Theor Appl Genet (2010) 120:1367–1380
DOI 10.1007/s00122-010-1261-5

ORIGINAL PAPER

Mapping of QTLs for morpho-agronomic and seed quality traits in a RIL population of common bean (*Phaseolus vulgaris* L.)

Elena Pérez-Vega · Astrid Pañeda · Cristina Rodríguez-Suárez · Ana Campa · Ramón Giraldez · Juan José Ferreira

Received: 1 April 2009 / Accepted: 27 December 2009 / Published online: 19 January 2010

Abstract The objective of this research was to determine the quantitative trait loci (QTLs) controlling phenological traits (days to flowering, days to end of flowering, days to harvest as green pod, and days to maturity), seed size traits (seed length, seed height, seed width, and seed weight), and seed quality traits (water absorption, and coat proportion), in common bean. A population of 104 F_7 recombinant inbred lines (RILs) derived from an inter-gene pool cross between Xana, and Cornell 49242, was used to develop a genetic linkage map including 175 AFLPs, 27 microsatellites, 30 SCARs, 33 ISSRs, 12 RAPDs, 13 loci codifying for seed proteins, and the four genes *Fin,fin* (growth habit); *Asp,asp* (seed coat shininess); *P,p* (seed color); and *I,i* (resistance to bean common mosaic virus). The map has a total length of 1,042 cM distributed across 11 linkage groups aligned to those of the core linkage map of bean using common molecular markers as anchor points. The QTL analyses were carried out over three environments using the mean environment data with composite interval mapping. Thirty-one QTLs for ten traits were found to be significant in at least one environment and in the mean environment data, the number of significant QTLs identified per trait ranging from two to five. Twenty-seven of these QTLs mapped forming clusters in eight different chromosomal regions. The rationale for this clustered mapping and the possible relationship between some QTLs for phenological traits and the genes *Fin* and *I* are discussed.

Communicated by H. T. Nguyen.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-010-1261-5) contains supplementary material, which is available to authorized users.

E. Pérez-Vega · A. Campa · J. J. Ferreira
Área de Cultivos Hortofrutícolas y Forestales,
SERIDA, Apdo. 13, 33300 Villaviciosa, Asturias, Spain

A. Pañeda · C. Rodríguez-Suárez · R. Giraldez (✉)
Department of Biología Funcional,
University of Oviedo, 33006 Oviedo, Spain
e-mail: giraldez@uniovi.es

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important legume for direct human consumption worldwide (Broughton et al. 2003). It is a diploid ($2n = 22$) and predominantly self-pollinating legume (Ferreira et al. 2000) with a relatively small genome (0.65 pg/1C; Aramuganathan and Earle 1991). The species was domesticated by Middle American and South American cultures (Gepts et al. 1986; Gepts 1998) and progressively dispersed worldwide from these regions. Two main gene pools, Middle American and Andean, associated with these two geographical areas, have been described in wild and cultivated common beans. As a result of the domestication process, a great number of varieties showing differences in morpho-agronomic quantitative traits including plant growing period, seed size, and seed quality, were obtained, and this variation has been extensively used in breeding programs or diversity studies. In particular, the estimates of the heritabilities of days to maturity, of different aspects related to seed size, and of seed water absorption, have been relatively high ($h^2 > 0.5$), suggesting an important genetic component in the expression of these quantitative traits (Polignano 1983; Conti 1985; Nienhuis and Singh 1988; Singh et al. 1991; Elia et al. 1997).

The discovery and improvement of DNA analysis techniques gave rise to the development of genetic maps

with a saturation degree appropriate for the mapping and dissection of quantitative trait loci (QTL). The first bean genetic maps, based on molecular markers, were developed by Vallejos et al. (1992), Nodari et al. (1993), and Adam-Blondon et al. (1994). The correspondence between these maps was later established in an integrated linkage map (Freyre et al. 1998) spanning 1,226 cM and including more than 550 markers assigned to 11 linkage groups (named B1–B11). A relatively large number of linkage maps have been subsequently developed, which, differ in the parents used, the type of segregating population analyzed, the traits evaluated and the number and type of molecular markers used (Beattie et al. 2003; Blair et al. 2003; Broughton et al. 2003; Kelly et al. 2003; Blair et al. 2006; Rodríguez-Suárez et al. 2007; Checa and Blair 2008; Grisi et al. 2007). Some of these maps have been used to analyze morpho-agronomic traits including days to flowering (Koinange et al. 1996; Tar'an et al. 2002; Blair et al. 2006), days to maturity (Koinange et al. 1996; Tar'an et al. 2002; Beattie et al. 2003; Blair et al. 2006), seed length (Park et al. 2000), seed height (Park et al. 2000), and seed weight (Koinange et al. 1996; Tsai et al. 1998; Park et al. 2000; Tar'an et al. 2002; Blair et al. 2006). In these publications, the maps used to identify QTLs differed in the parents used, in the number of molecular markers present, and had a relatively low degree of alignment with each other linkage map. Different number and relative positions of the QTLs involved in the genetic control of any given trait have been obtained in most of these cases. For example, concerning the phenological trait, days to maturity, Koinange et al. (1996) mapped three QTLs, two of them on linkage group B1 and the other on B8, while Tar'an et al. (2002) mapped two QTLs on B9 and B10, respectively, Beattie et al. (2003) mapped three QTLs for days to maturity on B4, B6 and B8, while and Blair et al. (2006) mapped two QTLs for the trait on B5 and B7. The mapping population used can be an important factor in these differences. Obviously, in a regular genetic analysis, only the QTLs for which the parents show differences can be identified.

The objective of this research was to identify QTLs for morpho-agronomic traits in common bean, using a RIL population derived from two genotypes (Xana and Cornell 49242), which exhibit differences in phenology, seed size, and seed quality traits, which are important to most common bean breeding programs in the world.

Materials and methods

Plant material

The mapping population used in the present study was formed by 104 F_7 recombinant inbred lines (RILs) developed from the cross Xana × Cornell 49242 by single seed descent (Fig. 1). Xana is a bean variety developed at Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA, Villaviciosa, Spain) from a cross between the two Andean genotypes Andecha and V203. It is a very large white-seeded line, having determinate, type I growth habit (Singh 1982), and belonging to the fabada market class. Cornell 49242, is a very small black-seeded line, having indeterminate postrate, type III growth habit (Singh 1982). It belongs to the Mesoamerican gene pool and is included in the market class black turtle. Cornell 49242 carries the dominant allele of the *I* gene, conferring resistance to the bean common mosaic virus (BCMV), whereas Xana is susceptible.

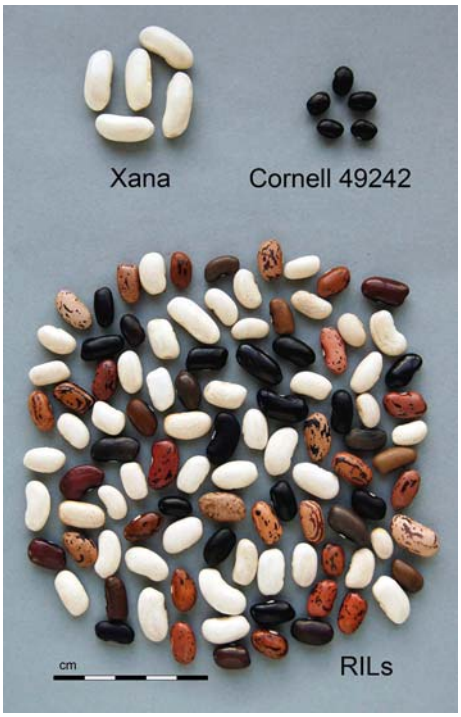

Fig. 1 Seeds of the parental lines Xana and Cornell 49242 and the 104 RILs analyzed (one seed of each RIL). *Bar* represents 5 cm

Field design

In the summer of 2004, 2005, and 2006, the 104 RILs, and the parents Xana and Cornell 49242, were grown in a randomized complete-block design with two replications at Villaviciosa, Spain (43°29′01″N, 5°26′11″W; elevation 6.5 m). Each experimental unit was a single 1.2-m row plot containing 15 plants. One meter spacing between rows was used to accommodate both climbing and prostrate RILs. Standard agronomic practices for tillage, fertilization, weed, and insect control were followed to ensure adequate plant growth and development.

Quantitative trait evaluation

Table 1 lists the quantitative traits measured on the RIL population. Frequency distributions for trait means were tested for normality with the Kolmogorov–Smirnov test, and a probability of $P < 0.05$ was used to indicate lack of fit. *t* tests were used to determine significant differences ($P < 0.05$) among means. Pearson's correlation coefficients among the traits were investigated using the mean phenotypic data for the 3 years. Statistic analyses were carried out using the SPSS V12 software.

Qualitative traits and seed protein analysis

Four qualitative traits were evaluated: indeterminate versus determinate growth habit (gene *Fin,fin*), shiny versus dull seed coat (gene *Asp,asp*), colored versus white seed coat (gene *P,p*), and resistance versus susceptibility to BCMV (gene *I,i*). Resistance to BCMV was evaluated in the experimental fields in the three seasons, 2004, 2005, and 2006. A RIL was considered resistant when none of their individual plants showed BCMV infection symptoms in the three seasons.

Seed protein patterns were analyzed in a bulk of five seeds per RIL using the SDS–PAGE system of Laemmli (1970) as modified by Ferreira et al. (2000). Proteins were extracted from four samples (0.01–0.02 g) taken from the suture end of each seed placed in a buffer solution (62 mM Tris–HCl pH 8.8, 2% (w/v) SDS, 10% glycerol and 0.005% (w/v) bromphenol blue) for 6 h at room temperature. The extracts were later reduced with one drop of 2-mercaptoethanol (2-ME). The mixture was heat-treated (100°C) for 5 min, centrifuged and the electrophoresis of the supernatant was carried out using 1-mm thin slab gels of 12 or 17% (w/v) polyacrylamide. The proteins were visualized using Coomassie Brilliant Blue R. The seed protein polymorphisms analyzed were controlled by 18 loci, 13 of which (the phaseolin locus, *Pha*, and 12 other loci, named *SpA* to *SpM*) were included in the genetic map (a figure of a polyacrylamide gel showing the polymorphic polypeptides corresponding to these loci is available upon request).

DNA isolation and molecular marker analysis

Genomic DNA from the parental genotypes and from each RIL was isolated from bulked young leaves of ten plants for each line, using the Nucleon™ PhytoPure™ Genomic DNA Extraction Kit (Amersham Biosciences, Fairfield, CT, USA) following the supplier's instructions. DNA concentration was measured by fluorometric analysis using a Q-bit Fluorometer (Invitrogen, Carlsbad, CA, USA). In order to develop the genetic maps, four types of molecular markers based on the polymerase chain reaction (PCR) were analyzed on the segregating population. PCR amplification was performed in a PCR System 9600 (Applied Biosystems, Foster City, USA) using a specific thermocycling profile for each type of marker.

Table 1 Quantitative traits measured on 104 RILs derived from the Xana × Cornell 49242 cross

Trait	Units	Description
Phenological traits		
Days to flowering (DF)	Days	Number of days from planting to 50% of the plants within a plot with flowers
Days to end of flowering (DE)	Days	Number of days from planting to 100% of the plants within a plot having ended flowering
Days to harvest as green pod (DG)	Days	Number of days from planting to 50% of the pods within a plot being harvested as snap beans
Days to maturity (DM)	Days	Number of days from planting to harvest maturity
Seed size trait[a]		
Seed length (SL)	mm	Average of ten randomly chosen seeds (measured parallel to the hilum)
Seed height (SH)	mm	Average of ten randomly chosen seeds (measured from hilum to opposite side)
Seed width (WI)	mm	Average of ten randomly chosen seeds
Seed weight (SW)	g	Average of 100 randomly chosen seeds
Seed quality traits[a]		
Water absorption (WA)	%	Average of three sets of 25 seeds (according to Castellanos et al. 1995)
Coat proportion (CP)	%	Average of three sets of 25 seeds. After soaking the seeds in water for 24 h, coats and embryos were separated, dried at 80°C for 8 days, and weighted (Castellanos et al. 1995)

[a] Moisture content of 14–15%

AFLP markers

AFLPs were generated using the kit of Applied Biosystems for small plant genomes (Applied Biosystems) following the supplier's instructions. Genomic DNA was double digested with the restriction enzymes *Eco*RI and *Mse*I (Vos et al. 1995). Selective amplification was conducted using the following ten primer pair combinations formed with *Mse*I-specific primers with three extra nucleotides (*Mse*-) and *Eco*RI-specific primers with two extra nucleotides (*Eco*-): *Mse*-CAG/*Eco*-AC, *Mse*-CAG/*Eco*-TC, *Mse*-CAT/*Eco*-AG, *Mse*-CAT/*Eco*-TA, *Mse*-CAT/*Eco*-TC, *Mse*-CTA/*Eco*-AT, *Mse*-CTA/*Eco*-TA, *Mse*-CTG/*Eco*-AC, *Mse*-CTG/*Eco*-AG, *Mse*-CTG/*Eco*-AT. Selective amplification products were separated and visualized using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). The size of AFLP fragments were estimated by means of Gene Scan software Version 2.1 (Applied Biosystems) using internal-lane size standards. AFLP markers were named by their corresponding primer pair combinations (M__E__), and their length in base pairs (superscript).

Microsatellites

Twenty-three microsatellite primer pairs obtained from genebanks coding sequences (Yu et al. 2000; Gaitán-Solís et al. 2002; Murray et al. 2002; Blair et al. 2003) showed polymorphism between the parental lines Xana and Cornell 49242 and were analyzed in the RILs. PCR reactions were performed according to the conditions described by their corresponding authors. The amplification products were resolved on 8% polyacrylamide gels with 1× TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA), stained with ethidium bromide and visualized under UV light. A 100-bp ladder (Amersham Biosciences) and the software PhotoCaptMw were used to measure the sizes of the amplification products. Microsatellites were named according to the respective authors (prefixes BM-, BMd-, Bng-, and PV-), followed by the estimated size of the fragment (superscript) when more than one locus was revealed with the same primer pair.

ISSR markers

A total of 20 ISSR primers showed polymorphism between the parental lines Xana and Cornell 49242 and were included in the map. PCR reactions were carried out in a total volume of 20 µl containing 50 ng of genomic DNA, 10 pmol of primer, 200 µl of each dNTP, 1× specific buffer, and 1 unit of *Taq* polymerase (Stoffel, Applied Biosystems). PCR amplification were performed under the following conditions: a hot start of 94°C for 5 min; followed by 35 cycles of denaturing at 94°C for 1 min; annealing at 45–55°C (depending on the primer) for 45 s; extension at 72°C for 80 s; and product extension for 6 min at 72°C. PCR products were resolved on 2% agarose gels (15 cm × 20 cm) run with 1× TAE buffer (40 mM Tris–HCl, 40 mM acetic acid, and 1 mM EDTA), stained with ethidium bromide and visualized under UV light. A 100-bp ladder (Amersham Biosciences) and the software PhotoCaptMw were used to measure the sizes of the amplification products. ISSR markers were named using the respective primer followed by the estimated size of the fragment (superscript).

SCAR markers

The following ten SCAR markers, previously described in common bean, were found to be polymorphic for the parent genotypes Xana and Cornell 49242: SBC6, SW13, SBA8, SW12, ROC11, SAS8, SI19, SBB14, Scareoli (for references see http://www.css.msu.edu/bic/) and, Bng21 (Murray et al. 2002). In addition, 19 SCAR markers developed by Pañeda et al. (2005), most of them obtained from RAPD fragments mapped by Rodríguez-Suárez et al. (2007), were included in this map: SAS15, SO15, SG5, SZ13, SD3, SD8, SE15, SF8, SG14, SH8, SH13, SH15, SCG5, SH18, SZ4, SR20, SY4, SU8, and SI19b (Pañeda et al. 2008). PCR reactions for the different markers were performed according to the conditions described by the corresponding authors. PCR products were resolved and visualized as indicated for the ISSR markers.

RAPD markers

A total of ten decamer primers obtained from Operon Technologies (Alameda, CA, USA) were used. PCR was performed in a 20-µl reaction solution according to Rodríguez-Suárez et al. (2007). PCR products were resolved and visualized as indicated for the ISSR markers. The name of each RAPD marker is derived from an 'O' prefix for Operon primers, the letters identifying the Operon kit, Operon primer number, and the approximate size (bp) of the marker (superscript).

Linkage analysis

Each marker was tested against the expected 1:1 segregation ratio using the Chi-square goodness-of-fit test. Markers showing highly significant deviation ($P < 0.01$) from this expectation were excluded from further analysis. The linkage analysis was performed on 104 RILs using the multipoint linkage analysis software JoinMap version 3.0 (van Ooijen and Voorrips 2001). The markers were sorted into distinct linkage groups using a LOD score of 2.5 as a linkage threshold with 0.25 as the maximum recombination

fraction. The loci order of each linkage group was established following the full multipoint analysis mapping method. Map distances (centiMorgans, cM) were calculated using the Kosambi mapping function. Linkage groups were identified by the presence of molecular markers common to previously published bean genetic maps (Vallejos et al. 1992; Nodari et al. 1993; Adam-Blondon et al. 1994; Freyre et al. 1998; Geffroy et al. 1998; Miklas et al. 2000, 2002; Blair et al. 2003; Kelly et al. 2003; Rodríguez-Suárez et al. 2007; Pañeda et al. 2008).

QTL mapping

Quantitative trait loci were located on the genetic map using QTL Cartographer V2.5 (Wang et al. 2005). QTLs were detected by composite interval mapping analysis (CIM) using a window size of 2 cM (walkspeed). A LOD score of 2.5 was used as the threshold to determine the presence of different QTLs. This value was estimated using a permutation analysis with 1,000 random data shuffles, as described by Churchill and Doerge (1994), to provide a genome-wide 0.05 significance level.

QTLs found at the same genomic location in different environments, and in the mean environment data, were considered a single QTL.

Results

Marker segregation analysis and map construction

After screening the parental lines Xana and Cornell 49242, 10 AFLP primer combinations, 23 microsatellite primer pairs, 29 SCAR primer pairs, 20 ISSR primers, and 10 RAPD primers, were selected for analysis of the RIL population. A total number of 481 polymorphic amplification fragments were analyzed. The segregation of 126 of these fragments (26.2%) deviated significantly from the expected 1:1 ratio, and they were not included in the linkage analysis. The 355 fragments that fit the expected 1:1 ratio generated 324 loci: 293 fragments showed the segregation corresponding to dominant loci (presence vs. absence of the fragment), and 31 pairs of fragments showing the co-segregation corresponding to codominant loci (presence in each RIL of only one of the two fragments of the pair). These 324 loci included 207 (202 dominant and 5 codominant) AFLP loci, 30 (16 dominant and 14 codominant) microsatellite loci, 40 (36 dominant and 4 codominant) ISSR loci, 30 (22 dominant and 8 codominant) SCAR loci, and 17 (all dominant) RAPD loci.

The seed protein polymorphisms analyzed were controlled by 18 loci (13 dominant and 5 codominant) including the phaseolin protein codominant locus *Pha* (Xana has the T type phaseolin, whereas Cornell 49242 has the S type). Four of the seed protein dominant loci (22.2%) deviated significantly from the 1:1 expectation and were not included in the linkage analysis.

The segregations of the four morphological loci analyzed (growth habit, gene *Fin*; seed coat shininess, gene *Asp*; seed coat color presence, gene *P*; resistance to BCMV, gene *I*) fit the expected 1:1 ratio.

Linkage analysis was performed on 104 F_7 RIL for 342 loci (306 dominant and 36 codominant). This resulted in the formation of 14 linkage groups with a number of loci ranging from 13 to 35, and 48 isolated loci (32 AFLP, 3 microsatellites, 7 ISSR, 5 RAPD, and 1 seed protein locus). The loci order of each linkage group was established following the full multipoint analysis mapping method. Later, correspondences of the 14 linkage groups obtained with the 11 linkage groups of the bean integrated linkage map (Freyre et al. 1998) were established using markers included in previous bean maps (Vallejos et al. 1992; Nodari et al. 1993; Adam-Blondon et al. 1994; Freyre et al. 1998; Geffroy et al. 1998; Miklas et al. 2000, 2002; Blair et al. 2003; Kelly et al. 2003; Rodríguez-Suárez et al. 2007; Pañeda et al. 2008) as anchor points. The resulting map (Fig. 2) includes 294 markers and covers 1,042 cM with an average marker distance of 3.53 cM.

QTL analysis

Figure 3 shows the distributions of the different evaluated quantitative traits in the RIL population. No significant deviations from the corresponding normal distributions were observed in all cases. Table 2 shows the phenotype for the different quantitative traits in the parents, Xana and Cornell 49242, as well as the corresponding mean and range values in the RIL population. Differences between the parents were significant in all cases except in the three phenological traits, days to flowering, days to end of flowering, and days to harvest as green pod. The comparison between the parents and the RILs showing the maximum and minimum values reveal the existence of significant transgressive segregants for phenological traits, and seed quality traits. Whereas in most seed size traits, transgressive segregants were not observed (Fig. 3).

Pearson phenotypic correlation coefficients for the ten quantitative traits found for trait pairs within the same group (phenological traits, seed size traits, and seed quality traits) were positive and significant in all cases (Table 3). Correlation coefficients for trait pairs belonging to different group were significant in only five cases: positive significant correlations were observed between days to maturity (DM) and seed length (SL), days to maturity (DM) and seed width (WI), and days to maturity (DM) and seed weight (SW), and negative significant correlations were

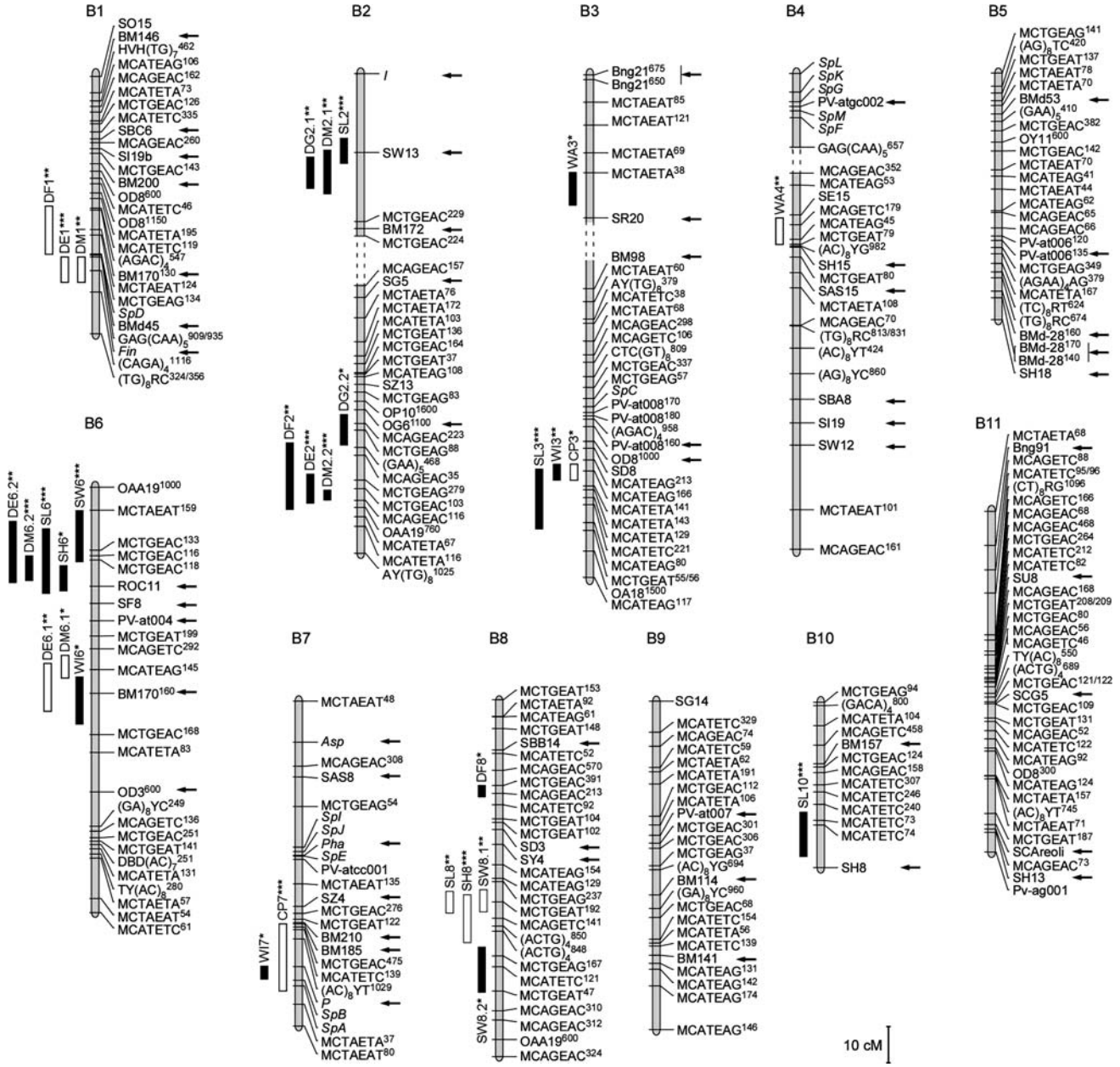

Fig. 2 Genetic linkage map for the RIL population developed from the cross Xana × Cornell 49242 showing the location of significant quantitative trait loci for days to flowering (DF), days to end of flowering (DE), days to harvest as green pod (DG), days to maturity (DM), seed length (SL), seed height (SH), seed width (WI), seed weight (SW), water absorption (WA), and coat proportion (CP). Vertical bars to the left of linkage groups indicate QTLs with a LOD value >2.5, detected in at least one environment and in the mean environment data. *Black and white vertical bars* represent QTLs in which the increase is supported by the alleles of Xana and Cornell 49242, respectively. AFLPs, microsatellites, ISSRs, SCARs, RAPDs, seed protein loci, and other qualitative trait loci are indicated with designations described in the text. *Arrows* indicate common loci to previously published maps (see text for references). The number of *asterisks* near the name of the QTL indicate the number of environments in which the QTL was detected

observed between seed width (WI) and coat proportion (CT), and between seed weight (SW) and coat proportion (CP).

Using CIM, a total of 72 different QTLs were detected (LOD >2.5) across all traits and the three environments. Of these, 50 (69.4%) were detected in only one environment, 11 (15.3%) were detected in two environments, and 11 (15.3%) were detected in the three environments. Of the 72 QTLs, 41 were not detected (LOD <2.5) using the mean environment data. These were considered further and were disregarded (data are available upon request). Table 4 shows the 31 QTLs that were significant using the mean environment data: 9 of them were detected in only one environment, 11 in two environments, and 11 in the three

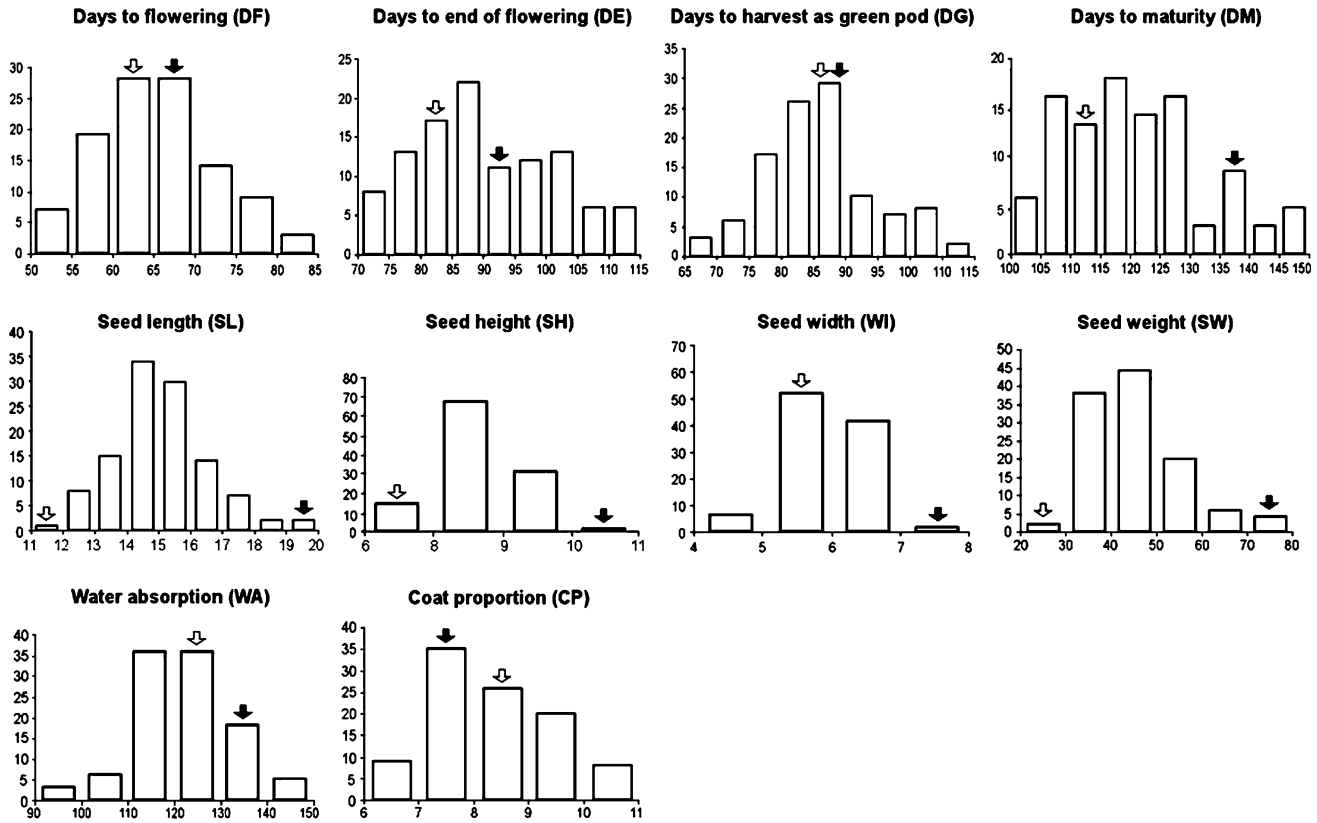

Fig. 3 Histograms showing the distributions (mean environment data) for the quantitative traits days to flowering, days to end of flowering, days to harvest as green pod, days to maturity, seed length, seed height, seed width, seed weight, water absorption, and coat proportion in the recombinant inbred lines derived from the cross Xana × Cornell 49242. *Black and white arrows* indicate the phenotypic values of parents Xana and Cornell 49242, respectively

environments. The length and the relative positions of these QTLs in the Xana/Cornell 49242 map are shown in Fig. 2. Significant QTLs were detected in all linkage groups except B5, B9, and B11. The number of significant QTLs for each individual trait ranged between 2 and 5. Considering the mean environment data, the amount of phenotypic variation (R^2) explained by the individual QTLs ranged from 7.3 to 55.3%.

Phenological traits

Three QTLs were detected for days to flowering (DF). One of them (DF1) was located in linkage group B1, the allele contributed by Xana decreasing DF. A second QTL (DF2) was located in linkage group B2, its increase in DF being produced by the allele contributed by Xana. The third QTL (DF8) was located in linkage group B8, the allele contributed by Xana increasing DF. Considering the mean environment data, the three QTLs, together, accounted for 85.5% of the phenotypic variation for days to flowering.

Four QTLs were detected for days to end of flowering (DE). One of them (DE1), in which the allele of Xana decreased the DE, was located in linkage group B1, in a position close to the QTL for days to flowering DF1. A second QTL (DE2) was located in linkage group B2, in a position close to the QTL for days to flowering DF2, its increase in DE being produced by the allele contributed by Xana. The remaining two QTLs detected for DE (DE6.1 and DE6.2) were located in B6 at relatively distant positions from one another. In these two QTLs, the allele contributions had opposite directions: in DE6.1 the allele contributed by Xana decreased DE, whereas in DE6.2 it increased DE. Considering the mean environment data, the four QTLs, together, accounted for 56.3% of the phenotypic variation for days to end of flowering.

Two QTLs were detected for days to harvest as green pod (DG). The QTLs (DG2.1 and DG2.2) were located in B2 at a relatively long distance from one another. In these two QTLs, the allele contributions had the same direction, with the alleles contributed by Xana increased DG. The QTL (DG2.2) was located in a position close to a QTL for days to flowering (DF2) and to a QTL for days to end of flowering (DE2). Considering the mean environment data, the two QTLs, together, accounted for 45.6% of the phenotypic variation for days to harvest as green pod.

Table 2 Mean values of the evaluated quantitative traits in the parents Xana and Cornell 49242 and in the RIL population

Trait	Parents			RILs				
				Mean	Range			
	Xana	Cornell 49242	t^a		Max.	t^b	Min.	t^c
Phenological traits								
Days to flowering	60.4 ± 0.8	57.4 ± 1.6	n.s.	60.4 ± 0.8	86.8 ± 5.6	*	46.0 ± 1.6	*
Days to end of flowering	85.6 ± 0.6	78.9 ± 2.3	n.s.	85.1 ± 1.1	111.0 ± 0.1	*	57.3 ± 4.2	*
Days to harvest as green pod	77.9 ± 2.6	77.6 ± 1.0	n.s.	80.3 ± 0.8	103.5 ± 6.5	*	62.4 ± 2.7	*
Days to maturity	130.2 ± 8.9	103.6 ± 2.7	*	115.4 ± 1.2	150.0 ± 0.9	*	92.5 ± 4.5	*
Seed size traits								
Seed length	22.4 ± 0.2	9.6 ± 0.1	**	14.1 ± 0.2	19.4 ± 0.3	*	11.4 ± 0.1	n.s.
Seed height	9.5 ± 0.1	6.6 ± 0.0	**	7.7 ± 0.1	8.9 ± 0.1	*	6.3 ± 0.1	*
Seed width	7.4 ± 0.1	5.5 ± 0.1	**	5.9 ± 0.1	7.1 ± 0.1	*	4.3 ± 0.1	**
Seed weight	85.3 ± 0.6	23.6 ± 0.1	**	45.2 ± 0.1	73.8 ± 0.2	*	30.9 ± 0.3	n.s.
Seed quality traits								
Water absorption	124.4 ± 3.4	114.5 ± 1.8	*	110.4 ± 3.2	136.5 ± 2.6	*	66.8 ± 3.3	**
Coat proportion	7.7 ± 0.2	8.0 ± 0.1	*	8.3 ± 1.2	12.6 ± 0.2	**	6.6 ± 0.1	**

[a] Comparison (t) between the parents

[b] Comparison between the RIL showing the maximum value and the parent showing the higher value

[c] Comparison between the RIL showing the minimum value and the parent showing the lower value

n.s. not significant ($P > 0.05$); * $0.01 > P < 0.05$; ** $P < 0.01$

Table 3 Pearson phenotypic correlation coefficients for ten quantitative traits using the mean environment data on 104 RILs derived from the cross Xana × Cornell 49242

Trait		Phenological traits				Seed size traits				Seed quality traits	
		DF	DE	DG	DM	SL	SH	WI	SW	WA	CP
Phenological traits											
Days to flowering	DF										
Days to end of flowering	DE	0.83**									
Days to harvest as green pod	DG	0.65**	0.72**								
Days to maturity	DM	0.67**	0.75**	0.74**							
Seed size traits											
Seed length	SL	n.s.	n.s.	n.s.	0.34**						
Seed height	SH	n.s.	n.s.	n.s.	n.s.	0.38**					
Seed width	WI	n.s.	n.s.	n.s.	0.21*	0.73**	0.49**				
Seed weight	SW	n.s.	n.s.	n.s.	0.31**	0.83**	0.74**	0.57**			
Seed quality traits											
Water absorption	WA	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
Coat proportion	CP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	−0.47**	−0.31**	0.23*	

n.s. not significant ($P > 0.05$); * $0.01 > P < 0.05$; ** $P < 0.01$

Five QTLs were detected for days to maturity (DM). One of them (DM1), in which the allele of Xana decreased the DM, was located in linkage group B1, in a position close to the QTL for days to flowering, DF1, and having the same location and length as the QTL for days to end of flowering, DE1. Two QTLs (DM2.1 and DM2.2), were located in B2 at relatively distant positions from one another, the alleles contributed by Xana increasing DM in both cases. DM2.1 was located close to the QTL for days to harvest as green pod, DG2.1. DM2.2 was located close to the above-mentioned QTLs, DF2, DE2, and DG2.2. The two QTLs DM6.1 and DM6.2 mapped to B6, and were located close to DE6.1 and DE6.2, QTLs for days to flowering. In these two QTLs, the allele contributions had

Table 4 Quantitative trait loci for phenological traits, seed size traits and seed quality traits, identified in at least one environment and in the mean environment data, by composite interval mapping analysis of the RILs derived from the cross Xana × Cornell 49242

QTL name	LG	Closest marker[a]	Environment									Mean environment data		
			Villaviciosa 2004			Villaviciosa 2005			Villaviciosa 2006					
			LOD	Percent var.[b]	Add. effect[c]	LOD	Percent var.[b]	Add. effect[c]	LOD	Percent var.[b]	Add. effect[c]	LOD	Percent var.[b]	Add. effect[c]
Phenological traits														
Days to flowering														
DF1	B1	BM170[130]	6.1	46.5	−8.44	4.6	14.9	−3.54	n.s.	–	–	7.1	55.3	−8.17
DF2	B2	MCTGEAG[279]	2.8	17.8	4.46	n.s.	–	–	3.8	11.0	2.45	3.8	19.2	4.96
DF8	B8	MCAGEAC[213]	n.s.	–	–	5.2	18.7	3.91	n.s.	–	–	3.0	11.0	2.69
Total[d]													85.5	
Days to end of flowering														
DE1	B1	Fin	5.2	14.1	−5.87	4.9	17.4	−4.61	7.6	22.1	−6.64	10.2	28.5	−6.07
DE2	B2	MCAGEAC[116]	4.2	10.7	2.78	3.1	8.7	4.39	6.3	17.3	5.74	5.2	12.1	3.89
DE6.1	B6	BM170[160]	2.7	7.9	−3.25	n.s.	–	–	3.4	12.1	−5.17	2.6	7.3	−3.27
DE6.2	B6	MCTGEAC[116]	2.5	6.8	3.10	n.s.	–	–	3.7	9.4	4.37	3.5	8.4	3.12
Total[d]													56.3	
Days to harvest as green pod														
DG2.1	B2	SW13	3.8	30.7	4.75	4.4	31.6	5.57	n.s.	–	–	4.8	33.7	4.75
DG2.2	B2	MCTGEAG[88]	3.1	11.1	2.75	n.s.	–	–	n.s.	–	–	3.2	11.9	2.85
Total[d]													45.6	
Days to maturity														
DM1	B1	Fin	4.4	12.3	−4.10	n.s.	–	–	3.7	9.1	−4.02	4.4	12.3	−4.22
DM2.1	B2	SW13	3.6	22.4	5.60	4.2	25.8	6.67	n.s.	–	–	3.8	24.3	5.70
DM2.2	B2	MCAGEAC[116]	5.3	18.8	4.58	3.3	14.1	4.93	7.2	19.2	5.56	6.3	17.5	4.98
DM6.1	B6	MCATEAG[145]	n.s.	–	–	n.s.	–	–	3.5	9.8	−4.26	4.1	11.2	−4.37
DM6.2	B6	MCTGEAC[118]	7.4	20.9	5.13	2.6	10.2	4.16	7.8	19.7	6.35	7.6	22.1	6.14
Total[d]													87.4	
Seed size traits														
Seed length														
SL2	B2	SW13	3.1	14.8	0.58	4.1	15.8	0.75	3.1	16.7	0.63	3.2	12.1	0.56
SL3	B3	MCATETC[221]	3.4	11.2	0.50	2.9	11.1	0.60	5.2	14.3	0.56	5.2	12.0	0.65
SL6	B6	ROC11	3.5	14.3	0.57	6.1	22.3	0.82	4.8	13.4	0.56	4.8	15.4	0.61
SL8	B8	MCTGEAT[192]	5.3	17.1	−0.99	3.6	18.7	−0.90	n.s.	–	–	3.4	20.2	−0.77
SL10	B10	MCATETC[73]	3.0	11.0	0.52	3.0	12.3	0.64	3.6	10.0	0.47	3.3	14.1	0.45
Total[d]													73.8	
Seed height														
SH6	B6	ROC11	n.s.	–	–	n.s.	–	–	4.9	13.1	0.25	7.8	21.8	0.30
SH8	B8	(ACTG)4[850]	3.1	24.6	−0.35	2.6	20.7	−0.34	3.5	22.8	−0.34	4.1	24.9	−0.32
Total[d]													46.7	
Seed width														
WI3	B3	MCATEAG[166]	3.6	11.1	0.24	4.2	13.7	0.26	n.s.	–	–	4.5	13.7	0.25
WI6	B6	BM170[160]	2.6	9.2	0.24	n.s.	–	–	n.s.	–	–	2.6	14.2	0.24
WI7	B7	P	4.0	13.3	0.26	n.s.	–	–	n.s.	–	–	3.5	12.1	0.21
Total[d]													40.0	

Table 4 continued

QTL name	LG	Closest marker[a]	Environment									Mean environment data		
			Villaviciosa 2004			Villaviciosa 2005			Villaviciosa 2006					
			LOD	Percent var.[b]	Add. effect[c]	LOD	Percent var.[b]	Add. effect[c]	LOD	Percent var.[b]	Add. effect[c]	LOD	Percent var.[b]	Add. effect[c]
Seed weight														
SW6	B6	MCGEAC[116]	8.8	23.1	0.65	5.0	19.0	0.49	5.5	14.5	0.43	6.4	17.8	0.49
SW8.1	B8	MCTGEAT[192]	3.6	17.2	−0.58	n.s.	–	–	2.8	16.0	−0.46	5.0	21.9	−0.58
SW8.2	B8	MCTGEAG[167]	2.9	11.5	0.54	n.s.	–	–	n.s.	–	–	3.9	14.3	0.54
Total[d]													54.0	
Seed quality traits														
Water absorption														
WA3	B3	MCTAETA[38]	n.s.	–	–	2.9	14.9	5.19	n.s.	–	–	3.7	16.3	4.99
WA4	B4	MCATEAG[45]	3.6	13.1	−5.41	4.8	20.3	−5.72	n.s.	–	–	2.7	10.0	−3.86
Total[d]													26.3	
Coat proportion														
CP3	B3	MCATEAG[166]	2.6	8.6	−0.32	n.s.	–	–	n.s.	–	–	2.7	8.1	−0.36
CP7	B7	P	7.2	23.9	−0.63	8.4	27.6	−0.79	6.4	23.9	−0.68	7.5	27.5	−0.65
Total[d]													35.6	

[a] Closest marker is the marker nearest to the peak LOD score

[b] Percent variance: the amount of phenotypic variation explained by each QTL

[c] Additive effect: value of the female parent allele (Xana)

[d] The amount of phenotypic variation explained by all significant QTLs found for a trait

n.s. not significant (LOD <2.5)

opposite directions: in DM6.1 the allele contributed by Xana decreased DM, whereas in DM6.2 it increased DM. Considering the mean environment data, the five QTLs together accounted for 87.4% of the phenotypic variation for days to maturity.

Seed size traits

Five QTLs were detected for seed length (SL). One of these (SL2) was located on linkage group B2, overlapping the QTLs DG2.1 and DM2.1. A second QTL (SL3) was located in linkage group B3 and a third one (SL6) was located in linkage group B6, overlapping the QTLs DE6.2 and DM6.2. The QTL SL8 mapped to B8. A fifth QTL (SL10) was located in linkage group B10. The alleles contributed by Xana for QTLs SL2, SL3, SL6 and SL10 increased SL, whereas that of SL8 decreased SL. Considering the mean environment data, the five QTLs together accounted for 73.8% of the phenotypic variation for seed length.

Two QTLs were detected for seed height (SH). One of them (SH6), in which the allele of Xana increased SH, mapped to B6, overlapping the QTLs SL6, DE6.2 and DM6.2. A second QTL (SH8), in which the allele of Xana decreased SH, mapped to B8, overlapping the QTL SL8. Considering the mean environment data, the two QTLs, together, accounted for 46.7% of the phenotypic variation for seed height.

Three QTLs were detected for seed width (WI). One of them (WI3), was located in linkage group B3, in a position close to the QTL for seed length, SL3. A second QTL (WI6) mapped to B6, overlapping the QTLs, DE6.1 and DM6. A third QTL (WI7) mapped to B7. The alleles contributed by Xana of QTLs, WI3, WI6, and WI7, increased WI. Considering the mean environment data, the three QTLs, together, accounted for 40.0% of the phenotypic variation for seed width.

Three QTLs were detected for seed weight (SW). The QTL SW6, in which the allele of Xana increased SW, mapped to B6, overlapping the QTLs, SL6, SH6, DE6.2, and DM6.2. The remaining two QTLs detected for SW (SW8.1 and SW8.2) were located in the linkage group B8: SW8.1 overlapped the QTLs SL8, and SH8 while SW8.2 was located in a position close to SW8.1. In these two QTLs, the allele contributions had opposite directions: in SW8.1 the allele contributed by Xana decreased SW, whereas in SW8.2 increased SW. Considering the mean environment data, the three QTLs together accounted for 54.0% of the phenotypic variation for seed weight.

Seed quality traits

Two QTLs were detected for water absorption (WA). One of these (WA3), was located in linkage group B3, the allele contributed by Xana increasing WA. The second QTL (WA4) was located in linkage group B4, with a decrease in WA being produced by the allele contributed by Xana. Considering the mean environment data, the two QTLs cumulatively accounted for 26.3% of the phenotypic variation for water absorption.

Two QTLs were detected for coat proportion (CP). One (CP3) mapped to B3, overlapping the QTLs SL3 and WI3. A second QTL (CP7) mapped to B7 and overlapped the QTL WI7. The alleles contributed by Xana for QTLs CP3 and CP7 decreased CP. Considering the mean environment data, together the two QTLs accounted for 35.6% of the phenotypic variation for coat proportion.

Discussion

The bean linkage map developed to date having the highest saturation degree is most likely that of Freyre et al. (1998), a core map assembled using the linkage maps of Vallejos et al. (1992), Nodari et al. (1993) and Adam-Blondon et al. (1994), that includes 563 markers arranged in 11 linkage groups, and spans 1,226 cM. The linkage map developed in the present study (Fig. 2) includes 294 markers, arranged in 11 linkage groups, and covers 1,042 cM, with an average marker distance of 3.53 cM. The genetic length as measured by cM of the present map compares well (85%) with that of the core map of Freyre et al. (1998). In its construction, 51 loci (46 molecular marker loci, 4 qualitative trait loci and the locus for the seed protein Phaseolin) included in previously published bean linkage maps (Vallejos et al. 1992; Nodari et al. 1993; Adam-Blondon et al. 1994; Freyre et al. 1998; Geffroy et al. 1998; Miklas et al. 2000, 2002; Blair et al. 2003; Kelly et al. 2003; Rodríguez-Suárez et al. 2007; Pañeda et al. 2008) were used as anchor points. In almost all cases (48 out of 50), the within-linkage group relative position of these loci is consistent with that of such maps. The exceptions are (Fig. 2) (1) the SCAR marker SBC6, described as linked to the rust resistance gene *Ur-6* (Park et al. 2003), and later suggested to be located in linkage group B11 (Park et al. 2004), is located in linkage group B1 in the current map; (2) the microsatellite BM172, located in linkage group B3 by Blair et al. (2003), is included in linkage group B2 in the current map; (3) Blair et al. (2003) mapped a locus for microsatellite marker BM170 on linkage group B6. In the present work, two loci were found for this microsatellite, one of them located in linkage group B6 (agreeing with Blair et al. 2003) and the other located in linkage group B1. The presence of some microsatellites amplifying more than one locus in common bean was detected by Blair et al. (2008) who indicated that duplicate loci could have resulted from segmental duplication events or similarity in primer sequences across more than one locus.

In the present study, of the 72 different QTLs detected across all traits and the three environments, 41 were detected in only one environment, and the remaining 31 were found to be significant in at least one environment and in the mean environment data. Inconsistent detection of QTLs has been reported in other studies in common bean (Tar'an et al. 2002; Beattie et al. 2003; Blair et al. 2006; Checa and Blair 2008). It seems likely that QTLs having a greater additive effect, and the corresponding greater explained variance, are more consistently detected across different environments. This tendency was observed by Tanksley (1993) and by Beattie et al. (2003), and could be noted in the present study (data are available upon request). High additive effect (and explained variance) and good consistency across different environments are critical attributes of QTL for success in developing marker-assisted breeding programs involving quantitative traits.

The 31 consistent QTLs found in the present work (Table 4; Fig. 2) map to 12 different chromosomal regions. In four cases (in linkage groups B3, B4, B8 and B10), such regions are occupied by single QTLs. Three regions are occupied by clusters including QTLs for traits belonging to the same group, phenological traits (in linkage groups B1 and B2) and seed size traits (in linkage group B8). The remaining five regions include clusters of QTLs for traits belonging to different groups based on phenology and seed size, on linkage groups B2 and B6 with seed size and coat proportion QTL being mapped to linkage groups B3 and B7. In most cases, the allelic effects of the QTLs having common or overlapping locations belonging to the same trait group have the same direction. This agrees with the positive correlations found between trait pairs within the same group (Table 3). Other studies in common bean have revealed QTLs controlling different traits mapping in the same chromosomal region (Tsai et al. 1998; Tar'an et al. 2002; Beattie et al. 2003; Blair et al. 2006). The clustering of QTLs can be explained by either the presence of different tightly linked genes or by pleiotropic effects of single genetic elements (Aastveit and Aastveit 1993). Pleiotropic effects can explain the present cases of clustered QTLs for traits belonging to the same group. This could also be the case in the clusters located in B3 and B7, in which the two QTLs found for coat proportion, CP3 and CP7, co-locate with the QTLs for seed width, WI3, and WI7, respectively. In both instances, the direction of the allelic effect of the QTLs for coat proportion is opposite to that in the QTLs for seed width. This is in agreement with the negative correlation found between these two traits (Table 3), and could be

explained as due to pleiotropic effects of a single genetic element. Obviously, the higher the seed width, the closer the seed shape approaches a sphere, which lowers the surface and coat proportions. Although it is difficult to imagine the physiological pathways supporting a pleiotropy that could explain the clustered organization of QTLs for phenology and seed size, this possibility cannot be disregarded. Interestingly, the two detected QTLs for water absorption, a trait apparently not related with the other analyzed traits, map in isolated locations in linkage groups B3 and B4.

The 14 mapped QTLs related to phenological traits (Table 4; Fig. 2) are clustered in 6 different locations in linkage groups B1, B2, B6, and B8. Three QTLs for days to flowering (DF1), days to end of flowering (DE1), and days to maturity (DM1), respectively, in which the increase in number of days is supported by the alleles of Cornell 49242, are clustered in linkage group B1, close to the *Fin* gene. This was expected due to the phenotypic effect of *Fin* conditioning indeterminate versus determinate growth habit. The recessive *fin* allele derived from Xana conditions determinate growth habit, the main stem ending its vegetative growth, and thus shortening the plant growing period. An association between quantitative phenological traits and the *Fin* gene in common bean was also observed by Koinange et al. (1996). Two QTLs for days to harvest as green pod (DG2.1), and days to maturity (DM2.1), respectively, in which the increase is supported by the alleles of Xana, are located near the upper end of linkage group B2, close to the *I* gene. This could also be expected due to the effect of the *I* gene. Cornell 49242 carries the dominant allele of this gene, conferring resistance to BCMV, whereas Xana is susceptible. The RILs having the allele proceeding from Xana would have a tendency towards a longer life period due to growing difficulties produced by the disease. The remaining detected QTLs for phenological traits could be loci involved in physiological aspects more directly related to growing period. They map in four locations, near the lower end of linkage group B2 (DF2, DE2, DG2.2, and DM2.2), near the upper end of linkage group B6 (DE6.2 and DM6.2), near the middle of linkage group B6 (DE6.1 and DM6.1), and in linkage group B8 (DF8), respectively. QTLs for phenological traits related to growing period have also been identified on linkage groups B1 and B8 by Koinange et al. (1996) in a RIL population from a cross between Midas (a cultivated common bean) and G12873 (a wild bean accession), on linkage groups B6 and B8 by Beattie et al. (2003) in a population of RILs derived from the cross WO3391 × OAC Speedvale, and on linkage groups B1, B2, and B6 by Blair et al. (2006) in an advanced backcross population derived from a cross between ICA Cerinza (a cultivated Andean bean) and G24404 (a wild common bean). In the present work, QTLs for phenological traits located near the lower end of linkage group B2, followed by QTLs located near the upper end of linkage group B6, show the highest additive effect (and corresponding percent variance) and the highest consistency across the three environments. The markers located in the lower end of linkage group B2 and near the upper end of linkage group B6 could be good candidates for assisting selection for traits related to growing period.

Seed size QTLs map in eight locations, near the upper end of linkage group B2 (SL2), near the lower end of linkage group B3 (SL3 and WI3), near the upper end of linkage group B6 (SL6, SH6 and SW6), near the center of linkage group B6 (WI6), near the lower end of linkage group B7 (WI7), near the center of linkage group B8 (SL8, SH8 and SW8.1), near the lower end of linkage group B8 (SW8.2), and near the lower end of linkage group B10 (SL10). Several of these QTLs are consistent with those detected in previous studies in common bean: Tsai et al. (1998) identified two QTLs for seed weight on linkage group B2 in F_3 families derived from the cross BAT-93 × Jalo EEP558, Park et al. (2000) found QTLs for seed length and seed weight on linkage groups B2, B3, B6, and B8 in a RIL population derived from the cross PC-50 × XAN-159, and Blair et al. (2006) found QTLs for seed weight on linkage groups B2, B3, B6, B8, and B10. The results obtained in the present work indicate that QTLs located near the center of linkage group B8, followed by QTLs located near the upper end of linkage group B6, show the highest additive effect (and corresponding percent variance) and the highest consistency across the three environments. The markers located in the center of linkage group B8 and near the upper end of linkage group B6, could be good candidates for assisting selection for traits related to seed size.

In summary, our research has identified several across-environment repeatable QTLs controlling important morpho-agronomic and seed quality traits in common bean. These QTLs were placed on a map consistent with the integrated linkage map for this species. Repeatability and map consistency are crucial QTL aspects to consider in the utilization of marker-assisted selection for complex traits.

Acknowledgments This work was supported by grants AGL2001-2676-C02 and AGL2007-66563-CO2-02 from the Ministerio de Ciencia y Tecnología, Spain. Elena Pérez-Vega and Ana Campa were recipient of salaries fellowship from the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA, Spain).

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