



Toward validation of QTLs associated with pod and seed size in common bean using two nested recombinant inbred line populations

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Abstract The common bean is an important legume worldwide. The aim of this study was to identify quantitative trait loci (QTLs) associated with seed and pod phenotypes and to evaluate the consistency of these QTLs across different environments and genetic backgrounds. Two nested recombinant inbred populations obtained from the crosses “Xana”/ “Cornell 4924” (XC) and “Xana”/ “BAT93” (XB) were used. The

populations were phenotyped with respect to pod and seed size and number of seeds per pod and seed weight over two (XB) or five seasons (XC) using a randomized complete block design. The XC population was re-genotyped, and an updated linkage map, with 732 markers and a total length of 1390 cM, was developed. The XB population was genotyped using genotyping by sequencing (GBS), and the corresponding genetic linkage map consisted of 497 single-nucleotide polymorphisms (SNPs) with a total length of 1547 cM. Altogether, 13 and 18 QTLs for pod traits and 21 and 25 QTLs for seed traits were detected in the XC and XB populations, respectively. In addition, 20 and 27 significant epistatic interactions between QTLs were detected in the XC and XB populations, respectively. The overlap among identified QTLs in the two nested populations was also investigated. Results revealed four overlapping regions for pod traits and eight for seed traits between the XC and XB populations. QTLs for seed or pod phenotypes detected on telomeric genomic regions of chromosomes Pv01, Pv05, Pv06, Pv07, Pv08, and Pv11 overlapped with QTLs associated with pod or seed phenotypes previously reported in other studies. The results showed the complex architecture of the genetic control of the pod and seed phenotype and the use of the bean genome for the integration and validation of QTLs.

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Abbreviations

cM CentiMorgan

GBS	Genotyping by sequencing
INDEL	Insertion-deletion polymorphisms
LG	Linkage group
LOD	Logarithm of odds score
Mb	Megabase
QTL	Quantitative trait loci
SCAR	Sequence-characterized amplified regions
SNP	Single-nucleotide polymorphisms
SSR	Simple sequence repeat
XB	Xana/BAT93 recombinant inbred population
XC	Xana/Cornell recombinant inbred population

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important legume crop for direct human consumption grown worldwide in a wide range of environments (FAOSTAT 2018). It is a predominantly self-pollinating species and has a relatively small diploid genome size ($2n = 22$; 587 Mb) (Schmutz et al. 2014). *P. vulgaris* germplasm is organized in two geographically and genetically differentiated gene pools, Middle American and Andean (Gepts et al. 1986; Kwak and Gepts 2009), which diverged from a common ancestral population (Bellucci et al. 2014; Mamidi et al. 2013).

Extensive phenotypic variation for seeds and pods has been reported in common bean for traits such as color, shape, size, dimension, and weight and use in human consumption (e.g., Voysest 2000). Depending on genotype, beans can be consumed as pods (green or snap beans), dry seeds after re-hydration (dry beans), or both, so the study of the genetic control of pod and seed traits is of great interest. Qualitative and quantitative loci (named as quantitative trait locus, QTL) are involved in the genetic control of seed and pod phenotypes. For example, the major genes *P* and *Asp* are involved in the genetic control of seed color and shine, while genes *Arg* and *Y* are associated with pod color (see bean gene list 2017 in http://arsftfbean.uprm.edu/bic/wp-content/uploads/2018/04/Bean_Genes_List_2017.pdf). With respect to traits such as seed and pod size or seed weight, qualitative inheritance has been reported, and different studies have analyzed the genetic control of seed width, seed length (González et al. 2016; Perez-Vega et al. 2010), seed weight (Park et al. 2000; Tar'an et al. 2002; Perez-Vega et al. 2010; González et al. 2016; Sandhu et al. 2018; Cichy et al. 2009; Correa et al. 2018

), and pod length, thickness, and width (González et al. 2016; Yuste-Lusbona et al. 2014; Hagerty et al. 2016). In these studies, biparental mapping populations obtained from different parental lines were analyzed. As a consequence, a large number of QTLs involved in the control of these characters has been reported, but few efforts have been carried out to establish the correspondence between the QTLs identified in different bean genotypes. On the other hand, 139 candidate genes were reported to be involved in seed size control in the common bean genome (Schmutz et al. 2014).

Traditional QTL analysis methods are based on mapping in genetic linkage maps developed from biparental populations like recombinant inbred line (RIL) populations. These biparental populations allow for the exploration of the variation found in each of the two parental genotypes. Moreover, this type of population can unmask putative epistatic interactions that affect the expression of the phenotype. Then, different segregating populations involving the same genotype (e.g., nested populations) can be analyzed for the validation of QTLs present in a specific genotype. QTLs need to be validated in different genetic backgrounds and environments before they are used in plant breeding or underlying candidate genes can be searched for. QTL validation can involve its identification in different mapping populations. However, the identification of a specific QTL in different mapping populations has been limited by the absence of common molecular markers. Now, the availability of the common bean genome sequence offers the opportunity to physically locate the QTLs from the underlying markers and to detect correspondences or overlaps among QTLs. The aims of this study were to identify and validate QTLs associated with seed and pod phenotypes in two nested RIL populations and to evaluate the consistency of these QTLs across different environments, studies, and genetic backgrounds.

Materials and methods

Plant material

Two RIL populations derived from two crosses between Middle American and Andean gene pools were used. The two populations were developed using the single seed descent method. The XC population (115 F_{7,8} lines) was obtained from the cross between the genotypes “Xana” and “Cornell 49242,” and the XB

population (145 F_{6,7} lines) was generated from the cross between the lines “Xana” and “BAT93.” “Xana” is a breeding line developed from a cross between the two Andean landraces, “Andecha” and “V203,” at Agri-Food Research and Development Regional Service (SERIDA, Asturias Government, Spain). The line “Xana” has pods of 12–15 cm length with 3–4 seeds, very large (100 g/100 seeds) white seeds (Fig. 1), and determinate growth habit and is a member of the fabada market class. “Cornell 49424” is a Mesoamerica genotype race that is classified into the black turtle market class. It has indeterminate prostrate growth habit, short pods with four to six very small black seeds (Fig. 1). “BAT93” is a breeding line developed at the Centro Internacional de Agricultura Tropical (CIAT, Colombia) from a double cross involving four Middle American genotypes (Veranic 2, PI 207262, Jamapa, and Great Northern Tara). “BAT93” has short pods with four to six seeds, small seeds (24 g/100 seeds; Fig. 1), and



Fig. 1 Phenotype of dry pod and seeds in the three parental genotypes used for the obtaining of the two nested recombinant inbred populations. (Left) Cornell49424; (central) position, Xana; (right), BAT93. White bar, 1 cm

indeterminate prostrate growth habit, and its genome was recently sequenced, although its functional annotation is not available (Vlasova et al. 2016).

Genotyping

Genomic DNA was isolated from young leaves of individual plants (F₇ or F₈) using the CTAB method described by Doyle and Doyle (1990). The concentrations of DNA were quantified photometrically (absorbance measurements between 260 and 280 nm) using a Biomate 3 ultraviolet-visible spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). The DNA quality was assessed in 1% agarose gels, stained in RedSafe (INtRON, Biotechnology, Gyunggi-Do, Korea), and visualized under ultraviolet light. The DNA samples were preserved at –80 °C.

The XC population was re-genotyped using the single-nucleotide polymorphism (SNP) supplied by the BARCBean6K_3 bean chip (Song et al. 2015). Four types of molecular markers were also used for linkage map construction: (i) InDel markers (insertion-deletion polymorphisms), which were selected based on their physical positions on the bean genome (Moghaddam et al. 2014); (ii) simple sequence repeat (SSR) molecular markers; (iii) sequence-characterized amplified regions (SCARs); and (iv) two seed proteins. Finally, five major genes controlling morphological traits were included: genes *P*, *Asp*, *Fin*, and R73 (resistance gene to anthracnose race 73) and the locus conferring resistance to anthracnose race 6, 38, and 39 (R6, R38, and R39) (Pérez-Vega et al. 2010; Pérez-Vega et al. 2012).

The XB population was genotyped by “genotyping by sequencing” (GBS) using the *ApeKI* restriction enzyme (Elshire et al. 2011). GBS was carried out at the Institute of Genomic Diversity (Cornell University, Ithaca, NY, USA). First, a GBS sequencing library was prepared by digesting DNA samples with *ApeKI* restriction enzyme, ligating them to two unique nucleotide adapters (barcodes) and amplified by PCR. Sequencing was performed using Illumina HiSeq2000. The sequencing reads from different genotypes were deconvoluted using the barcodes and aligned to the *Phaseolus vulgaris* L. reference genome (version 2.1 available at <https://phytozome.jgi.doe.gov/>). SNPs were extracted using the GBS pipeline implemented in TASSEL 5.0 software (Bradbury et al. 2007).

Genetic linkage map construction

The polymorphic markers were surveyed across the RILs of the respective population. Those heterozygous SNP markers with more than 10% missing data and a physical distance less than 0.1 Mb from a neighboring marker were removed. Markers showing significant deviation from the expected Mendelian segregation ratio (1:1) as determined by chi-squared analysis ($p < 0.05$) were not considered. Linkage map construction was carried out using OneMap package in R platform (Margarido et al. 2007; R core Team 2018). Linkage groups were established with a logarithm of the odds (LOD) threshold greater than 3.0 and a recombination fraction of 0.25. Marker order was estimated based on the rapid chain delineation algorithm (Doerge 1996) and ripple analyses. Map distances among the loci (cM) were calculated using the Kosambi mapping function. For co-segregating SNPs, only one SNP was used for map generation. The linkage groups were named according to the method described by Pedrosa-Harand et al. (2008).

Phenotyping

Both populations were phenotyped at Villaviciosa, Spain (43°2901 N, 5°2611 W; elevation 6.5 m). The XC population was phenotyped in crop field during the years 2004, 2005, and 2006 for seed (Pérez-Vega et al. 2010) and pod dimensions (unpublished) and re-phenotyped in crop field during the years 2013 and 2014 using a randomized complete block design with one replicate per line that contained ten plants distributed in a 1-m row plot. Standard agronomic practices for tillage, fertilization, weed, and insect control were followed to ensure adequate plant growth and development. Seed and pod traits were manually recorded (Table 1).

The XB population was characterized in the greenhouse (2015) and in the field (2016). A randomized complete block design was also used in both seasons. In greenhouse, one replicate of a single-1-m row plot containing ten plants per recombinant line was used. The crop field trial had two replicates per line, each consisting of a 1.5-m row plot containing 15 plants. One meter spacing between rows was used, and the same standard agronomic practices used in the XC population were followed. Seed and pod characters of each line were digitally recorded. Pod dimensions

(Table 1) were measured with the Tomato Analyzer Software (Brewer et al. 2006), while seed dimensions were analyzed with the SmartGrain software (Tanabata et al. 2012). The trait 25, seed weight, was manually recorded.

Data analysis

Frequency distributions for individual traits were tested for normality using the Shapiro-Wilk test. Student's tests were used to investigate significant differences ($p < 0.05$) between the parents for the evaluated traits. Pearson's correlation coefficients among the traits were determined. Statistical analyses were carried out using the mean phenotypic data obtained from all environments and conducted in R platform (R Core Team 2018). The package ggplot2 (Wickham 2016) was used to investigate the distributions of respective traits.

QTL analysis and validation

QTLNetwork 2.0 software (Yang et al. 2008) was used to detect QTLs with individual effects, epistasis (QTL x QTL), and QTL-environment (QTL x E) interactions. This software was used to estimate the broad-sense heritability (H^2) for each trait as variance of genetic main effects divided by phenotypic variance. The software also performs a one-dimensional genome scan using a mixed model based on composite interval mapping (MCIM; Zeng 1994) in order to identify individual QTLs and their interactions with the environment. A window size with a walking speed of 1 cM was used in this analysis. Significant QTLs were determined from the F value after 1000 permutation tests (Churchill and Doerge 1994) considering a confidence level of 95%, and among them, QTLs with an estimated heritability of additive effects (h^2) greater than or equal to 0.05 were considered. The effect of interactions (QTL x E) were determined by the Markov Chain Monte Carlo algorithm (Wang et al. 1994). QTLs showing epistatic interaction (QTL x QTL) were detected in the two-dimensional genome scan, and only interactions with a heritability of additive by additive effect (h^2) greater than or equal to 0.01 were considered. The QTLs were named as recommended by Miklas and Porch (2010). Individual QTLs also identified in the two-dimensional genome scans are indicated with an asterisk, and those QTLs only detected with a two-dimensional scan were

Table 1 List of quantitative traits measured on XC and XB RIL populations

Traits	Units	Description
Seed length ⁽²⁾⁽³⁾	SL mm	Measure in parallel to the hilum of 25 randomly chosen seeds
Seed width ⁽²⁾⁽³⁾	SWI mm	Measure from hilum to opposite side of 25 randomly chosen seeds
Seed thickness ⁽²⁾	ST mm	Measure perpendicular to the width of 25 randomly chosen seeds
Seed area ⁽³⁾	SA mm ²	Measure of 25 randomly chosen seeds
Seed perimeter ⁽³⁾	SP mm	Measure of 25 randomly chosen seeds
Seed weight ⁽⁴⁾	SW g	Measure of 4 sets of 25 seeds
Pod size traits		
Pod length ⁽²⁾⁽³⁾	PL cm	Measure from peduncle to the apex excluding the beak of ten randomly chosen pods
Pod width ⁽²⁾⁽³⁾	PWI cm	Measure from suture to opposite side in right angle of ten randomly chosen pods
Pod thickness ⁽²⁾	PT cm	Measure perpendicular to the width of ten randomly chosen pods
Pod area ⁽³⁾	PA cm ²	Measure of ten pods randomly chosen excluding the beak
Pod perimeter ⁽³⁾	PP cm	Measure of ten pods randomly chosen excluding the beak
Number of seeds per pod ⁽⁴⁾	NSP	Measure of 10 randomly chosen pods

¹ Seed moisture adjusted to 10–12%

² Manually measured in XC population using a digital caliper

³ Measured from digital images in XB population

⁴ Measured manually in both populations

named as E-QTL. Linkage genetic maps and QTLs were displayed using Mapchart v.2.2 (Voorrips 2002).

To investigate the correspondence or overlap between QTLs, the physical position of each QTL in the common bean genome was determined based on the positions of the flanking or underlying markers. Physical positions of marker loci were obtained by alignment between the sequences of the respective markers with the bean genome sequence v2.1 using the BLASTN algorithm (<https://phytozome.jgi.doe.gov/pz/portal.html>). Marker sequences were obtained from PhaseolusGenes (<http://phaseolusgenes.bioinformatics.ucdavis.edu/>) or tag sequences containing the SNP supplied by the GBS analysis. ShinyCircos package (Yu et al. 2018) was used to visualize the position of each QTL in the bean genome from the underlying markers.

Results

Update of XC linkage map

The updated version of the XC genetic map integrates SNP markers obtained from the BARCBear6K_3 BeadChip as well as INDEL, SSR, and SCAR markers are previously reported and mapped. The updated

version of the XC map contained 762 markers distributed across 11 linkage groups: 648 SNP, 43 INDEL, 39 SSR, 20 SCAR, 1 RAPD, 2 STS, 2 seed proteins loci, and 5 morphological traits (genes *P*, *Asp*, and *Fin*; genes providing resistance to anthracnose races 73 and 38). The total map length was 1390 cM, and the linkage groups ranged in size from 78.01 cM (Pv10) to 169.88 cM (Pv01) with an average of 126.39 cM per linkage group. The average distance between markers was 2.01 cM, and gaps with more than 15 cM were detected in LGs Pv01, Pv04, Pv06, and Pv10 (see Supplementary Fig. 1, Supplementary Table 1).

GBS analysis and XB linkage map construction

SNPs supplied by GBS analysis were filtered on the basis of data completeness (>90%), and heterozygous genotypes were removed. A total of 7145 SNPs was identified between the parental lines “Xana” and “BAT93,” of which 1555 SNPs were discarded due to their significant departure from a 1:1 monogenic segregation (< 0.05). As a result, 5590 SNPs were used to construct the map, with the final version of the XB linkage map containing 497 informative SNPs (showing recombination among them) distributed along the 11 linkage groups (see Supplementary Fig. 1, Supplementary Table 1). The genetic map had an

estimated total genetic length of 1547.6 cM, with an average distance between molecular markers of 3.10 cM. The sizes of LGs ranged from 89.72 cM (Pv07) to 184.94 cM (Pv08). Two regions containing loci with significantly distorted segregations were detected in LGs Pv07 and Pv10. Both regions had an excess of the “BAT93” parent alleles. The segregation distortion region in LG Pv07 covered the end of this chromosome (> 30 Mb), whereas the region with the distorted segregation in LG Pv10 covered the beginning of this chromosome (0–30 Mb). Eight gaps of more than 15 cM were detected involving the LGs Pv01, Pv02, Pv03, Pv04, Pv05, and Pv09.

Trait variation

A continuous distribution was detected for all traits (Supplementary Fig. 2). No significant deviations from the corresponding normal distributions were observed except for seed width, thickness, and weight in the XC population and pod width in the XB population. Significant differences between the parents (student’s test) were detected, and a wide variation was observed in both RIL populations for all traits evaluated (Supplementary Table 2).

Pearson’s correlation coefficients for seed traits were positive and significant in both populations (Table 2). Most pod traits were also significantly correlated. For most pairs of seed and pod traits, significant and positive correlations were observed. However, those correlations between the number of seeds per pod (NSP) and all seed traits and pod width or thickness were significantly negative.

QTLs for pod traits

The estimates of estimated broad-sense heritability (H^2) for pod traits ranged between 0.21 (pod thickness) and 0.39 (pod length) in the XC population, whereas, in the XB population, they varied between 0.31 (NSP) and 0.47 (pod area) (Supplementary Table 2). QTL analysis, using the MCIM method implemented in QTLNetwork software, revealed 31 single-locus QTLs for pod traits, 13 in the XC population, and 18 in the XB population (Tables 3 and 4; Supplementary Fig. 1). QTLs with significant QTL–environment interactions were not detected. In the XC RIL population, significant QTLs were located on chromosomes Pv01, Pv02, Pv03, Pv07, Pv08, and Pv11 (Table 3; Supplementary Fig. 1). Alleles

from the parent “Xana” increased the value (positive additive effect) for all phenotypes except for QTLs associated with NSP and the QTL PL1^{XC}. The estimates heritability of additive by additive effects (h^2) ranged between 0.05 (PL7^{XC*}) and 0.27 (PW17^{XC*}), and the highest values were noted for QTL PL2.2^{XC} (0.17) and PW17^{XC*} (0.27).

For the XB population, 18 QTLs involved in pod traits were detected (Table 4; Supplementary Fig. S1): four involved in the genetic control of pod perimeter, four for pod area, four for pod width, four for pod length, and two for the NSP. These QTLs were located on chromosomes Pv01, Pv03, Pv05, Pv06, Pv07, Pv09, and Pv11. Depending on the QTL, alleles contributed by the parental “Xana” increased or decreased the phenotypic value (see Table 4). Also, the alleles derived from the parent “Xana” decreased the NSP. The estimated heritability of additive by additive effects (h^2) for each QTL ranged between 0.05 (PP9^{XB*}) and 0.27 (PW17^{XB}), with the highest values being observed for QTLs PA7.2^{XB} (0.27), PA7.3^{XB} (0.24), PW17^{XB} (0.28), and NSP1.3^{XB} (0.19).

QTLs for seed traits

The estimates of H^2 for the seed traits ranged between 0.57 (seed weight) and 0.67 (seed length) in the XC population, whereas, in the XB population, H^2 varied between 0.56 (seed width) and 0.74 (seed length) (Supplementary Table S2). QTL analysis revealed a total of 21 single-locus QTLs with significant additive effects in the XC RIL population (Table 3; Supplementary Fig. S1): five for seed length, six for seed width, six for seed thickness and four for seed weight. These QTLs were located on bean chromosomes Pv02, Pv03, Pv05, Pv06, Pv07, Pv08, Pv10, and Pv11. No QTL–environment interactions involving QTLs with h^2 estimates higher than or equal to 0.05 were detected. In most QTLs, alleles contributed by the parent “Xana” increased the phenotypic value (positive additive effect), and the h^2 estimates ranged between 0.05 (SL11.1^{XC}, SW18^{XC}) and 0.24 (SL2.6^{XC}). The highest estimates for h^2 were found for the QTL SL2.6^{XC} (0.24), SL6^{XC} (0.19), SW6.2^{XC*} (0.15), and SW7^{XC} (0.19) which are associated with seed length (Table 3).

For the XB population (Table 4), 25 significant QTLs were detected for seed traits located on 8 of the 11 bean chromosomes (Pv01, Pv02, Pv03, Pv06, Pv07, Pv08, Pv09, Pv11); 6 for seed area; 5 for seed perimeter; 6 for seed length; 3 for seed width; and 5 for seed weight. In

Table 3 Single QTL with individual effects for seed and pod traits identified by mixed model based composite interval mapping analysis (MCIM)) in the XC RIL population using the QTL Network 2.0 software

QTL name (1)	Genetic position				QTL traits		
	LG	Position (cM)	Start	End	<i>F</i> -value	A	<i>h</i> ²
Pod size traits							
PL1 ^{XC*}	Pv01	157.0–166.8	IND1_516243	SNP_8	8.02	−0.42	0.15
PL2.1 ^{XC}	Pv02	76.5–80.3	SNP_586	SNP_688	9.40	0.33	0.06
PL2.2 ^{XC}	Pv02	145.2–153.9	SNP_1348	SNP_1722	10.91	0.52	0.17
PL7 ^{XC*}	Pv07	68.0–72.5	IND7_393007	SNP_1967	9.11	0.35	0.05
PW11 ^{XC*}	Pv01	158.0–163.8	IND1_516243	SNP_8	7.90	0.26	0.07
PW17 ^{XC*}	Pv07	48.4–52.0	SNP_195	SNP_2278	18.5	0.58	0.27
PT1.1 ^{XC}	Pv01	0.0–3.0	PVBR233	SNP_1252	7.60	0.21	0.08
PT1.2 ^{XC*}	Pv01	102.0–115.4	SNP_1846	SNP_2114	7.90	0.27	0.09
PT8 ^{XC*}	Pv08	94.4–101.4	SNP_1795	SNP_1355	7.70	0.26	0.07
NSP2 ^{XC}	Pv02	157.2–158.7	SNP_2018	SW13	7.20	−0.12	0.05
NSP3 ^{XC}	Pv03	16.4–26.9	SNP_965	SNP_705	8.32	−0.18	0.06
NSP7 ^{XC}	Pv07	45.7–52.9	SNP_195	SNP_2278	9.43	−0.24	0.10
NSP11 ^{XC}	Pv11	23.7–34.2	SNP_1657	SNP_1658	8.22	−0.12	0.06
Seed traits							
SL2.6 ^{XC}	Pv02	141.6–150.2	SNP_582	SNP_425	9.22	0.72	0.24
SL6 ^{XC}	Pv06	117.5–120.0	BMd37	SNP_1336	11.30	0.49	0.19
SL7.2 ^{XC}	Pv07	50.7–52.0	SNP_1000	SNP_642	10.71	0.26	0.07
SL10 ^{XC}	Pv10	61.1–70.0	SNP_1898	SH08_10	9.42	0.31	0.08
SL11.1 ^{XC}	Pv11	24.7–27.9	BMd33	Bng91	10.8	0.17	0.05
SW13 ^{XC*}	Pv03	24.0–29.1	SNP_1309	SNP_1227	8.92	0.14	0.07
SW15 ^{XC*}	Pv05	77.4–80.1	SNP_1644	SNP_11	6.95	−0.09	0.10
SW16.2 ^{XC}	Pv06	123.2–128.4	SNP_1	SNP_1602	8.93	0.16	0.12
SW17 ^{XC}	Pv07	50.7–52.0	SNP_1000	SNP_642	9.64	0.18	0.13
SW18 ^{XC}	Pv08	12.4–21.5	SNP_1406	78L17c	5.52	0.14	0.05
SW110 ^{XC}	Pv10	28.1–32.5	SNP_476	PVBR185	12.42	0.12	0.07
ST2.2 ^{XC*}	Pv02	144.2–150.6	SNP_582	SNP_425	8.14	−0.14	0.10
ST3.1 ^{XC}	Pv03	45.8–52.7	SNP_1279	SNP_1138	5.24	0.10	0.09
ST3.3 ^{XC}	Pv03	103.4–111.2	SNP_1915	SNP_1660	5.77	−0.13	0.09
ST5.1 ^{XC*}	Pv05	0.0–2.9	SNP_217	SNP_730	6.11	−0.15	0.08
ST6.2 ^{XC*}	Pv06	123.2–126.3	SNP_1	SNP_1602	9.85	0.17	0.07
ST7 ^{XC}	Pv07	61.2–62.5	SNP_1121	BM185	7.76	0.17	0.10
SW2.2 ^{XC*}	Pv02	111.4–130.4	IND2_42.5225	SNP_717	5.95	0.44	0.09
SW3 ^{XC}	Pv03	27.3–29.1	SNP_2338	SNP_130	7.65	0.65	0.08
SW6.2 ^{XC*}	Pv06	119.2–120.7	SNP_1336	ROC11(300)	15.62	0.93	0.15
SW7 ^{XC}	Pv07	50.7–52.0	SNP_1000	SNP_642	24.24	1.28	0.19

A, additive effects (positive values are contributed by alleles from the parent Xana); *h*², heritability of additive effect; *, indicate that the QTL also is involved in epistatic interactions. SL, seed length; SWI, seed width; ST, seed thickness; SA, seed area; SP, seed perimeter; SW, seed weight; PL, pod length; PWI, pod width; PT pod thickness; PA pod area; PP, pod perimeter; NSP, number of seeds per pod

all cases, alleles contributed by “Xana” increased the phenotypic value. The estimated *h*² values ranged

between 0.05 (SL1^{XB*}) and 0.29 (SW7.2^{XB}), with the highest *h*² estimates being found in the QTLs located on

Table 4 Single QTL with individual effects for seed and pod traits identified by mixed-model based composite interval mapping analysis (MCIM)) in the XB RIL population using the QTL Network 2.0 software

QTL name (1)	Genetic position				QTL traits		
	LG	Position (cM)	Start	End	<i>F</i> -value	A	<i>h</i> ²
Pod size traits							
PP1.1 ^{XB}	Pv01	43.1–69.5	SNP01_228	SNP01_243	11.04	−0.77	0.05
PP1.2 ^{XB}	Pv01	136.5–144.1	SNP01_562	SNP01_572	17.82	−0.94	0.10
PP7 ^{XB}	Pv07	54.9–63.3	SNP07_243	SNP07_247	21.52	1.42	0.13
PP9 ^{XB*}	Pv09	0.0–2.7	SNP09_023	SNP09_049	11.54	0.67	0.05
PA5.2 ^{XB}	Pv05	119.1–127.1	SNP05_290	SNP05_297	10.62	−0.55	0.10
PA7.2 ^{XB}	Pv07	41.5–49.7	SNP07_188	SNP07_204	10.77	0.69	0.27
PA7.3 ^{XB}	Pv07	57.1–61.1	SNP07_243	SNP07_247	36.92	0.57	0.24
PA9 ^{XB}	Pv09	0.0–2.7	SNP09_09	SNP09_023	9.13	0.37	0.06
PW15 ^{XB*}	Pv05	56.8–62.8	SNP05_106	SNP05_108	10.44	−0.02	0.07
PW16 ^{XB}	Pv06	18.1–23.0	SNP06_200	SNP06_247	12.71	−0.03	0.11
PW17 ^{XB}	Pv07	40.5–51.2	SNP07_175	SNP07_188	12.22	0.06	0.28
PW11 ^{XB*}	Pv11	59.3–72.6	SNP11_186	SNP11_202	16.42	0.03	0.06
PL1.1 ^{XB}	Pv01	59.6–63.7	SNP01_288	SNP01_295	10.93	−0.39	0.06
PL1.2 ^{XB*}	Pv01	133.6–136.5	SNP01_543	SNP01_562	11.64	−0.45	0.06
PL3.1*	Pv03	65.7–72.4	SNP03_515	SNP03_532	11.45	0.26	0.06
PL7 ^{XB}	Pv07	45.7–52.2	SNP07_188	SNP07_204	25.21	0.51	0.15
NSP1.2 ^{XB}	Pv01	46.6–63.7	SNP01_243	SNP01_251	8.94	−0.18	0.05
NSP1.3 ^{XB}	Pv01	135.6–144.1	SNP01_562	SNP01_572	16.92	−0.32	0.19
Seed traits							
SA1.2 ^{XB*}	Pv01	130.5–137.5	SNP01_543	SNP01_562	8.13	3.15	0.06
SA2.2 ^{XB}	Pv02	96.8–101.7	SNP02_172	SNP02_179	7.04	2.88	0.06
SA7.1 ^{XB}	Pv07	41.5–52.2	SNP07_175	SNP07_188	14.11	6.31	0.25
SA7.2 ^{XB}	Pv07	86.9–88.9	SNP07_316	SNP07_317	18.74	5.59	0.26
SA9 ^{XB*}	Pv09	4.1–8.3	SNP09_098	SNP09_119	15.44	3.57	0.07
SA11 ^{XB}	Pv11	58.3–71.4	SNP11_186	SNP11_202	10.23	3.90	0.07
SP2.1 ^{XB}	Pv02	96.8–102.3	SNP02_172	SNP02_179	11.22	1.09	0.06
SP7.1 ^{XB}	Pv07	40.5–50.2	SNP07_175	SNP07_188	10.13	1.52	0.20
SP7.2 ^{XB*}	Pv07	84.9–88.9	SNP07_316	SNP07_317	12.01	0.88	0.18
SP9 ^{XB}	Pv09	10.1–15.7	SNP09_129	SNP09_137	18.30	0.89	0.08
SP11.2 ^{XB*}	Pv11	67.4–72.6	SNP11_202	SNP11_219	16.03	1.25	0.11
SL1 ^{XB*}	Pv01	68.5–73.5	SNP01_348	SNP01_350	14.94	0.37	0.05
SL2 ^{XB}	Pv02	97.8–101.7	SNP02_172	SNP02_179	13.42	0.43	0.06
SL7.1 ^{XB*}	Pv07	39.5–51.2	SNP07_175	SNP07_188	9.22	0.49	0.19
SL7.2 ^{XB*}	Pv07	83.3–88.9	SNP07_316	SNP07_317	13.10	0.42	0.22
SL9 ^{XB}	Pv09	10.1–15.0	SNP09_129	SNP09_137	22.02	0.42	0.07
SL11 ^{XB*}	Pv11	67.4–72.6	SNP11_202	SNP11_219	26.52	0.43	0.11
SW11 ^{XB}	Pv01	132.2–138.5	SNP01_543	SNP01_562	10.62	0.23	0.11
SW13.1 ^{XB}	Pv03	18.9–37.9	SNP03_111	SNP03_122	6.73	0.22	0.11
SW17 ^{XB*}	Pv07	37.9–49.7	SNP07_164	SNP07_175	14.42	0.29	0.25
SW3 ^{XB*}	Pv03	24.5–33.7	SNP03_122	SNP03_145	7.75	0.25	0.06
SW6 ^{XB}	Pv06	69.7–77.0	SNP06_582	SNP06_612	14.64	0.64	0.09

Table 4 (continued)

QTL name (1)	Genetic position				QTL traits		
	LG	Position (cM)	Start	End	<i>F</i> -value	A	<i>h</i> ²
SW7.1 ^{XB}	Pv07	64.4–67.6	SNP07_271	SNP07_286	47.31	0.44	0.20
SW7.2 ^{XB*}	Pv07	84.9–88.9	SNP07_316	SNP07_317	12.91	1.09	0.29
SW8 ^{XB}	Pv08	162.7–167.5	SNP08_643	SNP08_652	9.63	0.66	0.10

A, additive effects (positive values are contributed by alleles from the parent Xana); *h*², heritability of additive effect; *, indicate that the QTL also is involved in epistatic interactions. SL, seed length; SWI, seed width; ST, seed thickness; SA, seed area; SP, seed perimeter; SW, seed weight; PL, pod length; PWI, pod width; PT pod thickness; PA pod area; PP, pod perimeter; NSP, number of seeds per pod

chromosomes Pv07 (SA7.1^{XB}(0.26), SA7.2^{XB}(0.26), SL7.2^{XB*}(0.22), SW7.1^{XB}(0.20), and SW7.2^{XB*}(0.29)).

Epistatic QTL

In all, 47 QTL × QTL significant interactions with heritability of additive by additive effects greater than or equal to 0.01 were detected in the two-dimensional genome scan, namely, 20 in the XC RIL population and 27 in the XB RIL population (Tables 5 and 6; Fig. 2). All of these interactions showed an additive effect and involved 49 QTLs in the XB population and 38 in the XC population. Most of these interactions involved QTL only detected in the two-dimensional genome scan (named as E-QTLs), 26 in XC and 33 in the XB population. Interactions detected could be classified into three types based the genome scan in which they were identified (one- or two-dimensional scans): E-QTL × E-QTL (24), E-QTL × QTL (13), and QTL × QTL (10). Most QTLs with epistatic interactions were only detected in the two-dimensional scan even though ten interactions involved QTLs are detected in the one-dimensional scan whose interaction contributed significantly to the expression of character (e.g., PT1.2XC*–PT8XC*; PL1.2^{XB*}–PL3.1^{XB*}; SL3^{XB*}–SL11^{XB*}). In general, the estimated heritability of additive by additive effects were low (0.01–0.07), even though three significant interactions exhibited values greater than 0.05, namely, E-SWI4^{XB}–SWI7^{XB*}, E-NSP1.1^{XB}–E-NSP11^{XB}, and E-SWI6.1^{XC}–E-SWI11^{XC}.

Shared QTLs between XC and XB RIL populations

In order to identify the main genomic regions (windows ± 1 Mb) involved in the control of pod and seed traits, overlapping QTLs detected in the two nested

populations were investigated, taking into account the physical position of the underlying markers QTLs. Results revealed four main genomic regions in which overlapping QTLs were involved for pod traits in the XC and XB populations (Fig. 3): chromosome Pv01, position 50.7–51.10 Mb, with the QTL PL1.2^{XB*}, NSP1.3^{XB}, PP1.2^{XB}, PL1^{XC*}, and PWI1^{XC*}; chromosome Pv02, 49.03–49.60 Mb, with NSP2^{XC} and E-PP2^{XB}; chromosome Pv07, 5.76–6.94 Mb, with PA7.2^{XB}, PL7^{XB}, and NSP7^{XC}; and chromosome Pv11, 3.59–4.52 Mb, with NSP11^{XC} and PWI11^{XB*}.

For the seed traits, eight overlapping regions were detected in the following positions (Fig. 3): chromosome Pv01, 50.51–51.10 Mb, with the QTL SA1.2^{XB*}, SWI1^{XB}, and E-ST1.2^{XC}; chromosome Pv02, 2.47–3.08 Mb, with E-SL2.2^{XC} and E-SA2.1^{XB}; chromosome Pv03, 3.7–5.52 Mb, with SWI3.1^{XB}, E-SL3.1^{XC}, and SW3^{XB*}; chromosome Pv05, 38.44–39.44 Mb, with E-SW5.2^{XB} and SWI5^{XC*}; chromosome Pv06, 28.52–29–42 Mb, with SL6.2^{XB*}, SW6^{XB}, and ST6.2^{XC*}; chromosome Pv07, 4–92–5.10 Mb, with SA7.1^{XB}, SL7.1^{XB*}, SP7.1^{XB}, and E-SL7.1^{XC}; chromosome Pv08, 61.08–61.92 Mb, with SW8^{XB} and E-ST8^{XC}; and chromosome Pv11, 3.6–4.5 Mb, with SL11.1^{XC}, E-SWI11^{XB}, SA11^{XB}, SL11^{XB*}, and SP11.2^{XB*}.

Finally, in agreement with the high correlations observed, three regions shown to be involved in the genetic control of both pod and seed traits (Figs. 2 and 3):

- i) The region at the end of chromosomes Pv01, between the physical position 50.51 and 51.10 Mb, in which eight QTLs were located (PL1.2^{XB*}, NSP1.3^{XB}, PP1.2^{XB}, PL1^{XC*}, PWI1^{XC*}, SA1.2^{XB*}, SWI1^{XB}, and E-ST1.2^{XC})
- ii) The region at the beginning of the chromosome Pv07, between the physical position 4.90 and 5.10 Mb (surroundings cluster phaseolin), in which

Table 2 Pearson phenotypic correlation coefficients for the quantitative traits using the mean environment data.

	Pod size traits					Seed size traits						
	PL	PWI	PT	PA	PP	NSP	SL	SWI	ST	SA	SP	SW
Pod length		0.21*	-0.28**			0.29**	0.40***	0.002 ns	-0.09 ns			0.22*
Pod width	0.29***		0.24*			0.52***	0.52***	0.57***	0.21*			0.50***
Pod thickness						-0.37***	0.18*	0.23*	0.49***			0.28*
Pod area	0.84***	0.69***										
Pod perimeter	0.96***	0.35***	0.89**									
N. seeds per pod	0.24**	-0.40***	0.06 ns	0.22**								
Seed length	0.44***	0.55***	0.55***	0.48***	0.48***	-0.48***	-0.42***	0.70***	0.24**			0.47***
Seed width	0.32***	0.71***	0.52***	0.33***	0.33***	-0.50***	0.82***	0.81***	0.44***			0.60***
Seed thickness												0.57***
Seed area	0.44***	0.64***	0.59***	0.47***	0.47***	-0.49***	0.97***	0.92***				
Seed perimeter	0.42***	0.59***	0.56***	0.45***	0.45***	-0.52***	0.98***	0.89***				
Seed weight	0.43***	0.56***	0.54***	0.46***	0.46***	-0.40***	0.87**	0.81***		0.97***	0.86**	

(Upper) Pearson correlation coefficients obtained from XC RIL population. (Bottom) Pearson correlation coefficients obtained from XB RIL population. ns, $p > 0.05$; **, $0.01 > p < 0.05$; ***, $0.001 > p < 0.01$; ****, $p < 0.001$

seven QTLs were located (SA7.1^{XB}, SL7.1^{XB*}, SP7.1^{XB*}, E-SL7.1^{XC}, PA7.2^{XB}, PL7^{XB}, and NSP7^{XC})

- iii) The region at the beginning of chromosome Pv11, between the physical positions 3.59 and 4.52 Mb, in which seven QTLs were located (SL11.1^{XC}, E-SWI11^{XB*}, SA11^{XB}, SL11^{XB*}, SP11.2^{XB*}, NSP11^{XC}, and PWI11^{XB*})

Discussion

Common bean pod and seed phenotypes are important traits because they distinguish different gene pools and races of the cultivated form of the species. In this work, we investigated the genetic control of pod and seed size (measured as length, width thickness, area or perimeter) as well as the number of seeds per pod and mean seed weight in two nested populations. The two populations have a common parent (“Xana”). Biparental populations offer the opportunity to investigate the variation between the parental genotypes involved in the cross, but when two populations are compared, shared genetic effects can be discovered. However, results can be confounded by the level of polymorphisms, the frequency of recombination, map saturation, and segregation distortions along the chromosomes of the RIL populations (McMullen et al. 2009). For instance, two regions in chromosomes Pv07 and Pv10 were not tagged due to significantly distorted segregations observed in them, and the putative QTLs located at these positions cannot be investigated. The same regions exhibiting distorted segregation were reported in other populations involving the parent “BAT93” (Freyre et al. 1998). Nested populations allow the verification of the genetic control in the recurrent genotype, which improves the accuracy of locating the loci because two sources of recombination (historical recombination in parents and recombination in the lines within the population) are included, and allow the investigation of the genetic control of more variation than is possible for a single population (Fragoso et al. 2017).

All traits showed continuous distributions in the two populations, and most traits were normally distributed. Also, most of the evaluated traits exhibited significant and mostly positive correlations in both populations, suggesting the involvement of the same genes affecting the trait. Interestingly, pod length and width were

Table 5 QTL with significant epistatic interaction detected in the RIL population XC.

E-QTL 1 (1)	LG	Position (cM)	Start	End	E-QTL 2	LG	Position (cM)	Start	End	AA ⁽¹⁾	h ²
E-SL1.1 ^{XC}	Pv01	99.3–105.0	SNP_1846	SNP_2114	E-SL2.2 ^{XC}	Pv02	20.3–28.7	SNP_646	SNP_485	0.18	0.02
E-SL2.1 ^{XC}	Pv02	0.0–5.7	SNP_983	SNP_1818	E-SL2.5 ^{XC}	Pv02	110.5–113.4	SNP_926	SNP_1836	0.36	0.04
E-SL2.3 ^{XC}	Pv02	61.6–65.9	SNP_923	SNP_741	E-SL3.2 ^{XC}	Pv03	38.6–43.4	SNP_173	SNP_2259	0.30	0.02
E-SL2.4 ^{XC}	Pv02	93.1–97.2	SNP_852	SNP_142	E-SL7.1 ^{XC}	Pv07	39.1–45.7	PV/atcc001	T/S_07	-0.20	0.03
E-SL3.1 ^{XC}	Pv03	20.1–24.9	SNP_705	SNP_1532	E-SL11.2 ^{XC}	Pv11	54.2–57.5	SNP_2392	0.23	0.02	0.02
SW13 ^{XC*}	Pv03	24.0–29.1	SNP_1309	SNP_1227	SW15 ^{XC*}	Pv05	77.4–80.1	SNP_1644	SNP_11	0.07	0.02
E-SW12 ^{XC}	Pv02	83.8–91.1	SNP_2302	SNP_1402	E-SW19 ^{XC}	Pv09	41.3–46.6	SNP_242	SNP_1352	0.11	0.04
E-SW16.1 ^{XC}	Pv09	10.7–12.8	BMb342	SNP_1071	E-SW11 ^{XC}	Pv11	58.5–64.6	IND11_16.3739	0.11	0.05	0.05
ST2.2 ^{XC*}	Pv02	144.2–150.6	SNP_582	SNP_425	E-ST8 ^{XC}	Pv08	94.4–102.8	SNP_447	SNP_1358	0.07	0.02
ST5.1 ^{XC*}	Pv05	0.0–2.9	SNP_217	SNP_730	E-ST8 ^{XC}	Pv08	94.4–102.8	SNP_447	SNP_1358	-0.09	0.02
E-ST5.2 ^{XC}	Pv05	80.1–90.0	SNP_1635	SNP_1382	E-ST10 ^{XC}	Pv10	27.1–35.5	SNP_476	PVBR185	0.08	0.03
E-ST1.1 ^{XC}	Pv01	96.0–99.3	SNP_1229	SNP_108	E-ST6.1 ^{XC}	Pv06	116.0–120.7	SNP_389	BMd37	0.09	0.03
E-ST1.2 ^{XC}	Pv01	152.1–162.0	IND1_516243	SNP_8	E-ST3.2 ^{XC}	Pv03	64.4–68.5	SNP_530	SNP_1808	0.07	0.02
E-ST2.1 ^{XC}	Pv02	137.8–143.7	SNP_1746	SNP_1854	E-ST10 ^{XC}	Pv10	27.1–35.5	SNP_476	PVBR185	-0.10	0.02
SW2.2 ^{XC*}	Pv02	111.4–130.4	IND2_42.5225	SNP_717	E-SW6.1 ^{XC}	Pv06	34.8–44.5	Pv67700b	BM187	0.44	0.02
E-SW2.1 ^{XC}	Pv02	96.2–98.9	SNP_1543	SNP_505	E-SW11 ^{XC}	Pv11	25.7–29.9	BMd33	Bng91	0.37	0.02
E-SW4 ^{XC}	Pv04	80.0–99.6	SAS15	SNP_2166	SW6.2 ^{XC*}	Pv06	119.2–120.7	SNP_1336	ROC11(300)	-0.70	0.04
PL1 ^{XC*}	Pv01	157.0–166.8	IND1_516243	SNP_8	PL7 ^{XC*}	Pv07	68.0–72.5	IND7_393007	SNP_1967	0.19	0.02
PW11 ^{XC*}	Pv01	158.0–163.8	IND1_516243	SNP_8	PW17 ^{XC*}	Pv07	48.4–52.0	SNP_195	SNP_2278	0.20	0.03
PT1.2 ^{XC*}	Pv01	102.0–115.4	SNP_1846	SNP_2114	PT8 ^{XC*}	Pv08	94.4–101.4	SNP_1795	SNP_1355	0.16	0.03

QTL named with the prefix E- only were detected in the two-dimensional genomes scan. E-QTL 1 and E-QTL 2 are the couple of QTL involved in the epistatic interaction. AA, estimated additive by additive epistatic effect in which positive values are provided by the parent Xana. h², heritability of additive by additive effect. *p* value < 0.001

Table 6 QTL with significant epistatic interaction detected in the RIL population XC

E-QTL 1	LG	Position (cM)	Start	End	E-QTL 2	LG	Position (cM)	Start	End	AA ⁽¹⁾	h ²
E-SL1.1 ^{XC}	Pv01	99.3–105.0	SNP_1846	SNP_2114	E-SL2.2 ^{XC}	Pv02	20.3–28.7	SNP_646	SNP_485	0.18	0.02
E-SL2.1 ^{XC}	Pv02	0.0–5.7	SNP_983	SNP_1818	E-SL2.5 ^{XC}	Pv02	110.5–113.4	SNP_926	SNP_1836	0.36	0.04
E-SL2.3 ^{XC}	Pv02	61.6–65.9	SNP_923	SNP_741	E-SL3.2 ^{XC}	Pv03	38.6–43.4	SNP_173	SNP_2259	0.30	0.02
E-SL2.4 ^{XC}	Pv02	93.1–97.2	SNP_852	SNP_142	E-SL7.1 ^{XC}	Pv07	39.1–45.7	PV/atcc001	T/S_07	-0.20	0.03
E-SL3.1 ^{XC}	Pv03	20.1–24.9	SNP_705	SNP_1532	E-SL11.2 ^{XC}	Pv11	54.2–57.5	SNP_2392	IND11_13.7411	0.23	0.02
SW13 ^{XC*}	Pv03	24.0–29.1	SNP_1309	SNP_1227	SW15 ^{XC*}	Pv05	77.4–80.1	SNP_1644	SNP_11	0.07	0.02
E-SW12 ^{XC}	Pv02	83.8–91.1	SNP_2302	SNP_1402	E-SW19 ^{XC}	Pv09	41.3–46.6	SNP_242	SNP_1352	0.11	0.04
E-SW16.1 ^{XC}	Pv09	10.7–12.8	BMb342	SNP_1071	E-SW11 ^{XC}	Pv11	58.5–64.6	IND11_16.3739	IND11_28.2988	0.11	0.05
ST2.2 ^{XC*}	Pv02	144.2–150.6	SNP_582	SNP_425	E-ST8 ^{XC}	Pv08	94.4–102.8	SNP_447	SNP_1358	0.07	0.02
ST5.1 ^{XC*}	Pv05	0.0–2.9	SNP_217	SNP_730	E-ST8 ^{XC}	Pv08	94.4–102.8	SNP_447	SNP_1358	-0.09	0.02
E-ST5.2 ^{XC}	Pv05	80.1–90.0	SNP_1635	SNP_1382	E-ST10 ^{XC}	Pv10	27.1–35.5	SNP_476	PVBR185	0.08	0.03
E-ST1.1 ^{XC}	Pv01	96.0–99.3	SNP_1229	SNP_108	E-ST6.1 ^{XC}	Pv06	116.0–120.7	SNP_389	BMd37	0.09	0.03
E-ST1.2 ^{XC}	Pv01	152.1–162.0	IND1_516243	SNP_8	E-ST3.2 ^{XC}	Pv03	64.4–68.5	SNP_530	SNP_1808	0.07	0.02
E-ST2.1 ^{XC}	Pv02	137.8–143.7	SNP_1746	SNP_1854	E-ST10 ^{XC}	Pv10	27.1–35.5	SNP_476	PVBR185	-0.10	0.02
SW2.2 ^{XC*}	Pv02	111.4–130.4	IND2_42.5225	SNP_717	E-SW6.1 ^{XC}	Pv06	34.8–44.5	Pv67700b	BM187	0.44	0.02
E-SW2.1 ^{XC}	Pv02	96.2–98.9	SNP_1543	SNP_505	E-SW11 ^{XC}	Pv11	25.7–29.9	BMd33	Bng91	0.37	0.02
E-SW4 ^{XC}	Pv04	80.0–99.6	SAS15	SNP_2166	SW6.2 ^{XC*}	Pv06	119.2–120.7	SNP_1336	ROC11(300)	-0.70	0.04
PL1 ^{XC*}	Pv01	157.0–166.8	IND1_516243	SNP_8	PL7 ^{XC*}	Pv07	68.0–72.5	IND7_393007	SNP_1967	0.19	0.02
PW11 ^{XC*}	Pv01	158.0–163.8	IND1_516243	SNP_8	PW17 ^{XC*}	Pv07	48.4–52.0	SNP_195	SNP_2278	0.20	0.03
PT1.2 ^{XC*}	Pv01	102.0–115.4	SNP_1846	SNP_2114	PT8 ^{XC*}	Pv08	94.4–101.4	SNP_1795	SNP_1355	0.16	0.03

QTL named with the prefix E only were detected in the two dimension genomes scan. E-QTL 1 and E-QTL 2 are the couple of QTL involved in the epistatic interaction. AA, estimated additive by additive epistatic effect in which positive values are provided by the parent Xana. h², heritability of additive by additive effect; *, indicate that the QTL also is involved in epistatic interactions

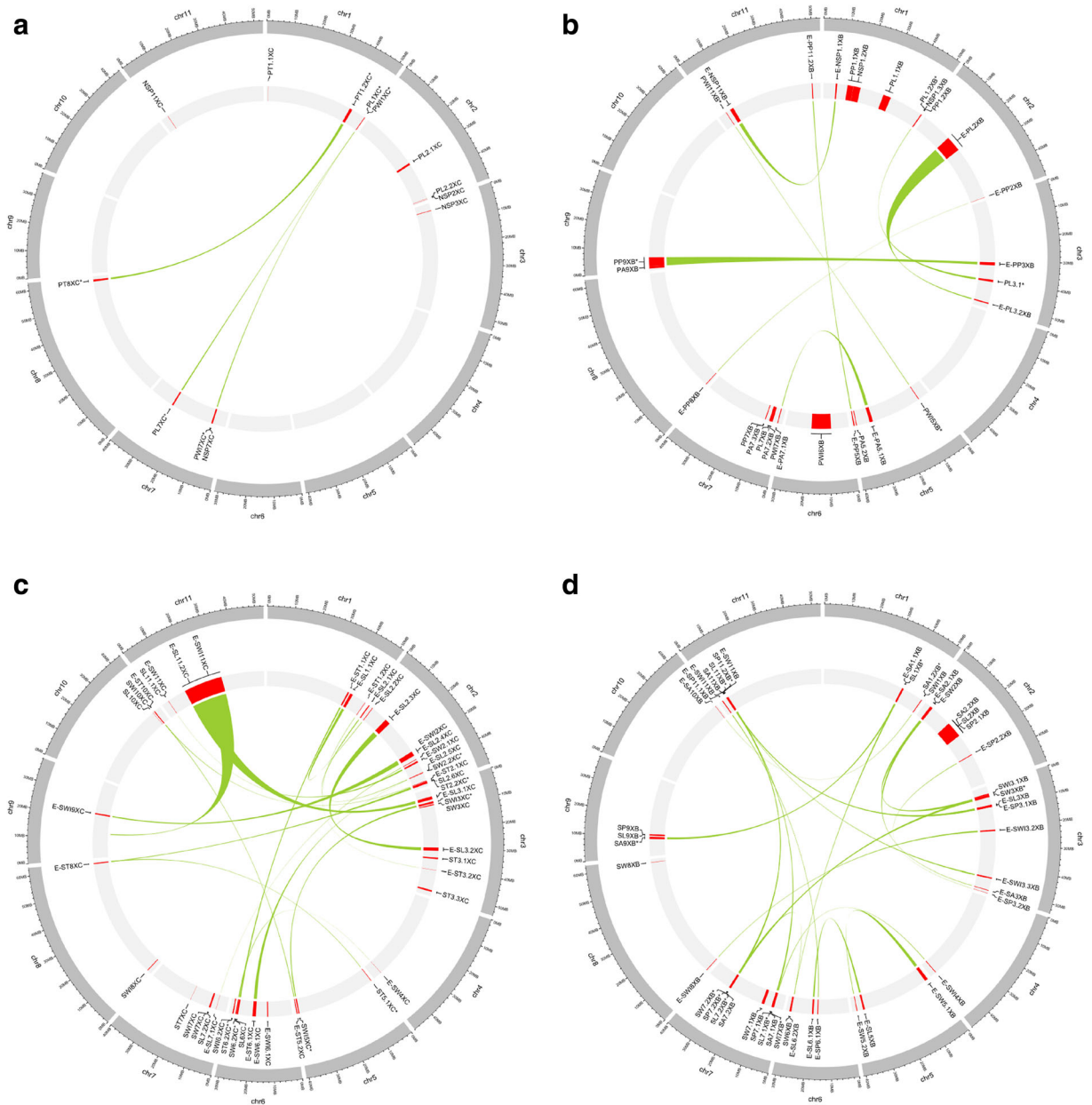


Fig. 2 Genetic map showing the physical positions of the significant single QTL and E-QTL detected for the pod and seed traits investigated in the XC and XB RIL populations. The QTL with epistatic interactions are connected with green lines. **(A)** QTL for

pod traits in XC RIL population. **(B)** QTL for pod traits in XB RIL population. **(C)** QTL for seed traits in XC RIL population. **(D)** QTL for seed traits in XB RIL population

positively correlated with seed length and width, while the NSP was negatively correlated to all seed traits. Seed weight and the NSP are components of seed yields (Adams 1967), and both traits exhibit moderate heritability (Coyné 1968). In fact, several studies have reported a co-location of yield and seed weight QTLs (Sandhu et al. 2018; González et al. 2016).

QTL analysis showed a total of 46 single QTLs (21 in XC + 25 in XB) associated with seed traits and 32 QTLs (13 in XC + 18 in XB) associated with pod traits in both RIL populations. Moreover, two-dimensional genome scans revealed a complex network with significant epistatic interaction between QTLs. A total of 47 epistatic QTL x QTL interactions were detected involving 59

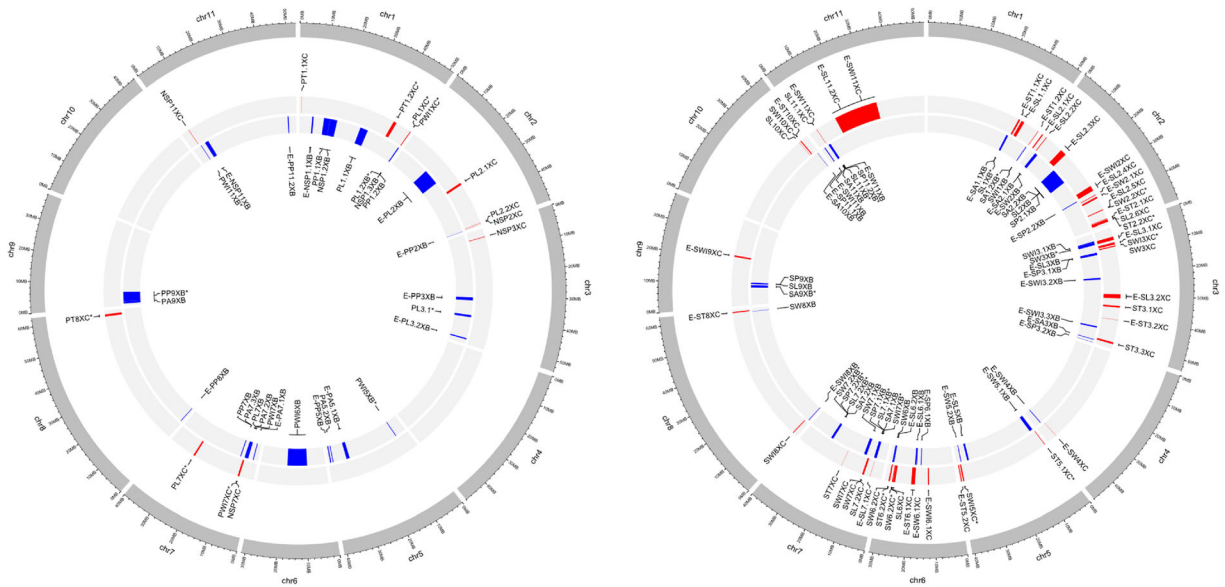


Fig. 3 Genetic map showing the physical positions of the QTL for the pod and seed traits detected in the XC and XB RIL populations. Red track, QTL detected in the XC RIL population; blue track, QTL detected in the XB RIL population

additional QTLs (26 in XC and 33 in XB). The vast majority of these interactions occurred between QTLs mapped onto different chromosomes and involved QTLs which are detected only in the two-dimensional scan, 24 of a total of 47 significant interactions. Significant QTL x QTL interactions have also been detected previously for pod and seed traits using low-density genetic maps (Johnson and Gepts 2002; González et al. 2016; Yuste-Lisbona et al. 2014). This study confirmed the role of QTL x QTL interactions in the control of seed and pod phenotypes and showed the complexity of the genetic control of the seed and pod phenotype in common bean.

Stable and repeatable QTLs are desirable for use in plant breeding or the identification of underlying candidate genes associated with observed quantitative variation. In common bean, several works have reported QTLs for seed size, the majority of which focused on seed weight (e.g., González et al. 2016; Pérez-Vega et al. 2010; Correa da Silva et al. 2018), while few studies have described QTLs for pod size or NSP (e.g., González et al. 2016; Yuste-Lisbona et al. 2014). However, few of the QTLs described previously have been validated in different genetic backgrounds or in different environments. QTL validation can be addressed through identification of some QTLs in different genotypes, namely, the same genetic or genomic region associated with the expression of a quantitative phenotypic trait. In

this work, we tried to validate QTLs associated with pod and seed traits through comparison of their physical positions, first, by using two nested populations and, second, by comparing the identified QTLs with previously reported QTLs on different mapping populations.

Until now, the identity of a specific QTL was based on its genetic position in the genetic linkage map. The comparison of two genetic maps requires the presence of common loci, but the XC and XB genetic maps did not share common loci. However, in both genetic maps, most loci are markers whose physical positions in the available bean genome are known (Schmutz et al. 2014). Comparison of physical positions in both nested populations revealed, at least eight genomic regions (± 1 Mb) with common QTLs involved in the phenotypic expression of pod or seeds (see Fig. 2 and Supplementary Fig. 3). Variation in the number and position of identified QTLs can be due to the polymorphism between both parents and the density of the markers labeling the 11 chromosomes. For example, the end of chromosome Pv07 in the XB population had few markers because of distorted segregation. Although no QTLs could be detected in this region, the gene *P* controlling the seed color was mapped to this region and shown to be associated with seed weight (McClellan et al. 2018).

The correspondence of the QTLs detected in this work in both nested populations with the QTLs reported

elsewhere for pod or seed traits was also investigated (see Supplementary Fig. 3). This analysis was not possible in all cases since the underlying marker sequences were either not available (e.g., marker loci type RFLP, RAPD, or AFLP) or the BLASTN exhibited several alignments with the bean genome. Moreover, some QTL studies were based on low-density genetic maps, and they were mapped in wide genetic regions tagged by few loci. Even so, it was possible to detect several genomic regions with overlapping QTLs between the present study and QTLs reported in previous studies. Most notable are the following regions of the chromosomes Pv01, Pv05, Pv06, Pv07, Pv08, and Pv11:

- The region tagged by the gene *fin* in chromosome Pv01 (candidate gene PvTFL1y ~44 Mb, Kwak et al. 2008) in which were mapped the QTLs SL-1.2^{MA} and eST-1^{AM} associated to seed size control as well as the QTLs PWI-1.1^{AM} and NSP-1^{AM} involved in the control of pod size and the number of seeds per pod, respectively (González et al. 2016)
- The region in the distal position of chromosome Pv01 (~50 Mb) with the QTL SL-1^{AM}, SW-1^{AM}, and SWI-2^{MA} involved in the control of seed size and PWI-1.2^{AM}, PWI-2^{MA}, and ePWI-2^{MA} involved in the control of pod size (González et al. 2016)
- The distal region of chromosome Pv05 with the QTLs SF5-1^{RA}, ST5^{MA}, SW5^{MA}, and SW5.1^{RA}, all of them are involved in the control of seed size (González et al. 2016; Correa da Silva et al. 2018)
- The region on which the gene *bc-3* on chromosome Pv06 was mapped (~27.20 Mb, Hart and Griffiths 2013) with the eSL6^{MA}, SW6^{XC}, sw6.1, SL6^{XC}, SH6^{XC}, all them involved in the control of seed size (Blair et al. 2006; Pérez-Vega et al. 2010; González et al. 2016)
- The region at the beginning of chromosome Pv07 which the genes *Asp* and phaseolin cluster were mapped (1.5–5 Mb; Pérez-Vega et al. 2010); with the SW_MO-16, SF7.1^{RA}ePWI-7^{MA}, sw7.1, and SW7.1^{RA} (Blair et al. 2006; Correa da Silva et al. 2018; González et al. 2016; Sandhu et al. 2018), all them associated to the control of seed size
- The distal region on chromosome Pv08 (~61 Mb) with the SWI-8^{MA}, eSWI-8^{MA}, and SW8.2^{XC}, all them involved in the control of seed size (González et al. 2016; Pérez-Vega et al. 2010)
- The beginning of chromosome Pv11 (1–4 to 5.33 Mb) with the SWI-11.1^{AM}, eST-11^{AM}, and

PL11^{PP}, all of which are associated to seed and pod size control (Pérez-Vega et al. 2010; Yuste-Lisbona et al. 2014)

The resolution of this QTL study was not sufficiently high for precise candidate gene identification. However, the sequencing of the bean genome revealed 139 candidate seed weight genes (Schmutz et al. 2014), and 36 of these genes were located (± 1 Mb) in the position of the 50 QTLs identified in this study (see Supplementary Table 3). With single QTL associated with seed traits, 28 genes co-located. Many of these genes show a functional annotation implicated with growth and development. These include Phvul.001G149400 and Phvul.007G170100 (involved in the pathway of zeatin biosynthesis, a phytohormone) or Phvul.002G152900, Phvul.005G144500, Phvul.006G077200, Phvul.008G034700, Phvul.008G248000, Phvul.009G142800, and Phvul.011G063800 encoding expansin proteins (involves in the cell wall extension). It is remarkable that the candidate genes Phvul.007G064800 (SA7.1^{XB}, SL7.1^{XB*}, SP7.1^{XB}), Phvul.007G166700 (SA7.2^{XB}, SL7.2^{XB*}), Phvul.007G167900 (SP7.2^{XB*}), and Phvul.007G170100 (SW7.2^{XB*}) are co-located with QTLs that explained very high percentages of phenotypic variation. The gene Phvul.007G064800 was located at the region of the cluster genes encoding the *phaseolin* seed storage protein (5 Mb), a seed protein traditionally associated with the seed weight and differentiation of the two main gene pools. Seeds of the Middle American genotypes are usually smaller than seeds of Andean genotypes, and the two phaseolin alleles are distinctively different (Gepts et al. 1986).

The results showed a complex architecture of the genetic control of pod and seed phenotypes. Many QTLs have small heritabilities or have epistatic interactions among them to control pod or seed size. However, this study identified some genomic positions with QTLs controlling these traits located at the ends of the chromosomes Pv01, Pv05, Pv07, Pv08, and Pv11. These regions could be used to accelerate bean breeding by means of marker-assisted selection in breeding programs involving the pod or seeds traits. This study also shows the value of the bean genome to physically position QTLs and validation of QTLs obtained in different studies or genetic backgrounds. Nested populations can also be used as a tool to identify and validate QTLs involved in the genetic control of complex traits.

Continuous efforts to reveal and locate robust QTLs controlling important for breeding traits should be carried out in order to identify candidate genes and their interactions. Such knowledge will also provide a precise form of plant breeding.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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