, and imbibed overnight in the dark at 25° C. After removing the seed coat, seeds were germinated in paper rolls before the seedlings were transferred to root plates as described by Singh et al. (2017). A single tank held 16 plates (1 seedling/plate), and a maximum of four tanks were placed in a Conviron E15 growth chamber. Growth conditions were set at a photon flux density of 400 μmol $m^{-2}s^{-1}$, a 12 h photoperiod with a coordinated 23° C/18° C thermoperiod, and 90% relative humidity. Thermochron data loggers were used to record temperature every 15 minutes, and photosynthetic active radiation was regularly monitored with a LiCor light meter. Details of the root phenotyping platform are described in Supplemental Fig. S7.

Experimental Procedure and Trait Measurements

Root images were acquired non-destructively 12 days following transplantation using an Epson scanner. These images were stored as Tiff files and analyzed with three software packages: WinRhizo Pro9a (Regent Inc. Canada), GiA Roots (Galkovskyi et al., 2012) and ImageJ (http://rsb.info.nih.gov/ij/). The bean root system comprises a primary root, several basal roots that arise from the upper part of the primary root, and the adventitious roots arising from the hypocotyl. The root-growing angle between primary and basal roots marks the spatial deployment of the roots and provides a distinct shape to every

root system. Root framework data were manually acquired using ImageJ. A calibration image was used for scaling (pixels/cm) in order to estimate root lengths in centimeters

Data for various shoot traits were collected non-destructively using the same plant from which root measurements were taken. Hypocotyl and epicotyl lengths were measured using a graded ruler. Hypocotyl and epicotyl diameter were measured with a Vernier caliper (resolution of 0.1 mm). Leaf images were captured by photography using a custom-built platform with a built-in scale and were analyzed using ImageJ. Leaf image analysis provided area, length, width, and axis ratio.

Dry weight measurements were taken from each seedling following the root scan. Seedlings were partitioned into leaves, roots, and stems. Seed coats were removed after seed imbibition and kept for dry weight measurement. Tissues were oven dried at 60^{o} C for 72 hrs. Afterwards, weights were obtained from the dry tissues equilibrated to room temperature. Root and shoot growth characters correspond to different aspects of organ size and morphology measurements (Supplemental Table S10).

Experimental Design and Statistical Analyses

Bean accessions and the RIF were grown and phenotyped using an incomplete block design. A sub-set of 168 recombinant inbred lines (RILs) was chosen at random, from the original 186 RIL set, to randomize across total 12 incomplete blocks in 3 growth chambers. Each incomplete block contained 16 genotypes (14 RILs and 2 parental lines). The experiments were replicated three times during May 2012, August 2012, and November 2012 using the same growth chambers and experimental conditions.

Quantitative trait measurements were first evaluated with univariate analyses using the following linear mixed-effect model:

$$y = \mu + SW + Rep + Rep.Iblock + Chamber.Row + RIL + \varepsilon$$

where y corresponds to the response variable; μ is the overall mean; SW is the covariate of seed weight (when incorporated in calculations); Rep is a fixed effect of replicate or block; Rep.Iblock is a random effect of incomplete block within replicate; Chamber.Row is a random effect of row within the test chamber; RIL is the fixed effect of a recombinant inbred line; and ε is the residual term. Residuals were spatially modelled based on a separate autoregressive of order 1 error structure for the X and Y coordinates of each plot, which considers a spatial correlation in each of these directions. The above model was fitted using ASReml v. 3.0 (Gilmour et al., 2009), which estimates variance components based on a Residual Maximum Likelihood (REML) estimation method. Two types of models were fitted: with and without the SW covariate, and the significance of this covariate was evaluated using an approximate

F-test ($\alpha = 0.05$). Also, the significance of the spatial correlations on row and column was evaluated using the Bayesian Information Criteria (BIC) fitness criteria and the likelihood ratio test ($\alpha = 0.05$) in order to select the most parsimonious model. Best linear unbiased estimations (BLUE) of each of the RILs were used to predict RIL adjusted mean values. This approach provides the genetic means of individual traits corrected for all environmental variation, which were used to generate histograms from the RIL population, to provide the average phenotypic values of parentals and RILs, and to assess the trait frequency distributions. The trait distributions were tested for normality using the Shapiro-Wilk normality test ($\alpha = 0.05$), and non-normal traits were normalized for quantitative genetic analysis. The same model presented above was also fitted with RIL as a random effect to calculate broad-sense heritability (H^2 = genotype variance component/ (variance component + error variance component)) for each trait.

Later, a bivariate model was used to estimate genetic correlations among pairs of traits using the following linear model:

$$y = \text{trait} + \text{trait.SW} + \text{trait.Rep} + \text{trait.Rep.Iblock} + \text{trait.Chamber.Row} + \text{trait.RIL} + \varepsilon$$

where y corresponds to the vector of two stacked response variables; trait.SW is the fixed effect of covariate of seed weight within each trait; trait.Rep is a fixed effect of replicate nested within trait; trait.Rep.Iblock is a random effect of incomplete block within replicate for each trait; trait.Chamber.Row is a random effect of row within test chamber for each trait; trait.RIL is the fixed effect of a recombinant inbred line within trait; and ε is the residual term. The random terms of trait.Rep.iblock and trait.Chamber.Row were modelled using a diagonal variance-covariance matrix with a different variance component for each trait. The trait.RIL and residual terms were modelled using a heterogeneous correlation structure that contains a different variance for each trait and genetic and residual correlations between traits.

Finally, additional statistics were obtained to gain a better insight of the data, including rank correlations using the genetic estimates, principal component analyses (PCAs), and hierarchical clustering, all of which were performed with the estimated means and using functions from the software package R 3.3.1 (http://www.r-project.org/).

DNA Extraction, Genotyping-by-Sequencing and Linkage Map Construction

DNA extractions and library preparations for genotyping-by-sequencing (GBS; Elshire et al., 2011) were performed as described by Bhakta et al. (2015). Two GBS library pools, each containing 96 genotypes, were sequenced using the Illumina 2000 platform. Raw reads in fastq format were processed using the Tassel GBS pipeline (Glaubitz et al., 2014). RIL-specific sequencing reads and those of the

parental lines were separated based on the unique barcode sequence. Sequence tags were aligned to the *P. vulgaris* reference sequence in Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html) with Bowtie2 using the default settings (Langmead and Salzberg, 2012). Single nucleotide polymorphisms (SNPs) were detected using the SNPcaller plugin, and duplicate SNPs were merged with the MergeDuplicateSNPs plugin in Tassel. SNP variants were recorded in Variant Call Format (VCF) and were further processed using vcftools (Danecek et al., 2011). Resulting SNPs were filtered for maximum amount of missing data (10%), minor allele frequency (10%), and minimum read depth of five.

PCR makers were developed to cover low-density marker regions in the GBS map. Reads from whole-genome sequencing of G23419 were aligned against low-density marker sectors in the reference genome to identify SNPs that could be detected through high-resolution melting of PCR products (Liew et al., 2004; Simko, 2016). Suitable SNP-flanking primers were designed to amplify short (<100 bps) sequences (Supplemental Table S16). PCR reactions were performed in 10 µl reaction volumes containing 1X PCR buffer, 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.1 µM forward and reverse primer each, 0.25 units of Taq DNA polymerase, 2 µM Syto82, and 4 ng of DNA. PCR reaction conditions were: 95°C for 2 minutes, followed by 45 cycles of 95°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds. High resolution melting analysis of PCR products was carried out between 68°C and 90°C with temperature increase rate of 0.02°C/sec in continuous acquisition mode.

A linkage map was constructed with Mapmaker 3.0 (Lander et al., 1987) as modified by Russell Malmberg (http://www.plantbio.uga.edu/007Erussell/) and described in Bhakta et al., (2015). Markers were sorted into the 11 linkage groups using an LOD of 8 units and a maximum distance of 35 cM (Kosambi mapping function). Markers within each group were ordered by progressively lowering the linkage criteria to an LOD of 3 units.

QTL Analyses

Windows QTL Cartographer (Wang et al., 2007) was used for composite interval mapping (CIM; Zeng 1993, 1994) and multiple interval mapping (MIM; Kao et al., 1999; Zeng et al., 1999). CIM LOD thresholds used to identify QTL at $\alpha = 0.05$ were derived from one thousand permutations for each trait (Churchill and Doerge, 1994). Forward and backward stepwise regressions ($\alpha = 0.05$) were performed to select cofactors that accounted for genetic background variation. The analysis was performed using a 10 cM window size on either size of the interval being tested using a 1 cM walk speed. MIM analysis was applied to increase the sensitivity of QTL analysis, and the CIM results were used as the initial model. New QTL were added to the model through recurrent searches using a minimum distance of 5 cM

between QTL until no new QTL was detected. QTL with significant effects were retained in the model based on critical threshold values derived from likelihood ratio tests. The best QTL models, including optimized QTL map positions, were selected using BIC.

The significance of QTL-by-QTL interactions in the best genetic regression model were evaluated using likelihood ratio tests. Main and epistatic QTL effects falling below the critical threshold level were removed from the model. Main-effect QTL that had significant epistatic interactions with other QTL were retained if the significant threshold level was attained by the interactive effect. In the end, each QTL was optimized for their map position in the context of the position of other QTL in the model; the position that conferred the maximum likelihood in the model was retained as the best QTL position.

Several QTL were detected with short spans along the chromosomes. To discern between QTLs with pleiotropic effects and those tightly linked, we used multiple-trait multiple interval mapping (MT-MIM) algorithm in Windows QTL cartographer. First, we manually identified overlapping sectors of QTL associated with different root and shoot trait and pooled them to perform MT-MIM analysis. Pairwise MT-MIM tests were performed for all QTL within a specific QTL cluster to estimate whether co-localized QTL had significantly different map positions (linkage) or not (pleiotropy). The significance of close linkage or pleiotropy was determined using likelihood ratio test statistics. A minimum LOD threshold of 3 units was used to consider significantly better fit of close linkage model. Pleiotropy was assumed when the LOD score fell below the threshold; optimal map positions were obtained for a single pleiotropic QTL.

Accession Numbers

The molecular marker data and accompanying marker-flanking sequences are provided in Supplemental Table S16. Marker and QTL data have been submitted to the Legume Information System (Dash et al., 2016).

Supplemental Data

The following supplemental information is available.

Supplemental Figure S1. Principal component analysis of root traits from wild and domesticated accessions.

Supplemental Figure S2. Hierarchical clustering of wild (W-) and domesticated (D-) accessions.

Supplemental Figure S3. Frequency distribution histograms of root traits from the recombinant inbred family.

516 **Supplemental Figure S4.** Frequency distribution histograms of shoot traits from the recombinant inbred
517 family.

518 **Supplemental Figure S5.** Principal component analysis of root and shoot traits from the recombinant
519 inbred family.

520 **Supplemental Figure S6.** A scatterplot showing the distribution of recombination hotspots.

521 **Supplemental Figure S7.** Details of the 2D root phenotyping platform.

522 **Supplemental Table S1.** Statistical comparisons of root traits between wild and domesticated accessions
523 of common bean.

524 **Supplemental Table S2.** Summary of principal component analysis of various root traits in a set of wild
525 and domesticated common bean genotypes.

526 **Supplemental Table S3.** Phenotypic correlations between root traits, including seed weight, measured in
527 a set of wild and domesticated accessions of the common bean - No seed weight covariate adjustment.

528 **Supplemental Table S4.** Phenotypic correlations between root traits, including seed weight, measured in
529 a set of wild and domesticated accessions of the common bean - With seed weight covariate adjustment.

530 **Supplemental Table S5**. Hierarchical clustering analysis of different wild and domesticated accessions
531 using root trait values before and after applying seed weight covariate adjustment.

532 **Supplemental Table S6.** Mean trait values of various root and shoot traits of G19833 and G23419 and
533 their heritability estimates based on data from their recombinant inbred family.

534 **Supplemental Table S7.** Summary of principal component analysis (PCA) using root and shoot growth
535 traits of the recombinant inbred family generated between the landrace G19833 and the wild accession
536 G23419.

537 **Supplemental Table S8.** Descriptors of the linkage map constructed with the recombinant inbred family
538 generated between the landrace G19833 and the wild accession G23419.

539 **Supplemental Table S9.** Results obtained with composite interval mapping of principal components
540 before and after adjusting the data with the seed weight covariate.

541 **Supplemental Table S10.** List of root, leaf, stem and seed traits. Root traits were measured in the
542 collection of wild and landrace accessions and in the recombinant inbred family.

543 **Supplemental Table S11.** Composite interval mapping (CIM) analysis of various root and shoot traits;
544 data were analyzed without using seed weight as a covariate.

Supplemental Table S12. Composite interval mapping (CIM) analysis of various root and shoot traits; data were analyzed using seed weight as a covariate.

Supplemental Table S13. Multiple interval mapping (MIM) analysis of root and shoot traits using data adjusted with the seed weight covariate.

Supplemental Table S14. MIM-based assessment of pleiotropy over linkage among closely linked QTLs.

Supplemental Table S15. List of wild and domesticated genotypes used for preliminary analysis of various root growth and architectural traits.

Supplemental Table S16. List of SNP markers.

Supplemental Table Legends

Supplemental Table S1. Statistical comparisons of root traits between wild and domesticated accessions of common bean. The standard error (in parenthesis) of the mean (bold) is listed below the means.

Supplemental Table S2. Summary of principal component analysis of various root traits in a set of wild and domesticated common bean genotypes. PCA analysis was conducted separately with and without seed weight as a covariate.

Supplemental Table S3. Phenotypic correlations between root traits, including seed weight, measured in a set of wild and domesticated accessions of the common bean - No seed weight covariate adjustment. The bottom left sector of the matrix contains the R2 values, and the top right sector of the matrix contains the corresponding *p*-values.

Supplemental Table S4. Phenotypic correlations between root traits, including seed weight, measured in a set of wild and domesticated accessions of the common bean - With seed weight covariate adjustment. The bottom left sector of the matrix contains the R2 values, and the top right sector of the matrix contains the corresponding *p*-values.

Supplemental Table S5. Hierarchical clustering analysis of different wild and domesticated accessions using root trait values before and after applying seed weight covariate adjustment.

Supplemental Table S6. Mean trait values of various root and shoot traits of G19833 and G23419 and their heritability estimates based on data from their recombinant inbred family.

Supplemental Table S7. Summary of principal component analysis (PCA) using root and shoot growth traits of the recombinant inbred family generated between the landrace G19833 and the wild accession G23419. PCA analyses were conducted separately using datasets analyzed before and after adjusting for the seed weight covariate.

Supplemental Table S8. Descriptors of the linkage map constructed with the recombinant inbred family generated between the landrace G19833 and the wild accession G23419. The physical information for SNP markers were obtained from the *P. vulgaris* (v1.0), and coordinates of the pericentromeric regions from the Legume Information System (Dash et al., 2016).

Supplemental Table S9. Results obtained with composite interval mapping of principal components before and after adjusting the data with the seed weight covariate. Threshold LODs (0.05) were estimated after 1,000 random permutations. QTL with overlapping chromosome segments are marked with the same superscript letter.

Supplemental Table S10. List of root, leaf, stem and seed traits. Root traits were measured in the collection of wild and landrace accessions and in the recombinant inbred family. Leaf and Stem traits were only measured in the mapping population.

Supplemental Table S11. Composite interval mapping (CIM) analysis of various root and shoot traits; data were analyzed without using seed weight as a covariate. The threshold values for significance (α=0.05) were obtained from CIM data after 1,000 permutations for each trait.

Supplemental Table S12. Composite interval mapping (CIM) analysis of various root and shoot traits; data were analyzed using seed weight as a covariate. The negative or positive additivity values indicate whether the G23419 (wild) or G19833 (landrace) alleles added value to the trait, respectively. The threshold values for significance (0.05) were obtained from CIM data after 1,000 permutations for each trait.

Supplemental Table S13. Multiple interval mapping (MIM) analysis of root and shoot traits using data adjusted with the seed weight covariate. Results from CIM analyses were used as starting models. The negative or positive additivity values indicate whether the G23419 (wild) or G19833 (landrace) alleles added value to the trait, respectively. The threshold values for identifying QTLs exceeded by 0.5 those determined for CIM analysis. The map interval for MIM QTL corresponded to segments that were within 1 LOD of the QTL peak. Effects are labeled as A for additive, and AA for epistatic interactions of the additive-by-additive type. Interacting QTLs are marked with the same superscript.

Supplemental Table S14. MIM-based assessment of pleiotropy over linkage among closely linked QTLs. QTLs were assigned to one of four categories: Root System Architecture (Rsa), Shoot and Root traits (SR), Shoot traits (SHO), and Seed Weight (Swg).

Supplemental Table S15. List of wild and domesticated genotypes used for preliminary analysis of various root growth and architectural traits.

Supplemental Table S16. List of SNP markers. The position of each marker in the linkage map, the coordinates of the genomic sequence, and 100-base sequence including the SNP are listed.

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Figure Legends

Figure 1. Box plots of root trait of wild and domesticated accessions of the common bean. Trait values were normalized as log100 using three replicas of each of the 16 wild and 28 cultivated accessions, before (A) and after (B) correcting for the seed weight covariate.

Figure 2. Principal component analysis using root trait means in wild and domesticated accessions of the common bean. The analysis was performed before (A) and after (B) the inclusion of seed weight as a covariate.

Figure 3. Hierarchical clustering of wild (W-) and domesticated (D-) accessions from the Mesoamerican and Andean gene pools based on 14 root traits. A, Dendrogram showing seven distinct clusters. Different genotype clusters were defined at a cut threshold of 8. The clustering height scale is shown at the bottom. B, Parallel coordinate plots showing mean normalized trait values of genotypes in each cluster.

Figure 4. Time series root scans of the landrace (G19833) and the wild (G23419) accession.

Figure 5. Genetic correlations between different root, shoot, seed, and dry weight traits of the recombinant inbred family (n=168; 3 replicas each), which was generated between the landrace (G19833) and the wild (G23419) accession. Correlations were calculated as described in the Materials and Methods section. Results obtained before (A) and after (B) the inclusion of seed weight as a covariate.

Figure 6. Principal component analysis using estimated means of root and shoot growth traits from the recombinant inbred population generated between the landrace (G19833) and the wild (G23419) accession. Bi-plots between first and second principal components before (A) and after (B) correcting for the seed weight covariate.

Figure 7. Linkage map based on the recombinant inbred family derived from a cross between the landrace G19833 and wild accession G23419. The map also marks genomic regions where quantitative analyses detected QTL associated with root system architecture (RSA), shoot (SHO), root/shoot (RS) and seed weight (SWG) traits.

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