LEONARDO CORRÊA DA SILVA

LINKAGE FINE-MAPPING, GWAS AND QTLs AFFECTING MORPHO-AGRONOMIC TRAITS OF A COMMON BEAN RIL POPULATION

Thesis submitted to the Genetic and Breeding Graduate Program of the Universidade Federal de Viçosa, in partial fulfillment of the requirements for degree of *Doctor Scientiae*.

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Ao ***Eterno***,

por me ajudar a ser o autor

da minha própria história!

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BIOGRAFIA

LEONARDO CORRÊA DA SILVA, filho de Vianelo Martins da Silva e Ana Corrêa da Silva, nasceu em Visconde do Rio Branco, Minas Gerais, em 14 de Dezembro de 1985.

No município de Ubá, Minas Gerais, cursou o Ensino Primário na Escola Estadual Professor José Gonçalves Sollero, de 1992 a 1996; o Ensino Fundamental na Escola Estadual Coronel Camilo Soares, de 1997 a 2000; e o Ensino Médio na Escola Estadual Senador Levindo Coelho, de 2001 a 2003.

Em maio de 2006, iniciou o curso superior na Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais. Foi Bolsista de Atividade no Laboratório de Microbiologia de Alimentos, sob liderança da professora Maria Cristina Dantas Vanetti, de agosto de 2006 a fevereiro de 2007. Foi estagiário e bolsista de Iniciação Científica nos Laboratórios Bioquímica e Genética de Plantas, Biologia Molecular e Filogeografia, e Genética Molecular de Plantas, sob liderança dos professores Everaldo Gonçalves de Barros, de quem foi orientado, e Maurilio Alves Moreira (*in memorian*), de março de 2007 a julho de 2011. Colou grau em 22 de julho de 2011 como Engenheiro Agrônomo.

Em agosto de 2011, iniciou o curso de Mestrado no Programa de Pós-graduação em Fitotecnia da UFV, na área de Melhoramento de Plantas, Recursos Genéticos e Biotecnologia. Desenvolveu seu projeto de mestrado no Programa Feijão, sob liderança dos professores José Eustáquio de Souza Carneiro e Pedro Crescêncio Souza Carneiro. Defendeu a dissertação intitulada ‘Caracterização fenotípica de RIL’s de feijão derivadas da população Rudá x AND 277’ em 16 julho de 2013.

Em agosto de 2013, iniciou o curso de Doutorado no programa de Pós-graduação em Genética e Melhoramento da UFV. Desenvolveu seu projeto de doutorado também no Programa Feijão e em parceria com a Embrapa Arroz e Feijão, em Santo Antônio de Goiás, Goiás. Defendeu a tese intitulada ‘Linkage fine-mapping, GWAS and QTLs affecting morpho-agronomic traits of a common bean RIL population’ em 20 de julho de 2017.

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ABSTRACT

SILVA, Leonardo Corrêa da, D.Sc., Universidade Federal de Viçosa, July, 2017. **Linkage fine-mapping, GWAS and QTLs affecting morpho-agronomic traits of a common bean RIL population.** Adviser: José Eustáquio de Souza Carneiro. Co-advisers: Cosme Damião Cruz; Everaldo Gonçalves de Barros; Pedro Crescêncio Souza Carneiro and Thiago Lívio Pessoa Oliveira de Souza.

Common bean (*Phaseolus vulgaris* L.) is one of the most cultivated and consumed legumes worldwide. It is a relatively inexpensive source of protein and nutrients, establishing itself as an important food in maintaining food security on the world. In this sense, genetic breeding is essential to obtain more productive cultivars, with plant architecture more adequate to the harvesting systems, with a cycle suitable to the regions of production, and grain type compatible with the requirements of the local market. An auxiliary tool in plant breeding is the DNA marker-assisted selection. Linkage mapping (LM) is the most common approach to detect molecular markers associated to quantitative trait loci (QTL). The abundance of molecular markers in the genome of the species made of association mapping (AM) a new methodology to QTLs detection. An important association mapping (AM) methodology is the genome wide association study (GWAS). In this context, the Common Bean Breeding Program of the Universidade Federal de Viçosa (UFV) developed a population consisting of 376 RILs, obtained from the crossing between Rudá and AND 277, to construct a genetic map and detect QTLs related to seven morpho-agronomic traits using these two methodologies. Another objective was to know the biological function of these QTLs by their location in relation to candidate genes with biological functions that related to the traits of these QTLs. The population was genotyped with 3,098 SNP (single nucleotide polymorphism) markers and phenotyped in the field conditions for the trais number of days to flowering (DF) and to maturity (DM), plant architecture (ARC), seed yield (YLD), degree of seed flatness (SF), seed shape (SS), and 100- seed weight (SW). A genetic map with 1,962 SNPs, spanning a total size of 1,065.48 cM, was obtained by linkage analysis. In addition, 29 QTLs were detected for the seven characteristics distributed on the 11 chromosomes, which explained from 3.83 to 32.92% of the phenotypic variation. In gene annotation, four sequences of SNPs identified as linked to QTLs were related to 18 genes with known biological function. 112 SNPs/QTLs related to the traits evaluated were detected in all chromosomes by genome wide association study (GWAS), except to chromosomes 06 and 07. Some of these QTLs were

positioned near or within candidate genes with biological function that were related to the morpho-agronomic traits evaluated. It is concluded that the population size of RA RILs (376 lines) allowed to obtain a genetic map with accurate estimates of recombination frequency. The number of markers used in this study provided good saturation in all chromosomes, which allowed the efficiently and reliably QTL detection by linkage mapping and GWAS. The candidate genes located in the regions of these QTLs corroborate their potential in the marker-assisted selection for these seven morpho-agronomic traits.

RESUMO

SILVA, Leonardo Corrêa da, D.Sc., Universidade Federal de Viçosa, julho de 2017. **Mapeamento fino, GWAS e QTLs relacionados a características morfo-agronômicas de uma população de RILs de feijão comum.** Orientador: José Eustáquio de Souza Carneiro. Coorientadores: Cosme Damião Cruz; Everaldo Gonçalves de Barros; Pedro Crescêncio Souza Carneiro e Thiago Lívio Pessoa Oliveira de Souza.

O feijão comum (*Phaseolus vulgaris* L.) é uma das leguminosas mais cultivadas e consumidas em todo o mundo. É uma fonte relativamente barata de proteínas e nutrientes, firmando-se como um importante alimento na manutenção da segurança alimentar no planeta. Nesse sentido, o melhoramento genético é fundamental para a obtenção de cultivares mais produtivas, com arquitetura de plantas mais adequada aos sistemas de colheita, com ciclo compatível com às regiões de produção e de aspecto de grãos que atenda às exigências do mercado consumidor. Uma ferramenta auxiliar no melhoramento genético de plantas é a seleção assistida por marcadores moleculares do DNA. O mapeamento de ligação (*Linkage Mapping*, LM) é a abordagem mais comum no melhoramento para detectar marcadores moleculares associados a QTLs (*Quantitative Trait Loci*, ou locos controladores de características quantitativas). A abundância de marcadores no genoma das espécies fez do mapeamento de associação (*Association Mapping*, AM) uma nova estratégia pra detecção de QTLs. Uma importante metodologia do mapeamento de associação é o estudo de associação genômica ampla (*Genome Wide Association Study*, GWAS). Neste contexto, o Programa de Melhoramento do Feijoeiro da Universidade Federal de Viçosa (UFV) desenvolveu uma população formada por 376 RILs (*Recombinant Inbred Lines*, ou linhagens endogâmicas recombinantes) de feijão comum, obtidas do cruzamento entre Rudá e AND 277, para a obtenção de um mapa genético e detecção de QTLs relacionados a sete características morfo-agronômicas usando essas duas metodologias. Essa população foi denominada de RILs RA. Outro objetivo foi conhecer a função biológica destes QTLs pela sua localização em relação a genes candidatos com função biológicas que se relacionassem às características destes QTLs. A população foi genotipada com 3.098 marcadores do tipo SNP (*Single Nucleotide Polymorphism*, polimorfismo a partir de um único nucleotídeo) e fenotipada em campo para as características número de dias até o florescimento (DF) e até a maturação (DM), arquitetura de plantas (ARC), produtividade de grãos (YLD), grau de achatamento (SF)

da semente, forma da semente (SS) e massa de cem grãos (SW). Pelo mapeamento de ligação (LM), foi obtido uma mapa genético com 1.962 SNPs e tamanho total de 1.065,48 cM. Também foram detectados 29 QTLs, para as sete características, distribuídos nos 11 cromossomos, que explicaram de 3,83 a 32,92% da variação fenotípica. Na anotação gênica, quatro sequências de SNPs identificados como ligados aos QTLs foram relacionados a 18 genes com função biológica conhecida. Pelo estudo de associação genômica ampla (GWAS), foram detectados 112 SNPs/QTLs em todos os cromossomos, com exceção dos cromossomos 06 e 07, relacionados a todas as características avaliadas. Alguns destes QTLs estavam posicionados próximos ou dentro de genes candidatos com função biológica que se relacionava com as características morfo-agronômicas avaliadas. Conclui-se que o tamanho da população de RILs RA (376 linhagens) permitiu a obtenção de um mapa genético com estimativas de frequência de recombinação acurada. O número de marcadores utilizados propiciou boa saturação em todos os cromossomos, o que permitiu a detecção de QTLs com mais eficiência e confiabilidade pelo mapeamento de ligação e pela GWAS. Os genes candidatos localizados nas regiões destes QTLs corroboram o potencial destes na seleção assistida por marcadores moleculares para as características morfo-agronômicas avaliadas.

GENERAL INTRODUCTION

The process of detecting the position and distance between molecular markers on chromosomes is called linkage mapping (LM) or genetic mapping, and the resulting product a genetic map. This mapping process is based on the principle that the genes and markers contained in the DNA molecule segregate by chromosomal recombination or crossing-over during meiosis and can be analyzed in the progenies (Paterson 1996). One of the main applications of genetic maps is the identification of chromosomal regions containing genes and QTLs (Quantitative Trait Loci) related to traits of agronomic interest (Collard et al. 2005).

The first genetic maps of common bean (Phaseolus vulgaris L.), published by Lamprecht (1961) and Vallejos et al. (1992), were based on phenotypic and molecular DNA markers, respectively. Other DNA marker-based maps were developed by Nodari et al. (1993) and Adam-Blondon et al. (1994). To align these last three maps, a genetic map called Core Linkage Map was constructed of a population consisting of 78 Recombinant Inbred Lines (RILs) derived from the cross BAT 93 × Jalo EEP558, known as BJ population in the scientific community (Freyre et al. 1998).

For genetic mapping in plants, different population types with specific characteristics can be used, according to the researcher's objectives. These can be backcross, F2 and Fn populations, populations derived by selfing from the F2 generation, or RIL, double haploid (Schuster and Cruz 2004), and NIL (Nearly Isogenic Line) populations (Semagn et al. 2006). However, the parents of the mapping population must be genetically contrasting, to allow the identification of polymorphic markers and subsequent construction of the genetic map. Once polymorphic markers are identified, they can be used to genotype the entire population, including the parents (Collard et al. 2005).

Since RILs consist of homozygous plants only, they can be multiplied and repeatedly evaluated in different environments, so that the traits to be mapped can be estimated more precisely (Schuster and Cruz 2004). Recombinant inbred lines are reported in the literature, some derived from parents of the same gene pool (Jung et al. 1996; Jung et al. 1997; Cichy et al. 2009; Galeano et al. 2009; Blair et al. 2011; Blair et al. 2012; Yuste-Lisbona et al. 2012) and others from different gene pools (Freyre et al.

1998; Blair et al. 2003; Frei et al. 2005; Ochoa et al. 2006; Blair et al. 2009; Pérez-Vega et al. 2010).

The markers used for genetic mapping include phenotypic, isoenzymatic, RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeat, also called microsatellites), Sequence Characterized Amplified Regions (SCAR), and Sequence-Tagged Site (STS) markers.

With the advent of Next-Generation Sequencing (NGS), the development of SNP markers became a common practice in the scientific community (Galeano et al. 2009; Shi et al. 2011; Souza et al. 2011). Single nucleotide polymorphism (SNP) markers are based on the detection of polymorphisms resulting from the alteration of a single base in homologous regions in the genome. To be considered a SNP, this change must occur in at least 1% of the analyzed population. These markers are bi-allelic and abundant in the eukaryotic genome and can occur in both expressed and non-expressed regions. They allow the construction of high-resolution genetic maps, since the SNP density can be measured at the kilobase scale, while most of the current genetic maps were developed at the megabase scale (Caixeta et al. 2006; Hyten et al. 2010).

Although SNP detection is relatively expensive, especially for species with still incomplete genome sequencing, the high-throughput methods developed for SNP genotyping reduced genotyping costs, making the technique more widely accessible for research (Hyten et al. 2010). Thus, these markers are promising for the development of saturated genetic maps of populations from different gene pools and even of those of a same pool, in which genetic variability is lower.

An alternative approach to linkage mapping (LM) in the detection of QTLs is association mapping (AM). This technique is based on the existence of natural populations or planned plant populations, aside from the biparental populations, and requires no linkage maps (Pasam et al. 2012). One of the most commonly used AM methodologies is the Genome-Wide Association Study (GWAS). This technique depends on a high number of genome-wide distributed markers, generating an increased probability that the QTLs of interest are in strong linkage disequilibrium with the markers and thus detected (Rafalski et al. 2010). The abundance of DNA markers in the common bean genome indicates GWAS as a potential tool for marker-assisted breeding (Cichy et al. 2015; Moghaddam et al. 2016).

Although GWAS of unstructured populations allows a better understanding of the different allelic forms involved in the trait control, biparental populations are still the most indicated to study rare alleles involved in the genetic control of some traits, mainly of disease resistance (Rafalski et al. 2010). According to Casañas et al. (2013), a combination of mapping methods such as GWAS with the gene expression study is essential to confirm QTLs detected by linkage mapping (LM) in biparental populations.

Several QTLs related to morpho-agronomic traits in common bean have been reported, including QTLs for days to flowering and to maturity (Tar'an et al. 2002; Beattie et al. 2003; Pérez-Vega et al 2010a), plant architecture (Tar et al. 2002; Blair et al. 2006), traits related to seed size and chemical composition (Park et al. 2000, Cichy et al. 2009a; Cichy et al. 2009b; Yuste-lisbona et al. 2014), and to grain yield (Blair et al. 2006; Blair et al. 2012. Other QTLs for resistance to the major diseases-causing pathogens of common bean were detected, e.g., for white mold (Sclerotinia sclerotiorum) (Kolkman and Kelly 2003; Maxwel et al. 2007), angular leaf spot (Pseudocercospora griseola) (Oblessuc et al. 2012), and anthracnose (Colletotrichum lindemuthianum) resistance (González et al. 2015).

By sequencing the common bean genome (Schmutz et al. 2014), each QTL detected can be physically located in the genome. The QTL region may be in or close to, in linkage disequilibrium, of a candidate gene. A candidate gene is a sequenced gene with a known biological function, i.e., it is directly or indirectly involved in the phenotypic expression (Cichy et al. 2015; Hoyos-Villegas et al. 2015; Moghaddam et al. 2016).

Data banks such as Phytozome (Goodstein et al. 2012), where plant genome data are deposited, have been used for the physical location of QTLs in the genome and the search for candidate genes (Perseguine et al. 2016; Zuiderveen et al. 2016). In addition, the functionality of these genes can be identified in studies of the respective species or by comparisons with others. In such comparisons, the gene of the species under study is considered a candidate gene when its sequence has a high degree of homology to a gene characterized in another species, for example in Arabidopsis thaliana. Moghaddam et al. (2016) identified a QTL for plant architecture within the genomic region of the candidate gene Phvul.007G246700, on P. vulgaris chromosome 07 (Pv07), whose Arabidopsis homolog (AtPME4I) encodes an enzyme involved in altering cell wall

rigidity, which may therefore be involved in breeding plants with a more erect architecture.

Some candidate genes close to QTLs related to phenological traits have also been reported for common bean. Gene Phvul.001G221100 on chromosome 11 (Pv11), close to the QTL for number of days to flowering, is homologous to phyA (Phytochrome A) and GmPhyA3 genes, involved in photoperiod sensitivity and flowering of Arabidopsis and soybean (Glycine max), respectively (Kamfwa et al. 2015a). The gene Phvul.011G158300, close to QTL for number of days to harvest, is homologous to the SHL gene of Arabidopsis, involved in flowering and senescence (Moghaddam et al. 2016). Other QTLs close to or within regions of candidate genes were reported as related to drought tolerance mechanisms (Hoyos-Villegas et al. 2015), cooking time (Cichy et al. 2015), seed weight (Moghaddam et al. 2016), symbiotic nitrogen fixation (Kamfwa et al. 2015b), and resistance to pathogens causing anthracnose and angular leaf spot in common bean (Perseguine et al. 2014; Zuiderveen et al. 2016).

The size of the RIL populations developed so far for genetic mapping and QTL detection ranged from 70 (Jung et al., 1997) to 185 RILs (Yuste-Lisbona et al., 2012) and are considered small, with exception of a 346-RIL population described by Oblessuc et al (2012). Small mapping populations affect the accuracy of recombination estimates between loci and the detection and estimation of QTL effects (Collard et al. 2005; Casañas et al. 2013). In a simulation study, Silva et al. (2007) reported that populations of 200, 300 and 500 RILs, respectively, are required to obtain reliable maps with a high (5 cM mean distance between markers), medium (10 cM mean distance between markers) and low (20 cM mean distance between markers) genome saturation level.

In this context, the Common Bean Breeding Program of the Universidade Federal de Viçosa (UFV) developed a RIL population of approximately 500 lines derived from the cross between Rudá and AND 277, currently in the F10 generation, named RA RILs (Sanglard et al.2013; Silva et al. 2016). This cross was promising because it involved parents from different gene pools, making it possible to obtain a population with broad genetic variability, as described by Silva et al. (2016).

The objective of this study was to construct a saturated genetic map using SNP markers of the RA RILs to map QTLs linked to morpho-agronomic traits of this

common bean population by linkage mapping (LM) and association mapping (AM) or genome-wide association studies (GWAS), and to identify candidate genes related to these QTLs.

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CHAPTER I

Linkage fine-mapping and QTLs affecting morpho-agronomic traits of a Mesoamerican × Andean RIL common bean population

Abstract

This paper proposes the construction of a genetic linkage map with 376 recombinant inbred lines (RILs) derived from a cross between Mesoamerican × Andean common bean (*Phaseolus vulgaris* L) parents based on single nucleotide polymorphism (SNP) markers; and to detect quantitative trait loci (QTLs) associated with seven morpho-agronomic traits: number of days to flowering (DF), number of days to maturity (DM) or crop cycle; plant architecture (ARC); seed yield (YLD); degree of seed flatness (SF); seed shape (SS); and 100-seed weight (SW). A total of 3,060 polymorphic SNP markers were used and 2,041 segregated at a 1:1 ratio in the RIL population, as expected. These markers were subjected to linkage analysis in each chromosome. The genetic linkage analysis resulted in linkage maps with a total of 1,962 markers spanning 1,079.21 cM. A total of 29 QTLs associated with seven morpho-agronomic traits were detected on the 11 chromosomes, which explained between 3.83 and 32.92% of the phenotypic variation in DF. A total of 18 candidate genes associated with the detected QTLs were identified and related with biological processes, molecular functions and cellular components.

Keywords: Recombinant inbred line; Single nucleotide polymorphism; Quantitative trait loci; Gene annotation.

1. Introduction

The first genetic maps of common bean (*Phaseolus vulgaris* L), based on phenotypic and molecular markers, were published by Lamprecht (1961) and Vallejos et al. (1992), respectively. Linkage maps with additional DNA markers were developed by Nodari et al. (1993), Adam-Blondon et al. (1994), and Freyre et al. (1998).

Different types of populations have been used for genetic mapping in plants. These include backcross, early segregation population, RIL, double haploid (Schuster and Cruz 2004), and nearly isogenic line (NIL) populations (Semagn et al. 2006). According to Schuster and Cruz (2004), an advantage of RILs is the possibility of being propagated and evaluated in replications by different research groups without altering the genetic structure, making a more consistent phenotyping of the traits possible. The development of RIL populations of common bean derived from parents of a same gene pool (Jung et al. 1996; Galeano et al. 2009; Yuste-Lisbona et al. 2012) and from different gene pools (Freyre et al. 1998; Ochoa et al. 2006; Blair et al. 2009; Pérez-Vega et al. 2010) have been reported.

The population size reported in the previous studies ranged from 70 (Jung et al. 1997) to 185 RILs (Yuste-Lisbona et al. 2012), and the genetic maps were based on phenotypic, isozyme, RFLP (Restricted Fragment Length Polymorphism), RAPD (Amplified Fragment Length Polymorphism), SCAR (Sequence Characterized Amplified Region), STS (Sequence Tagged Site), and SSR (Simple Sequence Repeat) markers.

The development of SNP (Single Nucleotide Polymorphism) markers by next-generation sequencing has become a common practice in the scientific community (Galeano et al. 2009; Hyten et al. 2010; Souza et al. 2011, Song et al. 2015). In plant genomes, SNP markers are abundant and polymorphic (Hyten et al. 2010), and thus indicated as source for the construction of saturated genetic maps. One of the key applications of genetic maps is the identification of QTLs (Quantitative Trait Loci) associated with agronomic traits to be explored in marker-assisted selection. This selection technique is time-saving and increases the efficiency of identifying superior genotypes.

There are many reports on the identification of QTLs controlling agronomic traits in common bean, e.g. days to flowering, days to maturity or harvest (Tar'an et al. 2002; Blair et al. 2006; Pérez-Vega et al. 2010), plant architecture (Tar'na et al. 2002;

Blair et al. 2006), and seed (Park et al. 2000; Cichy et al. 2009; Yuste Lisbona et al. 2014), and yield-related traits (Blair et al. 2006; Blair et al. 2012). The mapping populations used in these studies were derived from parents from different gene pools, with different population sizes, and genetic linkage maps containing different numbers and types of markers. Thus, differences were also observed with regard to the number and relative position of the QTLs associated with the same traits.

In general, the RIL populations used for genetic mapping of common bean are restrictive due to their small size. A small population size reduces the accuracy of recombination rate estimates and consequently the accuracy of the genetic linkage map. In a simulation study, Silva et al. (2007) reported that populations of 200, 300 and 500 RILs are necessary to obtain reliable maps at a genome saturation level that can be high (mean distance between markers 5.0 cM), medium (mean distance between markers 10.0 cM) or low (mean distance between markers 20.0 cM), respectively. The Common Bean Breeding Program of the Universidade Federal de Viçosa (UFV) (Viçosa, MG, Brazil) developed a population of 500 RILs from a cross between the lines Rudá (Mesoamerican) and AND 277 (Andean), called RA RILs. In this context, we aimed to construct a genetic map using SNP markers of the RA RIL population and detect QTLs associated to agronomic traits of this population.

2. Material and methods

2.1 Plant material

The cross between cultivar Rudá and landrace AND 277 was performed and the F_1 hybrid plants were identified by the flower color, which is white (recessive phenotype) in female parent Rudá and pink (dominant phenotype) in the male parent AND 277. The F_1 seeds were sown in a greenhouse, and F_2 plants were advanced to the F_{10} generation by the single seed descent method (Sanglard et al. 2013).

Cultivar Rudá (Landrace A285) of Mesoamerican origin was developed at CIAT (International Center for Tropical Agriculture, Cali, Colombia) from the cross Carioca × Rio Tibagi. It was introduced in Brazil by Embrapa Arroz e Feijão (Santo Antônio de Goiás, GO, Brazil) in 1995 (Embrapa, 2014). Rudá belongs to the carioca grain class, which is the most widely consumed bean type in Brazil (market share of 70%). Landrace AND 277 was also developed at CIAT by crossing [(Cargabello × (Pompadour Checa × Linea 17)) × (Linea 17 × Red Cloud)]. It is a source of the gene of

resistance to angular leaf spot (gene *Phg-1*) and an Andean red-mottled bush bean (Aggarwal et al. 2004). The cross of Rudá × AND 277 involved parents from two different gene pools and two genetically divergent parents in terms of agronomic traits (Silva et al. 2016) and molecular characterization (Grisi et al. 2007; Souza et al. 2012).

2.2. Morpho-agronomic characterization

The field test of RA RILs was carried out at an experimental station of the Department of Plant Science of the Universidade Federal de Viçosa, in Coimbra, Minas Gerais, Brazil (latitude 20°50'30" South, longitude 42°48'30" West, 720 m asl) during the winter of 2012. A total of 395 plots including 393 RILs and the parents Rudá and AND 277 were field-tested using a randomized block design with additional controls with three replications. Each experimental plot consisted of 30 plants, distributed in two 1.0-m rows each, spaced 0.5 m apart and with 15 plants per row.

The following morpho-agronomic traits were evaluated: number of days to flowering (DF); number of days to maturity (DM) or crop cycle; and seed yield (YLD), in Kg ha^{-1}, at physiological maturity (when 90% of the pods were yellow-green to brown); 100-seed weight (SW) in gram, randomly chosen per plot; degree of seed flatness (SF), given by the ratio between the seed thickness and width; and seed shape (SS), given by the ratio between the seed length and width of five randomly chosen seeds per plot. The plant architecture (ARC) in each plot was evaluated at physiological maturity, based on a scale proposed by Collicchio et al. (1997), by which more upright plants received lower scores. The data of days to flowering, days to maturity, seed yield, and plant architecture were based on evaluations of all plants in a plot.

2.3. Genotyping and alignment of SNP markers

Plants of RA RILs and their parents were grown in a greenhouse. Only 376 of all 393 RILs could be genotyped. The DNA was extracted from bulk samples consisting of the leaf tissue of 10 plants for each RIL and parents. The commercial Invisorb® Spin Plant Mini Kit was used for DNA extraction and purification, according to the manufacturer's instructions. The plants were genotyped in the Soybean Genomics and Improvement Laboratory, USDA-ARS/BARC-W (Beltsville, MD, USA), using the BARBean6K_3 Illumina BeadChip consisting of 5,398 SNPs. The procedures of genotyping with the Illumina Infinium® HD Assay Ultra protocol were applied as

described by Song et al. (2015). The SNP allele for each genotype was called using software Genome Studio v2011.1 (Illumina, San Diego, CA, USA). To obtain pre-information about the chromosome to which each SNP marker was linked, the sequences containing informative SNPs were aligned against the common bean reference genome (genotype G19833) (Schmutz et al. 2014), available at Phytozome (http://www.phytozome.net/commonbean.php) by version 1.0 BlastN of the CLC Genomics Workbench version 5.5. The resulting physical SNP map agreed fully with the mapping results reported by Song et al. (2015).

2.4. Segregation test and marker mapping

The goodness-of-fit of markers to an expected segregation ratio of 1:1 in the RIL mapping population was chi-square tested at a probability of 5%, and genetic mapping was carried out using software Genes (Cruz 2013). The distances between pairs of markers were calculated by the maximum likelihood method, and the genetic map was constructed by establishing a minimum log-of-odds (LOD) threshold of 3.0 and maximum recombination frequency of 30%. The Kosambi function was used to convert recombination frequencies into genetic distances in centiMorgans (cM) (Kosambi 1944). In the case of more than one linkage group per chromosome, as well as the existence of unlinked markers, the map with the highest number of markers was selected to represent the chromosome (Pv) in question (Arumuganathan and Earle 1991; Pedrosa et al. 2003). Any number of completely linked (co-segregating) markers was considered a single locus.

2.5. Analysis of phenotypic data, QTL detection and gene annotation

Analysis of variance was carried out for each trait and the significance of differences among the RILs were tested at 5% probability. Population distribution was evaluated for normality. The mean of each RIL for the traits was compared with the parent mean by Dunnett's test at 5% probability. All analyses of phenotypic data were carried out with software Genes (Cruz 2013).

The markers on the final genetic map obtained with the 376 RILs and their means of the traits were used for QTL detection using software Genes (Cruz 2013). Simple interval mapping (SIM) (Lander and Botstein 1989) was performed with a multiple linear regression model (Halley and Knot 1992; Martinez and Curnow 1992),

at distance intervals of 0.1 cM. The QTL position was defined by a LOD threshold of 3.0. The proportion of phenotypic variation explained by each QTL was determined by the R^2 value of the regression model. We chose this method given its simplicity and the high saturation of the genetic map. Under these conditions, the SIM ensures the control of false positives of QTL detection. The QTLs were identified as recommended by Miklas and Porch (2010), in that the QTL nomenclature was composed of the abbreviation of the trait (e.g., DFL); then, the linkage group number, and finally, the serial number (e.g., DFL1.1).

The flanking sequences of the SNP markers were used to identify the physical position of the QTLs for the morpho-agronomic traits. In this context, gene annotation was performed using software BLAST2GO, as described by Conesa et al. (2005).

3. Results

3.1. Selection of SNP markers

From a total of 5,398 SNP markers used in the genotyping of the RA RILs, 60.91% (3,288) were informative, for being polymorphic in the mapping population and due to their high allele calling quality (> 0.91). Of these, 228 SNPs (6.93%) were eliminated for being monomorphic in the parents, resulting in 3,060 SNPs.

The number of polymorphic SNPs varied from 172 (Pv06) to 353 (Pv05), with an average of 278 per chromosome (Table 1). In the segregation test, a total of 1,019 SNPs (33.3%) did not fit the expected 1:1 segregation ratio in the RILs; of these, 686 SNPs (67.32%) had predominant alleles from parent Rudá, and 333 SNPs (32.68%) predominant alleles from parent AND 277. There was a predominance of alleles from parent Rudá on the chromosomes Pv01, Pv04, Pv07, Pv08, Pv10, and Pv11, and from parent AND 277 on Pv02, Pv05, Pv06, and Pv09.

Table 1 Number of SNP markers mapped in the RA RIL population across the 11 common bean chromosomes

Chromosome	Total number of SNPs	No. SNPs with distorted segregation	No. SNPs with high frequency of alleles from		No. SNPs fit 1:1 segregation ratio	No. linkage groups	No. SNPs in each linkage group	No. unlinked SNPs
			Rudá	AND 277				
Pv01	327	47 (14.37[a])	42	5	280 (85.63[a])	2	278 - 2	0
Pv02	321	136 (42.37)	41	95	185 (57.63)	5	164 - 17 - 2 -1 -1	2
Pv03	199	10 (5.03)	5	5	189 (94.94)	3	183 - 5 - 1	1
Pv04	313	83 (26.52)	82	1	230 (73.48)	4	226 - 2 -1 - 1	2
Pv05	353	18 (5.10)	7	11	335 (94.90)	2	310 - 25	0
Pv06	172	52 (30.24)	1	51	120 (69.76)	1	120	0
Pv07	249	12 (4.82)	12	0	237 (95.18)	2	236 - 1	1
Pv08	232	65 (28.02)	65	0	167 (71.98)	2	166 - 1	1
Pv09	276	162 (58.70)	0	162	114 (41.30)	5	108 - 2 - 2 - 1 - 1	2
Pv10	283	255 (90.11)	255	0	28 (9.89)	5	19 - 5- 2 - 1 -1	2
Pv11	335	179(53.43)	176	3	156 (46.57)	4	152 - 2 - 1 - 1	2
Total	3,060	1,019 (33.3)	686	333 (32.68 [a])	2,041 (66.7)	35	-	13
Mean	278.18	93	-	-	185.55	-	-	-

[a] Equivalence in percentage

Linkage analysis of the 2,041 SNP markers that segregated at the expected ratio of 1:1 was performed for individual chromosomes (Table 1). On chromosome Pv06, all 120 SNPs were on a single linkage map, however, on other chromosomes, two or more linkage groups were established due to the scarcity of markers in some of the genomic regions of the chromosomes, e.g. SNPs in Pv01, Pv05, Pv07, and Pv08 formed two linkage groups in each chromosome, and five per chromosome in Pv02 and Pv09. At most, two SNPs were unlinked to the linkage maps for chromosomes Pv02, Pv04, Pv09, Pv10, and Pv11. Only the linkage maps with the highest numbers of SNPs per chromosome were used for further analysis, which contained a total of 1,962 SNPs.

3.2. Linkage map

Genetic linkage analysis of 1,962 SNPs in the 11 chromosomes (Pv) (detailed in Table 2) resulted in a genetic map with a total length of 1,081.98 cM (Table S1). The number of SNPs on each linkage map varied from 19 (0.97% of 1,962; Pv10) to 310 (15.8%; Pv05) and the mean was 178 (9.09%).

The percentage of bins containing a single marker (PB1) was 79.14% (990 loci) of all loci and ranged from 72.54 (140 loci on Pv05) to 88.24% (15 loci on Pv10) among chromosomes. The proportion of bins containing two or more markers (PB2) was 20.86% (261) and varied from 11.76 (2 loci on Pv10) to 27.46% (53 loci on Pv05) among chromosomes.

The length of linkage maps ranged from 70.80 (Pv10) to 146.90 cM (Pv07), with a mean of 98.36 cM. The longest distance between two markers (Lon) ranged from 6.54 (Pv11) to 31.06 cM (Pv10). The mean distance between markers (MDM) ranging from 0.32 (Pv05) to 3.93 cM (Pv10) and the mean distance between markers considering all Pvs was 0.87 cM. The mean distance between loci (MDL) ranged from 0.51 (Pv05) to 4.43 cM (Pv10) and the mean distance between loci considering all Pvs was 1.20 cM. The percentage of distances between loci shorter than or equal to 5.0 cM ($D \leq 5$) considering the 11 Pvs was 95.13%, and ranged from 75.0 (Pv10) to 99.48% (Pv05).

Table 2 Distribution of 1,962 SNPs across the 11 common bean chromosomes in the genetic map constructed using the RA RIL mapping population

Chromosome	No. SNPs	PB1[a]	PB2[b]	Length (cM)	Lon[c]	MDM[d]	MDL[e]	D≤5[f]
Pv01	278 (14.17[g])	75.0 (135[h])	25.0 (45[h])	119.96 (11.09[g])	16.46	0.43	0.68	98.88
Pv02	164 (8.36)	77.14 (81)	22.86 (24)	71.02 (6.56)	6.84	0.44	0.68	96.15
Pv03	183 (9.33)	80.34 (94)	19.66 (23)	105.03 (9.70)	12.66	0.58	0.91	97.41
Pv04	226 (11.52)	74.53 (79)	25.47 (27)	103.54 (9.56)	17.37	0.46	0.99	97.14
Pv05	310 (15.80)	72.54 (140)	27.46 (53)	98.20 (9.07)	7.80	0.32	0.51	99.48
Pv06	120 (6.12)	86.76 (59)	13.24 (9)	75.94 (7.02)	26.41	0.64	1.13	97.01
Pv07	236 (12.03)	86.67 (130)	13.33 (20)	146.90 (13.57)	28.30	0.63	0.98	97.32
Pv08	166 (8.46)	82.5 (99)	17.5 (21)	102.83 (9.50)	16.03	0.62	0.86	96.64
Pv09	108 (5.50)	75.95 (60)	24.05 (19)	96.97 (8.96)	27.68	0.90	1.24	94.87
Pv10	19 (0.97)	88.24 (15)	11.76 (2)	70.80 (6.54)	31.06	3.93	4.43	75.00
Pv11	152 (7.75)	84.48 (98)	15.52 (18)	90.79 (8.39)	6.54	0.60	0.79	96.52
Total	1,962	79.14 (990)	20.86 (261)	1,081.98	-	-	-	-
Mean	178.36 (9.09)	-	-	98.36 (9.09)	-	0.87	1.20	95.13

[a] Percentage of bins containing a single marker (PB1)
[b] Percentage of bins containing two or more markers (PB2)
[c] Longest distance between two markers (Lon) in cM
[d] Mean distance of marker (MDM) in cM
[e] Mean distance of loci (MDL) in cM
[f] Percentage of distances between loci less than or equal to 5 cM (D≤ 5)
[g,h] Equivalence in percentage and absolute values, respectively

3.3. QTL analysis and gene annotation

All the traits measured were normally distributed, suggesting that all of them were inherited in a quantitative manner (Fig. 1). High narrow-sense heritability was observed for all traits, ranging from 82.81 for ARC to 97.09% for SW (Table 3). The variation coefficients (CV%) varied from 2.22 for DM to 17.58% for ARC. The differences among RILs were significant for all traits ($P < 0.05$). The effect of the source of variation of the parents was significant, except for the traits DM and YLD. The contrast of RILs versus parents was significant ($P < 0.01$) for the traits DF, MD, YLD, and SW, but not significant for ARC, SF and SS. The means of the 393 RILs for the seven traits under study were compared to the means of the parents (Rudá and AND 277) by Dunnett's test at 5% probability, and grouped in mean classes statistically equal to or different from those of the parents (Table 4). Only for trait SW there was no transgressive segregation, once there was no RIL that exceeded the limits of the parents.

A total of 29 QTLs were detected for the seven traits (Table 5), ranging from 3 (DF, DM, YLD and SS) to 7 QTL (SW) per trait analyzed. The number of QTLs per chromosome varied from one (Pvs 02, 04, 06, 10, and 11) to 5 (Pv03). The maximum and minimum proportion of phenotypic variance explained by each QTL ranged from 3.83 to 32.92%, both for DF. Contributions of alleles from both parents to the QTL effects were either positive or negative, except for SS, to which only parent AND 277 contributed alleles with positive effect.

Three QTLs were detected for the trait DF ($DF1.1^{RA}$, $DF3.1^{RA}$, and $DF9.1^{RA}$), which explained 3.83 to 32.92% of the phenotypic variation. The QTL DF1.1 had the highest R^2 value of all detected in the 11 Pvs. Three QTLs ($DM1.1^{RA}$, $DM3.1^{RA}$ and $DM9.1^{RA}$), were also detected for DM, which explained between 4.38 and 13.79% of the phenotypic variation. The position of the QTLs detected for this trait is very close or identical to that for DF. The reduction in number of days to flowering (DF) and number of days to maturity (DM) by these QTLs was associated to alleles of parent AND 277 on Pv01 and Pv03 and to the alleles of parent Rudá on Pv09.

The four QTLs detected for ARC ($ARC1.1^{RA}$, $ARC5.1^{RA}$, $ARC7.1^{RA}$, and $ARC8.1^{RA}$) explained 4.48 to 27.45% of the phenotypic variation. The QTL ARC1.1 (52.21 cM) was positioned between the QTLs DF1.1 (51.20 cM) and DM1.1 (52.60 cM) on Pv01. The allele of Rudá of ARC7.1 reduced the value of this trait, while the allele of AND 277 of ARC1.1, ARC5.1 and ARC8.1 influenced the trait negatively.

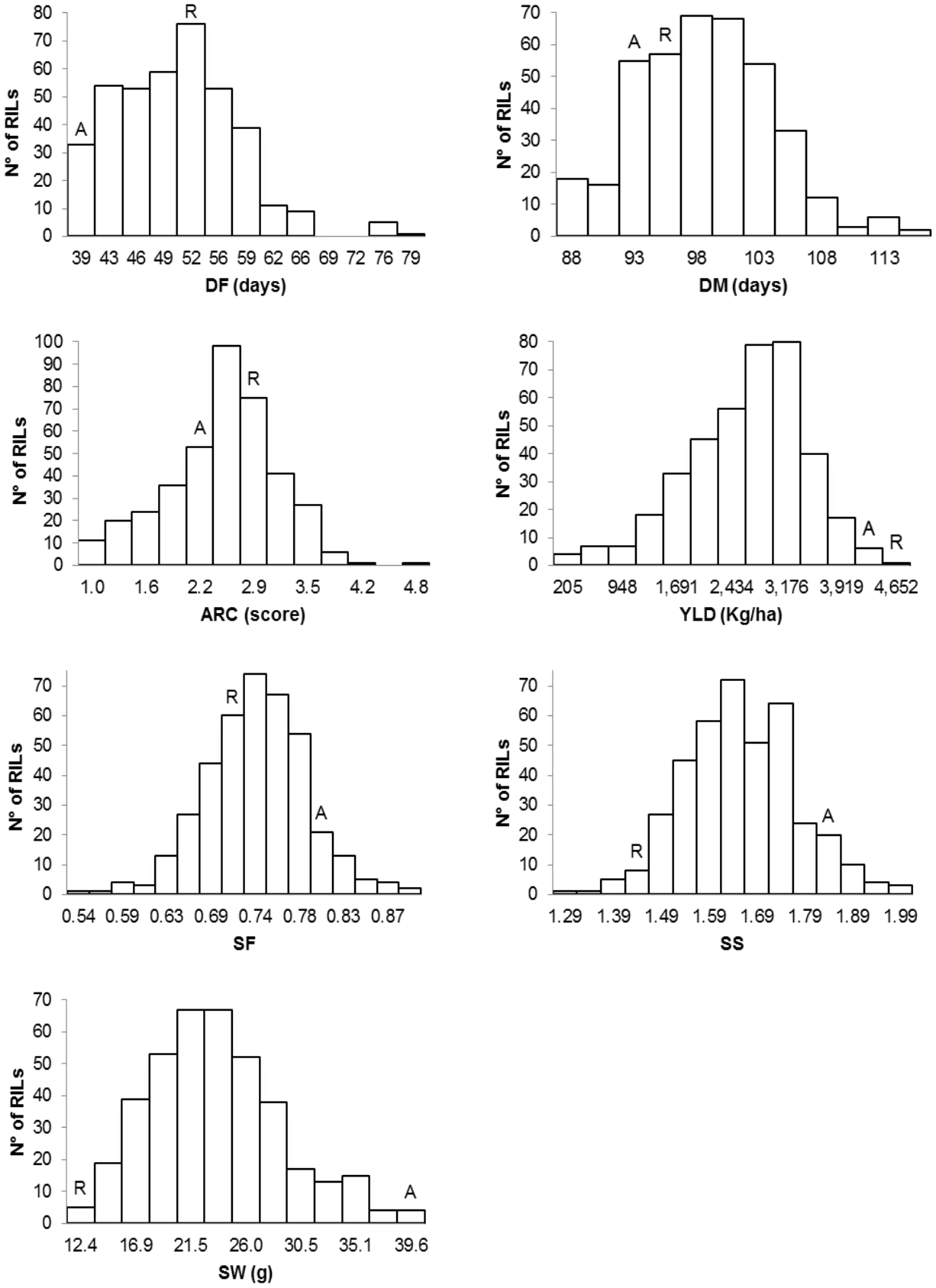

Fig. 1 Population distributions for the seven morpho-agronomic traits in the RA RIL population. Letters 'A' and 'R' indicate the phenotypic means of parents AND 277 and Rudá, respectively

Table 3 Mean square among blocks and RILs, estimated mean of the RILs (μ RILs) and parents (μ Parents), and heritability, based on the mean of the RILs (H^2) for each trait

SV	df	Mean squares[a]						
		DF	DM	ARC	YLD	SF	SS	SW
Blocks	2	164.57	45.23	7.17	12,313,995.90	0.0067	0.019	70.67
RILs	392	156.47**	82.16**	1.32**	1,935,032.23**	0.0089**	0.041**	89.21**
Parents (Pa)	1	240.67**	2.67^{ns}	1.04*	37,040.82^{ns}	0.0136**	0.235**	2003.27**
RILs *vs.* Pa	1	86.89**	88.48**	0.012^{ns}	18,122,690.18**	0.0021^{ns}	0.0002^{ns}	689.06**
Error	788	6.39	4.99	0.23	210,563.99	0.0011	0.0032	2.59
CV %		4.79	2.22	17.58	16.16	4.52	3.34	6.50
μ RILs		52.82	100.52	2.70	2,831.45	0.75	1.69	24.72
μ Parents		49	96.67	2.75	4,573.81	0.77	1.68	35.46
H^2 %		95.91	93.93	82.81	89.12	87.27	92.23	97.09

**, *: significant at 1 and 5% probability, respectively, by the F test
ns: not significant

Table 4 Grouping of RILs in different mean classes in relation to the parent mean, by the Dunnett test for each of the morpho-agronomic traits in the morpho-agronomic characterization of the RA RILs

	Number of RILs							
	Equal[a]			Different[b]			Parent mean	
Traits	A	B	AB	Greater	Smaller	Between A-B	AND 277 (A)	Rudá (B)
DF	127	221	0	37 > (B)	0 < (A)	8	42.67	55.33
DM	7	44	196	134 > (B)	12 < (A)	0	96.00	97.33
ARC	63	53	267	2 > (B)	8 < (A)	0	2.33	3.17
YLD	32	0	63	0 > (B)	298 < (A)	0	4,495.24	4,652.38
SF	55	140	186	3 > (A)	9 < (B)	0	0.81	0.72
SS	127	105	0	1 > (A)	1 < (B)	159	1.88	1.48
SW	0	100	0	0 > (A)	0 < (B)	293	53.73	17.19

[a]Equal: number of lines with means statistically equal to the parents AND 277 (A), Rudá (B) and both (AB) (Dunnett, P <5%)

[b]Different: number of lines with mean greater than the mean of the parent with larger mean value (Greater); number of lines with mean smaller than mean of the parent with smaller mean value (Smaller); and number of lines with mean between the mean of the two parents (Between A-B) (Dunnett, P < 5%)

Table 5 QTLs detected for each morpho-agronomic trait

QTL ID[a]	Chromosome	Closest Marker ID[b]	Position (cM)[c]	LOD[d]	Effect of the allele in Rudá[e]	Phenotypic variation explained (%)
DF1.1^{RA}	1	76	51.20	38.882	4.14	32.92
DF3.1^{RA}	3	111	49.93	3.151	1.39	3.83
DF9.1^{RA}	9	96	43.92	3.289	-1.40	3.99
DM1.1^{RA}	1	78	52.60	12.71	1.9	13.79
DM3.1^{RA}	3	54	49.33	3.634	1.07	4.38
DM9.1^{RA}	9	96	43.92	10.547	-1.74	11.75
ARC1.1^{RA}	1	78	52.21	30.063	0.35	27.45
ARC5.1^{RA}	5	280	43.70	7.017	0.19	8.3
ARC7.1^{RA}	7	69	65.30	4.215	-0.15	5.1
ARC8.1^{RA}	8	86	48.23	3.656	0.14	4.48
YLD3.1^{RA}	3	130	45.93	4.088	-174.85	4.89
YLD4.1^{RA}	4	174	4.40	10	-266.67	11.23
YLD8.1^{RA}	8	91	32.20	22.972	378.71	22.77
SF2.1^{RA}	2	155	42.28	3.107	-0.01	3.84
SF5.1^{RA}	5	50; 52	73.20	12.105	-0.02	13.16
SF7.1^{RA}	7	49	119.10	5.216	0.01	6.17
SF8.1^{RA}	8	11	44.73	7.904	-0.01	9.16
SF9.1^{RA}	9	39	88.37	3.489	-0.01	4.23
SF10.1^{RA}	10	14	23.00	5.731	-0.01	6.78
SS3.1^{RA}	3	74	32.87	4.708	-0.03	5.68
SS7.1^{RA}	7	80	87.50	6.036	-0.03	7.12
SS9.1^{RA}	9	58	20.02	5.043	-0.02	6.03
SW1.1^{RA}	1	151	109.51	7.904	-1.96	9.07
SW3.1^{RA}	3	36	63.53	10.714	-1.88	12.03
SW5.1^{RA}	5	9	82.50	3.853	1.18	4.72
SW6.1^{RA}	6	169	24.70	5.185	-1.35	6.13
SW7.1^{RA}	7	88	109.90	22.494	-2.60	22.02
SW8.1^{RA}	8	13	45.03	5.459	1.38	6.56
SW11.1^{RA}	11	4	3.30	8.716	-1.83	10.03

[a] QTL identification related to the traits

[b] Closest marker is the marker nearest to the peak LOD score. A match between 'Marker ID' and the 'SNP ID' from Song et al. (2015) is detailed in supplementary material (Table S1)

[c] Position of the QTL on the genetic map

[d] Logarithm (base 10) of odds (LOD value) associated with the QTL detection

[e] Positive and negative additive effect corresponds to the allele contribution of parent Rudá in increasing and reducing the trait value, respectively

For YLD, three QTLs (YLD3.1^{RA}, YLD4.1^{RA} and YLD8.1^{RA}) were detected, which accounted for 4.89 to 22.77% of the phenotypic variation. The position of the QTL YLD3.1 (45.93 cM) was very close to the position of the QTLs DF3.1 (49.93 cM) and DM3.1 (49.33 cM) on Pv03. The increase in seed yield was linked to the QTL on Pv03 and Pv04 with alleles from AND 277, and to the QTL on Pv08 with alleles from Rudá. The effect of these QTL on yield ranged from 174.85 to 378.71 kg/ha.

Six QTLs were detected for the trait SF (SF2.1^{RA}, SF5.1^{RA}, SF7.1^{RA}, SF8.1^{RA}, SF9.1^{RA}, and SF10.1^{RA}), which explained 3.84 to 13.16 % of the phenotypic variation. The QTL SF8.1 (44.73 cM) is near QTL ARC8.1 (48.23 cM), on Pv08. The three QTLs detected for the trait SS (SS3.1^{RA}, SS7.1^{RA} and SS9.1^{RA}) explained between 5.68 and 7.12% of the phenotypic variation. All QTL alleles detected for increasing SF and SS were from AND 277, except for the QTL allele of SF7.1.

The highest number of QTLs (7) was detected for trait SW (SW1.1^{RA}, SW3.1^{RA}, SW5.1^{RA}, SW6.1^{RA}, SW7.1^{RA}, SW8.1^{RA}, and SW11.1^{RA}), which explained between 4.72 and 22.02% of the phenotypic variation. The QTL SW8.1 at 45.03 cM was near QTL SF8.1 (44.73 cM), on Pv08. The increase in the SW value was linked to alleles of Rudá in QTLs of Pv05 and Pv08, and to alleles of AND 277 in QTLs of Pv01, Pv03, Pv06, Pv07, and Pv11.

Of the 30 SNP sequences identified as linked to the QTLs for morpho-agronomic traits, four linked to DM1.1^{RA}, ARC1.1^{RA}, SS7.1^{RA}, and SW11.1^{RA} were related to genes involved in biological processes (nine genes), molecular functions (three genes), and cellular components (six genes) (Table 6).

Table 6 QTLs for agronomic traits detected in the RA RIL mapping population annotated based on the available Phytozome reference common bean genome v 1.0 (http://www.phytozome.net/commonbean.php), using the 2nd level GO terms, including terms based on biological processes, molecular functions and cellular components

Main term	GO term	QTLs
Biological Process	Cellular process	DM1.1^{RA}, SS7.1^{RA}, ARC1.1^{RA}
	Single-organism process	DM1.1^{RA}, SS7.1^{RA}, ARC1.1^{RA}
	Metabolic process	DM1.1^{RA}, SS7.1^{RA}, ARC1.1^{RA}
	Cellular component organization	SS7.1^{RA}
	Multi-organism process	SS7.1^{RA}
	Response to stimulus	SS7.1^{RA}
	Growth	SS7.1^{RA}
	Immune system process	SS7.1^{RA}
	Biological regulation	SW11.1^{RA}
Molecular Function	Catalytic activity	DM1.1 ^{RA}, SS7.1 ^{RA}, ARC1.1 ^{RA}
	Molecular function regulator	SW11.1 ^{RA}
	Linkage	SS7.1^{RA}
Cellular Component	Cell part	DM1.1^{RA}, SS7.1^{RA}, ARC1.1^{RA}
	Cell	DM1.1^{RA}, SS7.1^{RA}, ARC1.1^{RA}
	Organelle	DM1.1^{RA}, ARC1.1^{RA}
	Extracellular region	SW11.1^{RA}, SS7.1^{RA}
	Membrane	SS7.1^{RA}
	Membrane part	SS7.1^{RA}

4. Discussion

4.1. Marker quality and distortion analysis

The percentages of monomorphism between parents from different gene pools of common bean were 44.0 and 59.0%, as reported by Blair et al. (2003) and Jung et al. (1997), respectively. The percentages exceeded that found in this study (6.93%). Even higher percentages of monomorphism between parents from the same than between parents from different common bean gene pools were reported elsewhere, as for example 69.0 (Cichy et al. 2009), 89.4 (Blair et al. 2011), and 92.8% (Yuste-Lisbona et al. 2012). It is worth mentioning that SNP markers were not used in those studies. The low monomorphism rate between Rudá and AND 277 confirms the potential of these populations for linkage mapping and QTL detection. The divergence between these parents had already been demonstrated by Sanglard et al. (2013) with SNP and SSR markers, and by Silva et al. (2016) with the phenotypic characterization of these parents.

For QTL detection, Shi et al. (2011) analyzed different commercial common bean landraces with 132 SNP markers and reported that 56.8% of the SNPs were informative. Yuste-Lisbona et al. (2012) genotyped 185 RILs with 251 SNP markers. Of these, 233 SNPs were monomorphic (92.8%) and of the remaining 18, only 13 SNPs were mapped. In this study, 3,288 SNPs (60.91%) were observed among the RA RILs, of which only 228 SNPs (6.93%) were monomorphic.

The segregation distortion percentage observed in the analysis of 3,060 markers was 33.30% (1,019 markers), which was relatively higher than in other populations genotyped with different types of markers e.g, the percentage in the studies discussed above was 17.5 (Hanai et al. 2010), 23.0 (Blair et al. 2003), 26.0 (Tar'an et al. 2002), and 31.0% (Jung et al. 1997). According to Nodari et al. (1993), segregation distortions are possibly caused by a small population size and/or genetic factors affecting the inheritance of these markers. The segregation of SNPs in the RA RILs population seems most likely influenced by genetic factors, according to Vallejos et al. (1992), and according to Jung et al. (1996), the higher frequency of marker distortion in populations derived from crosses between Andean and Mesoamerican common bean was the result of selection at gametogenesis, fertilization and seed and/or plant development.

With regard to the RA RILs, alleles derived from the Mesoamerican parent (Rudá) were predominant in 67.32% (686 SNPs) of the distortions, possibly because the RILs carrying the alleles of this parent are more adapted than those with alleles of the

Andean parent. In a study of an F_2 population (Nodari et al. 1993) and a RIL population (Blair et al. 2003), the distortions were distributed almost equally between the alleles of Andean and Mesoamerican parents. However, in the RILs obtained from the F_2 population analyzed by Nodari et al. (1993), 53.33% of the markers with distortion contained excess alleles of the Andean parent (Freyre et al. 1998). However, Hanai et al. (2010) saturated the 'core map' obtained by Freyre et al. (1998) with SSR markers derived from EST (Expressed Sequence Tags), RGA (Resistance Gene Analogs), and AFLP (Amplified Fragment Length Polymorphism), using the same BJ RILs, and observed an excess of alleles of the Mesoamerican parent (BAT 93).

4.2. Linkage map with SNP markers

The quality of genotyping of SNP markers, polymorphism between parents, segregation ratio of SNPs and number of linkage maps were factors to ensure the map accuracy of the RA linkage maps with 1,962 SNPs. These markers were well distributed in the 11 Pvs and the total length of the genetic map was 1,081.98 cM, similarly to that estimated initially for the common bean genome, of approximately 1,200 cM (Vallejos et al. 1992).

Common bean maps with a length shorter than 1,200 cM have been reported (Vallejos et al. 1992; Jung et al. 1997; Beattie et al. 2003; Cichy et al. 2009; Blair et al. 2011; Yuste-Lisbona et al. 2012). Common bean maps with length greater than 1,200 cM were also reported (Freyre et al. 1998; Tar'an et al. 2002; Blair et al. 2003; Galeano et al. 2009). In none of those maps SNP markers were used, except in the one constructed by Yuste-Lisbona et al. (2012), which however contained only 13 SNPs. The size of the RIL populations used in the cited studies ranged from 70 (Jung et al. 1997) to 185 RILs (Yuste-Lisbona et al. 2012), different from the number reported by Silva et al. (2007) as necessary to obtain reliable maps.

In this study, six Pvs (Pv02, Pv06, Pv08, Pv09, Pv10, and Pv11) had a lower number of markers than the mean among all chromosomes (178 markers, 9.09% of 1,962). Six Pvs (Pv02, PV05, Pv06, Pv09, Pv10, and Pv11) were shorter than the mean length of linkage groups (98.36 cM, 9.09% of 1,081.98 cM). In spite of the relatively high variation of the greatest distance between two markers in the Pv, from 6.54 (Pv11) to 31.06 cM (Pv10), the variation of the mean distance between markers (MDM) among the Pvs was small, between 0.32 (Pv05) and 3.93 cM (Pv10). The map constructed by

Blair et al. (2012) had on average one marker every 5.9 cM, with a variation between the highest and the lowest saturations, from one marker every 4.6 to one marker every 11.9 cM, respectively, considering each of the Pvs. Although the map of Blair et al. (2012) was not saturated, as in other maps with a saturation of one marker every 3.54 cM (Pérez-Vega et al. 2010), every 4.3 cM (Yuste-Lisbona et al. 2012) and even every 15.0 cM (Tar'an et al. 2002), QTLs were still detected.

The proportion of bins formed by a single marker (79.14%) was lower than that found by Shi et al. (2011) of 84.41%. These authors used only 75 SNP markers and identified 14 markers associated with traits of interest in common bean. In the map obtained with the RA RILs, the highest mean distance between loci (MDL) was 4.43 cM (Pv10), which is shorter than that reported in other studies (Galeano et al. 2009; Blair et al. 2012). In addition, the percentage of distances between loci of less than or equal to 5 cM was 95.13, indicating the good saturation of this map.

Clusters with co-segregating markers of 2 to 10 were detected in all chromosomes by Adam-Blondom et al. (1994). Blair et al. (2003) reported the occurrence of microsatellite clusters on most chromosomes, except for two with a low marker density. Similar results were found in this map, where Pv05 accounted for the highest number of markers (310 - 15.80%) and the highest percentage of loci containing two or more markers (27.46%); Pv10 was the group with the smallest number of markers (19 - 0.97%) and the lowest percentage of loci containing two or more markers (11.76%). In the literature, different hypotheses were used to explain the occurrence of these clusters. According to Pedrosa et al. (2003), the clusters of RFLP markers were nothing more than identical fragments, very close to each other, or the result of recombination suppression resulting from the chromosomal rearrangement (Pedrosa et al. 2002).

The high number of SNPs (1,962) increased the map marker density and the likelihood to detect QTLs controlling agronomic traits of common bean.

4.3. QTL analysis

The CV values estimated for the traits ranged from 2.22 to 17.58% (Table 3), indicating that the precision and accuracy of analyses and estimates based on these data were high. The existence of genetic variability among the RA RILs for the evaluated traits (Table 3) justified the QTL detection for these traits.

At Pv01, Pv03 and Pv09, three QTLs were detected for DF and DM at the same position or in very close positions (Table 5), accounting for 3.99 to 32.92% of the phenotypic variation. The alleles for the QTLs DF1.1, DM1.1, DF3.1, and DM3.1, which contributed to reduce the crop cycle, were from parent AND 277, whereas the alleles for the QTLs DF9.1 and DM9.1, also contributing to cycle reduction, were from parent Rudá. The proximity of QTLs related to the traits DF and DM was also observed in other RIL populations. Pérez-Vega et al. (2010) detected QTLs for these traits on Pv01, Pv02 and Pv06, and Blair et al. (2012) on Pv06. According to Aastveit and Aastveit (1993), these very close QTLs on the same chromosome and related to traits of the same group, e.g., phenological traits, may be different genes located very close to each other on the chromosome or genes with pleiotropic effects.

The four QTLs detected for ARC in the RA RILs explained 4.48 to 27.45% of the phenotypic variation (Table 5). For these QTLs, three of the alleles that contributed to the reduction of the trait value were from parent AND 277 and one from parent Rudá, since the lower ARC values correspond to more upright plants. The development of upright plants could facilitate mechanical harvesting with reduced losses and lower incidence of diseases such as white mold, by diminishing the soil-pod contact. The study of Blair et al. (2006) is one of the few that included plant architecture in the QTL analysis, although plant architecture was determined based on plant height and width. It should be emphasized that these authors used backcrosses in advanced generations as mapping populations, and detected seven QTLs for these two traits on Pv01, Pv06 and Pv07, which explained 8 to 19% of the phenotypic variation.

For YLD, three QTLs were detected, which explained 4.89 to 22.77% of the phenotypic variation (Table 5). Although the YLD of AND 277 was lower than that of Rudá, the Andean parent contributed with two QTLs (YLD3.1 and YLD4.1) to increase yield and Rudá with a single QTL (YLD8.1). For YLD, Blair et al. (2006) found four QTLs on Pv04 and highlighted the possible relationship of these QTLs with blight resistance genes, in view of the maintenance of the yield level even though disease incidence was high in the environment of evaluation.

For SF, SS and SW, the 16 detected QTLs explained 3.84 to 22.02% of the phenotypic variation (Table 5). These QTLs were distributed on 10 of the 11 common bean chromosomes. Only the QTL SF8.1 (44.73 cM) was near QTL SW8.1 (45.03 cM). All alleles for these QTLs that contributed to increase these traits were from parent

AND 277, except for the QTLs SF7.1, SW5.1, and SW8.1, whose alleles were from parent Rudá. Several authors detected QTLs related to seed length, width and thickness, aside from the ratio of some of these measures. On Pv02, Pv03, Pv04, Pv06, Pv08, and Pv11, Park et al. (2000) detected 10 QTLs, which explained from 2.4 to 16% of the phenotypic variation. On Pv 02, Pv03, Pv06, Pv07, Pv08, and Pv10, Pérez-Vega et al. (2010) detected 10 QTLs, which explained 12 to 24.9% of the phenotypic variation. On Pv01, Pv02, Pv06, Pv07, Pv09, and Pv10, Yuste-Lisbona et al. (2014) detected 14 QTLs, which accounted for 0.3 to 12.4% of the phenotypic variation. In these studies, the QTLs for these traits were very close to each other and close to the QTLs related to SW.

In the RA RILs, the highest number of QTL was detected for the trait SW. The same result was reported by Blair et al. (2006), Blair et al. (2009) and Blair et al. (2012). However, different results were obtained by Pérez-Vega et al. (2010), Blair et al. (2010) and Yuste-Lisbona et al. (2014). According to Blair et al. (2010), the small variation in seed weight between the parents, both of the Mesoamerican gene pool, resulted in RILs with little variation in this trait, limiting QTL detection. In this study, there was a significant difference for SW between Rudá (17.29 g) and AND 277 (53.73 g) (Table 4).

In the RA RILs, there was a predominance of allelic contribution of the Andean parent (AND 277) to QTLs related to higher values of seed-related traits. Similar results were observed in other maps of populations whose parents belonged to different gene pools (Blair et al. 2006; Blair et al. 2009; Perez-Vega et al. 2010). In the studies in which the parents of the mapping populations were from a same gene pool, the allele distribution for the identified QTLs was more balanced (Park et al. 2000; Blair et al. 2010; Blair et al. 2012; Yuste Lisbona et al. 2014).

The QTL YLD3.1 was also found near the QTLs for DF and DM on this chromosome, as also detected in RILs by Tar'an et al. (2002), but on Pv09. These QTLs sharing a same chromosome region and related to different traits may be different genes, very close on the chromosome, or genes with pleiotropic effects. Pleiotropy may explain the proximity of QTLs related to similar traits, such as DF and DM (Aastveit and Aastveit 1993).

4.4. Gene annotation

In general, the SNPs linked to the QTLs DM1.1, SS7.1, ARC1.1, and SW11.1 were related to 38.89, 83.33, 38.88, and 16.67% of the 18 genes with described ontology, respectively (Table 6). It was observed that the SNPs associated with the QTLs DM1.1, SS7.1 and ARC1.1 were found in a set of genes linked to all three main functions, biological process, molecular function, and cellular component, indicating that these QTLs share the same gene ontology. The QTLs DM1.1 and ARCARC1.1 are very close on Pv01.

Similar distribution functions were observed by Valdisser et al. (2016) in an annotation analysis of 1,032 sequences containing RAD (Restriction-associated DNA) SNPs of common bean. However, these sequences were randomly taken from the genome, not described as linked to genes or QTLs, containing SNPs used by the authors in genetic diversity studies and analysis of population structure.

In the studies of Pérez-Vega et al. (2010) and Blair et al. (2012), as well as in other papers cited previously by these authors, QTLs were identified for agronomic traits in common bean. However, there was no gene annotation of the sequences containing the molecular markers linked to the QTLs, as the sequence of the common bean genome was unavailable.

Overall, it was concluded that the population size of RA RILs (376 lines) allowed the construction of a genetic map with accurate frequency estimates of recombination. The number of markers used ensured good saturation of all Pvs, allowing an efficient and reliable QTL detection. Moreover, this map can be further developed and aligned with other genetic maps containing other classes of molecular markers.

5. Additional file

Supplementary Table S1 Linkage group and linkage position (cM) of markers in the Rudá x AND 277 (RA) RIL Linkage Map: RA RIL linkage group; Marker ID and order in the Linkage Map (*markers with asteristic are nearest to the peak lod score of QTL); Match between 'Marker ID' and 'NCBI ssID' obtained from Song et al. (2015); Marker position (cM) in the RA RIL Linkage Map.

RA RIL Linkage Group	Marker ID and order in the RA RIL Linkage Map*	Match between 'Marker ID' and the 'NCBI ssID' obtained from Song et al. (2015)	RA RIL linkage map position (cM)	RA RIL Linkage Group	Marker ID and order in the RA RIL Linkage Map*	Match between 'Marker ID' and the 'NCBI ssID' obtained from Song et al. (2015)	RA RIL linkage map position (cM)
1	245	715650354	0	5	318	715643151	42.5
1	246	715650356	0.3	5	235	715649305	42.5
1	199	715648970	0.3	5	344	715644427	42.5
1	198	715648969	0.4	5	271	715641053	42.5
1	22	715645301	2.7	5	345	715644495	42.5
1	23	715645303	3	5	283	715650721	42.5
1	2	715645250	4.1	5	234	715640354	42.5
1	20	715645293	6.1	5	264	715649927	42.5
1	21	715645299	7.9	5	143	715639730	42.5
1	14	715645277	19.6	5	199	715640124	42.5
1	64	715645919	24.1	5	180	715639968	42.5
1	15	715645280	28.9	5	313	715643009	42.6
1	10	715645267	29.3	5	349	715644983	42.7
1	12	715645273	29.4	5	346	715644508	42.7
1	17	715645285	30	5	339	715644017	42.7
1	58	715645910	30.8	5	336	715643868	42.7
1	73	715645935	30.8	5	335	715643867	42.7
1	32	715645856	30.9	5	327	715643429	42.7
1	37	715645863	31	5	321	715643172	42.7
1	30	715645853	31.1	5	301	715642429	42.7
1	36	715645862	31.2	5	298	715642279	42.7
1	39	715645866	31.6	5	297	715642278	42.7
1	38	715645864	31.7	5	294	715642253	42.7
1	41	715645868	32	5	293	715642252	42.7
1	43	715645883	32.8	5	296	715642264	42.7

1	44	715645885	32.9	5	292	715642250	42.7
1	47	715645892	33.3	5	272	715641075	42.7
1	50	715645898	34.3	5	308	715642599	42.7
1	53	715645903	35.1	5	232	715649292	42.7
1	55	715645906	35.5	5	291	715642185	42.7
1	54	715645904	35.8	5	230	715640339	42.7
1	56	715645907	36.1	5	231	715640340	42.7
1	57	715645908	36.5	5	229	715649291	42.8
1	67	715645925	37.1	5	350	715645000	43.4
1	66	715645924	37.1	5	329	715643535	43.7
1	68	715645926	37.1	5	312	715642794	43.7
1	71	715645931	37.4	5	280*	715641675	43.7
1	72	715645934	37.4	5	111	715639487	44.1
1	75	715645939	37.7	5	113	715639488	44.4
1	104	715646591	38	5	249	715649615	45
1	109	715646601	38.1	5	352	715645072	45.3
1	110	715646604	38.1	5	324	715643323	45.3
1	108	715646599	38.2	5	274	715650235	45.4
1	107	715646595	38.3	5	248	715649613	45.4
1	103	715646590	38.6	5	212	715640196	45.4
1	100	715646585	38.9	5	211	715640195	45.4
1	101	715646586	39.2	5	112	715647140	45.5
1	102	715646589	39.2	5	304	715642505	45.8
1	99	715646582	39.6	5	299	715642351	45.9
1	98	715646578	40	5	305	715642506	46
1	95	715646565	40.3	5	130	715639603	46
1	96	715646567	40.4	5	333	715643646	46.1
1	97	715646571	40.4	5	242	715649463	46.2
1	82	715646301	40.5	5	241	715649462	46.2
1	86	715646309	41.6	5	224	715649222	46.2
1	80	715646299	42	5	223	715649221	46.2
1	81	715646300	42	5	276	715641338	46.5
1	84	715646304	42	5	240	715640474	47.1
1	79	715646298	42	5	303	715642447	47.4
1	83	715646302	42	5	302	715642446	47.7
1	269	715650809	42.8	5	273	715650220	47.8
1	271	715650818	43.6	5	183	715648648	47.9
1	173	715648273	44.4	5	182	715648647	47.9
1	172	715648272	44.5	5	181	715648643	47.9

1	120	715639411	45.3	5	219	715649137	48.2
1	118	715639409	45.4	5	309	715642669	49
1	119	715639410	45.4	5	239	715640455	49.1
1	121	715646885	45.8	5	289	715642093	49.1
1	129	715647097	47.4	5	265	715640860	49.2
1	139	715647366	49.5	5	133	715639628	49.3
1	140	715639536	49.8	5	290	715650866	49.6
1	142	715647370	50.8	5	220	715649151	49.6
1	141	715647368	50.8	5	221	715649152	49.6
1	76*	715639271	51.2	5	165	715639856	51.4
1	78*	715639272	52.6	5	166	715639858	51.7
1	77	715646075	52.7	5	167	715639859	51.8
1	182	715639957	54.2	5	129	715647500	55.5
1	181	715639956	54.7	5	109	715646996	57.1
1	195	715648889	57.1	5	233	715649300	60.9
1	117	715646869	58.6	5	51	715645400	68.7
1	187	715640009	59.1	5	48	715645395	68.8
1	197	715640152	60.1	5	172	715648405	69.2
1	131	715639491	62.2	5	170	715648403	69.3
1	259	715650604	62.3	5	54	715645412	69.9
1	286	715651021	62.4	5	57	715645426	71
1	130	715639489	63.6	5	171	715648404	72.6
1	238	715641050	64.2	5	34	715645373	72.6
1	159	715639753	65	5	49	715645396	72.7
1	132	715639492	65.3	5	50*	715645398	72.8
1	158	715647963	65.4	5	52*	715645405	73.2
1	196	715640118	65.7	5	53	715645406	73.2
1	322	715644923	66.3	5	55	715645416	73.7
1	113	715639382	67.5	5	56	715645421	74.1
1	230	715640828	67.5	5	64	715645439	75.9
1	112	715639381	67.6	5	68	715645444	76.3
1	231	715640829	67.6	5	69	715645445	76.3
1	114	715639383	67.7	5	66	715645441	76.9
1	228	715640803	67.8	5	72	715645449	77.2
1	307	715644397	67.8	5	62	715645436	77.6
1	229	715640804	67.8	5	59	715645431	78
1	308	715644398	67.8	5	58	715645430	78.1
1	111	715639380	68.6	5	61	715645434	78.2
1	218	715640468	69	5	65	715645440	78.3

1	239	715641107	69	5	63	715645437	78.3
1	240	715641108	69	5	67	715645443	78.4
1	298	715643538	69	5	70	715645446	78.5
1	256	715641602	69	5	71	715645447	78.5
1	305	715644156	69	5	73	715645455	79.3
1	267	715641997	69.6	5	74	715645456	79.8
1	268	715641999	69.6	5	2	715645319	81.6
1	276	715642149	69.6	5	4	715645321	81.6
1	161	715648078	69.9	5	5	715645322	81.6
1	194	715640100	70.5	5	3	715645320	81.9
1	227	715649751	70.8	5	8	715645325	82.2
1	263	715641845	70.9	5	9*	715645326	82.5
1	138	715639532	71	5	7	715645324	82.6
1	184	715639989	71.3	5	6	715645323	82.7
1	266	715641960	71.4	5	11	715645330	83.1
1	265	715641959	71.4	5	10	715645327	83.4
1	122	715639437	71.7	5	13	715645337	84.4
1	251	715641497	71.7	5	14	715645339	84.7
1	137	715639531	71.8	5	15	715645340	84.8
1	180	715639912	72.2	5	18	715645343	84.8
1	275	715642098	72.2	5	17	715645342	85.2
1	279	715642259	72.2	5	16	715645341	85.5
1	212	715649290	72.3	5	19	715645344	85.5
1	310	715644440	72.4	5	20	715645346	85.8
1	202	715640223	72.5	5	21	715645347	86.3
1	235	715640978	72.8	5	23	715645349	86.6
1	124	715639443	73.3	5	22	715645348	86.6
1	205	715640290	73.4	5	25	715645354	86.7
1	176	715639850	73.4	5	24	715645352	86.8
1	123	715639440	73.4	5	26	715645359	87.4
1	171	715639829	73.4	5	27	715645362	87.7
1	188	715640013	73.4	5	28	715645363	88
1	247	715641411	73.4	5	30	715645366	88.6
1	250	715641433	73.4	5	29	715645365	89.2
1	287	715642916	73.4	5	32	715645370	90
1	301	715643820	73.4	5	35	715645374	90
1	216	715640372	73.4	5	31	715645368	90.1
1	314	715644520	73.4	5	33	715645371	90.1
1	255	715641601	73.4	5	37	715645379	90.4

1	217	715640376	73.4	5	36	715645377	90.5
1	261	715641748	73.4	5	38	715645380	90.6
1	280	715642281	73.4	5	39	715645382	91
1	282	715642662	73.4	5	40	715645383	91
1	237	715641042	73.4	5	41	715645384	91
1	302	715644079	73.5	5	42	715645385	92
1	257	715641607	73.5	5	43	715645386	92
1	313	715644519	73.5	5	47	715645392	92.4
1	311	715644514	73.5	5	45	715645389	92.7
1	292	715643138	73.5	5	44	715645387	93
1	306	715644264	73.5	5	201	715648989	93.6
1	318	715644596	73.6	5	200	715648987	94.8
1	309	715644409	73.6	5	206	715648996	94.9
1	204	715640288	73.7	5	203	715648992	94.9
1	278	715642235	73.8	5	202	715648991	95
1	293	715643183	73.8	5	207	715648997	95
1	315	715644542	73.8	5	204	715648993	95
1	294	715643202	73.8	5	205	715648994	95
1	299	715643727	73.8	5	79	715646689	96.2
1	243	715641225	73.9	5	82	715646694	96.3
1	249	715641431	74	5	85	715646699	96.3
1	316	715644559	74.4	5	90	715639375	96.3
1	323	715644929	74.4	5	95	715646716	96.3
1	317	715644560	74.5	5	96	715646719	96.3
1	149	715639583	75.1	5	89	715646703	96.4
1	150	715639588	75.1	5	93	715646712	96.4
1	290	715642995	75.1	5	84	715646697	96.5
1	148	715639582	75.2	5	87	715646701	96.5
1	289	715642958	75.7	5	88	715646702	96.5
1	304	715644121	75.7	5	91	715646708	96.5
1	312	715644516	75.7	5	92	715646711	96.5
1	168	715648173	75.8	5	94	715646714	96.5
1	258	715641622	75.9	5	97	715646720	96.5
1	167	715639810	75.9	5	100	715646729	96.8
1	288	715642917	76	5	98	715646723	96.8
1	320	715644770	76	5	101	715646732	96.9
1	215	715640371	76.1	5	105	715646739	97.2
1	244	715641240	76.1	5	80	715646692	98.2
1	254	715641553	76.1	6	132	715650171	0

1	143	715639556	76.4	6	131	715641022	0
1	190	715648729	76.4	6	155	715642419	0
1	206	715640294	76.4	6	126	715649916	0.1
1	281	715642648	76.4	6	151	715641566	0.5
1	191	715640034	76.4	6	136	715641131	0.5
1	169	715639811	76.5	6	137	715650242	0.5
1	303	715644102	76.5	6	84	715648112	0.5
1	325	715644975	76.6	6	161	715643008	0.6
1	272	715650841	76.6	6	157	715650985	1
1	128	715647050	76.9	6	158	715650986	1.1
1	125	715647042	77	6	152	715641707	1.2
1	127	715647046	77	6	153	715650621	1.5
1	248	715650462	77	6	159	715650987	1.6
1	126	715647044	77.1	6	154	715650668	1.6
1	203	715649131	77.1	6	145	715650345	1.6
1	220	715649599	77.1	6	94	715648496	1.6
1	213	715649311	77.2	6	105	715649155	1.6
1	296	715651090	77.2	6	93	715648495	1.6
1	232	715649950	77.2	6	91	715648440	1.6
1	234	715649953	77.2	6	90	715648436	1.6
1	264	715641865	77.2	6	89	715648431	1.6
1	233	715649952	77.3	6	88	715648429	1.6
1	214	715649314	77.3	6	118	715649469	1.9
1	236	715650123	77.3	6	150	715650478	2
1	242	715650308	77.3	6	144	715650330	2
1	273	715650848	77.3	6	134	715650196	2
1	291	715643080	77.3	6	117	715649467	2
1	327	715645107	77.4	6	112	715649360	2
1	200	715648977	77.7	6	163	715643220	2.3
1	274	715650867	77.7	6	162	715651082	2.3
1	201	715648978	77.8	6	149	715650477	2.3
1	284	715642717	77.9	6	160	715642881	2.3
1	283	715642716	77.9	6	147	715641373	2.3
1	260	715641703	78	6	142	715650311	2.3
1	209	715649190	78.4	6	138	715641156	2.3
1	207	715649186	78.4	6	127	715650103	2.3
1	208	715649187	78.5	6	148	715650416	2.3
1	147	715647482	78.6	6	143	715650313	2.3
1	144	715647470	78.7	6	141	715650310	2.3

1	189	715648728	78.7	6	133	715641024	2.3
1	210	715649279	78.7	6	128	715650107	2.3
1	211	715649281	78.8	6	125	715640783	2.3
1	226	715649726	78.9	6	124	715640782	2.3
1	252	715650531	79.3	6	120	715649682	2.3
1	192	715648764	79.6	6	119	715640681	2.3
1	186	715648627	79.6	6	123	715649858	2.3
1	297	715643343	79.6	6	116	715649447	2.3
1	157	715647952	79.7	6	106	715649163	2.3
1	154	715647941	79.8	6	115	715649446	2.3
1	156	715647944	79.8	6	101	715648844	2.3
1	155	715647942	79.8	6	104	715648910	2.3
1	185	715648626	80.1	6	102	715648850	2.3
1	295	715651078	80.6	6	103	715648908	2.3
1	88	715639329	81.6	6	78	715647845	2.3
1	94	715639335	81.7	6	77	715647844	2.3
1	89	715646539	82.1	6	79	715639702	2.3
1	90	715646540	82.4	6	156	715642453	2.4
1	91	715639331	82.5	6	130	715650114	2.4
1	92	715646541	82.9	6	129	715650113	2.4
1	93	715646545	82.9	6	164	715651131	2.5
1	177	715648371	83.3	6	110	715649336	2.5
1	179	715648383	83.4	6	87	715648363	2.6
1	178	715648373	83.5	6	72	715647422	3.2
1	174	715648275	83.6	6	74	715647426	4.4
1	175	715648277	83.7	6	73	715647425	4.5
1	116	715646777	84.3	6	96	715648561	5.1
1	115	715639386	85.3	6	95	715648560	5.4
1	166	715639795	85.7	6	139	715650278	6
1	165	715648162	87.1	6	28	715645954	7.8
1	162	715648157	87.4	6	108	715640328	10.4
1	163	715648158	87.8	6	76	715639700	10.7
1	164	715648159	87.8	6	107	715640326	10.8
1	24	715645589	88.8	6	109	715640330	10.9
1	25	715645591	89.1	6	62	715646829	13.3
1	26	715645596	90.1	6	135	715641090	13.4
1	27	715645597	90.5	6	68	715647363	13.9
1	28	715639223	91.1	6	69	715639530	14.2
1	219	715640553	94.1	6	67	715639529	14.6

1	324	715644931	94.2	6	146	715641313	15.2
1	170	715648188	95	6	75	715639609	15.8
1	222	715649659	95.6	6	86	715639790	18.9
1	221	715649655	95.9	6	97	715640039	19.3
1	160	715648030	96.7	6	98	715648752	19.6
1	326	715645089	96.8	6	66	715647260	20.8
1	152	715647677	97.1	6	64	715647252	21.1
1	193	715648855	98.6	6	65	715647257	21.2
1	153	715647678	99.2	6	113	715649417	21.3
1	151*	715647675	99.5	6	114	715649420	21.7
1	146	715647476	115.96	6	61	715646823	23.3
1	145	715647472	116.06	6	59	715646818	23.4
1	224	715649713	116.06	6	60	715646822	23.5
1	285	715651004	116.16	6	169*	715645033	24.7
1	223	715649692	116.16	6	85	715639786	26.3
1	241	715650284	116.16	6	140	715650285	26.6
1	225	715649716	116.26	6	71	715647380	28.7
1	277	715650900	117.76	6	166	715644535	29
1	319	715644658	119.96	6	70	715647379	29.4
2	218	715649479	0	6	167	715644685	29.8
2	253	715641034	1.1	6	99	715648769	30.2
2	251	715641032	1.7	6	44	715646421	36.13
2	252	715641033	1.8	6	47	715646427	36.63
2	216	715640457	1.9	6	16	715645756	39.73
2	43	715639371	7.02	6	25	715645785	40.03
2	44	715639372	7.32	6	15	715645752	40.43
2	45	715646675	11.13	6	23	715645782	40.83
2	204	715640279	12.13	6	27	715645794	41.13
2	152	715639809	14.84	6	26	715645793	41.13
2	208	715640358	14.84	6	24	715645783	41.13
2	210	715640360	15.44	6	19	715645767	42.33
2	69	715639436	16.04	6	20	715645768	42.33
2	209	715649308	17.04	6	22	715645770	42.33
2	67	715646980	19.14	6	18	715645765	42.43
2	68	715639434	20.74	6	21	715645769	42.53
2	96	715647231	25.86	6	17	715645760	43.13
2	94	715647229	25.96	6	63	715647110	44.63
2	95	715639495	26.06	6	14	715645677	47.03
2	93	715647228	26.16	6	13	715645673	48.43

2	92	715647225	26.26	6	55	715646671	74.84
2	91	715647222	27.36	6	57	715639369	75.94
2	97	715647234	27.76	7	165	715649169	0
2	38	715646371	29.16	7	3	715639206	28.3
2	102	715639502	34.58	7	2	715645213	37.2
2	103	715647299	35.38	7	1	715645208	41.8
2	104	715647302	36.18	7	246	715644981	46.1
2	105	715647305	36.58	7	4	715645235	46.4
2	121	715647668	37.18	7	31	715646009	46.8
2	119	715647659	37.98	7	30	715646008	46.8
2	120	715647662	38.08	7	33	715646012	47.4
2	133	715647803	39.18	7	32	715646011	47.7
2	122	715639620	39.78	7	35	715646019	49.1
2	270	715641291	40.68	7	34	715646018	49.5
2	153	715639855	41.28	7	37	715646022	50.3
2	154	715648336	41.68	7	36	715646021	50.4
2	155*	715648338	42.28	7	39	715646027	51.2
2	124	715647710	42.88	7	40	715646028	51.6
2	279	715641612	43.28	7	41	715646029	51.6
2	229	715640819	43.88	7	38	715646025	52.2
2	256	715641094	43.98	7	42	715646030	52.8
2	226	715649765	43.98	7	45	715646035	53.1
2	221	715640643	43.98	7	43	715646033	53.1
2	222	715640656	44.28	7	131	715648570	53.2
2	214	715640404	44.68	7	46	715646036	53.3
2	315	715644726	45.18	7	44	715646034	53.4
2	318	715645138	45.18	7	135	715648576	53.5
2	301	715642938	45.28	7	132	715648573	53.8
2	297	715642724	45.38	7	137	715648579	53.9
2	298	715642725	45.48	7	136	715648577	54
2	129	715647790	45.58	7	134	715648575	54
2	128	715647789	45.58	7	133	715648574	54
2	258	715641120	45.58	7	129	715648565	54
2	233	715649944	45.58	7	130	715648566	54
2	130	715647791	45.58	7	156	715649073	54.1
2	131	715639680	45.58	7	155	715649072	54.1
2	280	715641676	46.18	7	154	715649069	54.1
2	228	715640776	46.18	7	28	715645849	58.1
2	202	715640222	46.58	7	29	715645850	58.1

2	37	715639292	46.58	7	27	715645848	58.4
2	188	715640056	46.88	7	26	715645847	59.2
2	190	715640058	46.98	7	25	715645846	59.5
2	289	715642165	47.28	7	23	715645843	59.6
2	288	715642164	47.38	7	24	715645845	59.9
2	187	715640055	47.38	7	22	715645842	60.7
2	189	715640057	47.38	7	21	715645840	61.1
2	236	715649961	47.68	7	109	715648044	62.2
2	140	715647859	48.28	7	69*	715646526	65.3
2	139	715647854	48.28	7	71	715646611	65.3
2	309	715644091	48.68	7	74	715646613	65.4
2	310	715644181	48.68	7	70	715646609	66.2
2	285	715650785	48.68	7	73	715646612	67
2	304	715651109	48.78	7	113	715648289	69.7
2	302	715651095	48.78	7	112	715648287	69.8
2	291	715642426	48.78	7	104	715647732	70.4
2	244	715640941	48.78	7	102	715647728	70.4
2	230	715640834	48.78	7	103	715647729	70.4
2	212	715640365	48.78	7	105	715647734	70.4
2	284	715641966	48.78	7	194	715650389	70.5
2	255	715650206	48.78	7	138	715639980	71.1
2	239	715640909	48.78	7	8	715639236	71.7
2	231	715649907	48.78	7	11	715639239	72.3
2	193	715648830	48.78	7	9	715639237	73.5
2	185	715640032	48.78	7	10	715639238	74.5
2	211	715640363	48.88	7	5	715639231	75.3
2	206	715649196	48.88	7	7	715639235	76.1
2	192	715640079	48.88	7	6	715639234	76.7
2	184	715640031	48.88	7	248	715645135	78.3
2	191	715640077	48.88	7	84	715639468	78.6
2	243	715650059	49.28	7	83	715647088	79
2	249	715650118	49.68	7	247	715645036	81.9
2	201	715649059	49.78	7	95	715647649	83.4
2	294	715642615	49.88	7	94	715647648	83.5
2	308	715651158	49.98	7	242	715644502	83.9
2	295	715650973	49.98	7	170	715640487	84.2
2	303	715643357	49.98	7	163	715640270	84.8
2	311	715651192	49.98	7	162	715649164	85.1
2	286	715641994	49.98	7	164	715640271	85.1

2	261	715650301	49.98	7	120	715639847	85.5
2	117	715647653	49.98	7	121	715639848	85.5
2	118	715647656	49.98	7	118	715648307	85.8
2	200	715649058	50.08	7	119	715639846	85.8
2	227	715649798	50.18	7	77	715639387	87.2
2	277	715641598	50.58	7	78	715639388	87.3
2	238	715649999	50.58	7	79	715646778	87.4
2	273	715641350	50.58	7	158	715649094	87.5
2	263	715650319	50.68	7	80*	715639389	87.5
2	50	715639400	50.68	7	160	715640251	87.8
2	113	715639568	50.78	7	161	715640252	88.1
2	51	715639401	50.78	7	53	715639295	88.6
2	219	715640499	50.78	7	171	715640488	88.7
2	213	715640388	50.78	7	159	715640250	89
2	114	715639571	50.78	7	203	715641677	89.1
2	49	715639398	50.78	7	122	715648341	89.2
2	299	715642829	51.38	7	202	715641587	89.2
2	225	715649701	51.38	7	235	715643737	89.3
2	197	715640140	51.48	7	211	715650773	89.6
2	282	715650638	52.28	7	188	715650218	89.9
2	281	715650636	52.68	7	106	715647800	90
2	287	715650858	52.68	7	249	715645144	90.3
2	274	715650443	53.08	7	189	715641085	90.6
2	307	715643844	53.18	7	199	715641485	90.9
2	72	715647026	53.28	7	139	715639985	91.5
2	71	715647020	53.38	7	110	715639778	91.5
2	73	715647035	53.78	7	117	715639834	91.5
2	183	715648633	53.88	7	140	715639986	91.5
2	181	715648628	53.88	7	143	715640084	91.5
2	182	715648630	53.88	7	147	715640138	91.5
2	171	715648524	54.28	7	168	715640351	91.5
2	175	715639924	54.38	7	172	715640515	91.5
2	6	715645827	54.48	7	175	715649568	91.5
2	2	715645819	54.58	7	144	715640085	91.5
2	3	715645820	54.58	7	178	715640661	91.5
2	1	715645818	54.68	7	179	715640699	91.5
2	5	715645825	54.98	7	181	715640736	91.5
2	4	715645824	55.08	7	180	715640700	91.5
2	259	715641175	55.18	7	197	715641464	91.5

2	172	715648525	55.48	7	191	715641149	91.5
2	173	715648526	55.48	7	187	715640950	91.5
2	176	715648529	55.88	7	193	715641292	91.5
2	246	715650085	56.48	7	205	715641713	91.5
2	74	715647055	57.08	7	206	715641715	91.5
2	76	715647070	57.08	7	195	715641347	91.5
2	77	715647071	57.08	7	201	715641572	91.5
2	78	715647072	57.08	7	209	715641880	91.5
2	79	715647074	57.08	7	213	715642107	91.5
2	80	715647078	57.18	7	208	715650711	91.5
2	150	715648143	57.18	7	210	715641918	91.5
2	81	715647081	57.28	7	222	715642839	91.5
2	151	715648149	57.28	7	223	715642863	91.5
2	82	715639466	57.58	7	227	715643101	91.5
2	257	715650231	57.88	7	224	715642965	91.5
2	108	715647375	58.18	7	215	715642225	91.5
2	53	715646834	58.48	7	217	715642445	91.5
2	54	715646835	58.58	7	214	715642111	91.5
2	180	715639972	58.98	7	216	715642392	91.5
2	61	715646945	58.98	7	220	715642535	91.5
2	109	715647376	59.38	7	229	715643188	91.5
2	267	715650341	66.22	7	218	715642480	91.5
2	275	715641420	66.72	7	219	715642495	91.5
2	223	715649648	67.92	7	221	715642793	91.5
2	250	715650148	68.22	7	225	715642986	91.5
2	278	715650567	69.02	7	234	715643688	91.5
2	156	715639860	71.02	7	226	715643037	91.5
3	9	715646083	0	7	233	715643508	91.5
3	10	715646086	1	7	228	715643128	91.5
3	1	715645576	11.97	7	232	715643503	91.5
3	199	715645123	14.77	7	237	715644042	91.5
3	35	715647339	15.97	7	236	715643917	91.5
3	38	715647429	19.47	7	241	715644463	91.5
3	41	715639553	20.07	7	239	715644097	91.5
3	40	715639552	20.07	7	244	715644839	91.5
3	39	715647430	20.07	7	90	715639574	91.6
3	89	715640114	20.47	7	91	715639575	91.6
3	90	715640115	20.77	7	92	715647457	91.6
3	88	715648884	20.77	7	152	715640194	91.6

3	19	715639319	20.87	7	185	715640903	91.6
3	20	715639320	21.67	7	192	715650353	91.6
3	22	715639322	22.27	7	207	715641798	91.6
3	21	715639321	22.27	7	184	715649926	91.7
3	23	715639323	23.07	7	93	715647458	91.7
3	7	715639253	24.17	7	196	715650406	91.7
3	8	715639254	25.57	7	204	715641695	91.7
3	145	715650580	26.57	7	238	715644076	92
3	132	715641141	27.37	7	145	715640087	92.3
3	3	715639243	28.47	7	243	715644698	92.6
3	195	715644902	29.27	7	169	715649327	93.8
3	5	715639247	30.07	7	177	715649622	94.2
3	4	715639246	30.67	7	176	715640625	94.7
3	18	715639317	30.97	7	108	715647989	95
3	75	715639895	32.87	7	107	715647988	95
3	74*	715639894	32.87	7	141	715648792	95
3	29	715639422	45.53	7	142	715648793	95
3	130*	715650182	45.93	7	157	715649089	95.1
3	27	715646624	46.33	7	198	715641468	95.4
3	26	715646619	46.73	7	96	715639621	96
3	54	715647689	49.33	7	97	715647672	97
3	80	715648538	49.63	7	100	715647725	97.3
3	111*	715649522	49.93	7	101	715647726	97.4
3	65	715648025	50.03	7	98	715647723	97.7
3	34	715639501	50.03	7	99	715647724	97.8
3	32	715647091	50.13	7	87	715639538	98.9
3	70	715639854	53.23	7	86	715647218	100.1
3	71	715639864	53.83	7	173	715649511	101.2
3	72	715639865	54.13	7	174	715649512	101.2
3	141	715641537	54.53	7	85	715647158	101.7
3	51	715639608	55.73	7	212	715650843	102.1
3	50	715639606	55.83	7	182	715640748	102.6
3	118	715649832	56.13	7	183	715640749	102.9
3	61	715647850	56.93	7	151	715648962	104.7
3	60	715647848	57.23	7	150	715640158	104.8
3	42	715647432	60.03	7	148	715648961	105.3
3	147	715650616	60.43	7	149	715640157	105.7
3	68	715648169	61.53	7	200	715650514	106.3
3	67	715639807	61.53	7	82	715647037	106.3

3	163	715642631	62.33	7	186	715650029	106.4
3	167	715642813	62.73	7	81	715639459	107.8
3	92	715640189	63.03	7	89	715639547	108.4
3	179	715643803	63.43	7	245	715644972	109.5
3	36*	715639511	63.53	7	88*	715639546	109.9
3	37	715639512	63.63	7	146	715648885	112.3
3	93	715640190	63.93	7	59	715646462	114.1
3	136	715641305	64.03	7	54	715646456	114.5
3	122	715649919	66.13	7	56	715646458	114.5
3	153	715641926	66.53	7	55	715646457	114.5
3	103	715640379	68.83	7	57	715646459	114.5
3	144	715641636	68.93	7	58	715646461	114.5
3	102	715640378	68.93	7	61	715646466	115.1
3	146	715641700	69.03	7	62	715646470	115.4
3	190	715644651	71.23	7	60	715646465	115.5
3	116	715640753	72.43	7	63	715646475	116.1
3	188	715644367	73.83	7	64	715639324	116.2
3	180	715643813	74.23	7	167	715649276	116.3
3	183	715643996	74.23	7	166	715649271	116.4
3	178	715643657	74.23	7	52	715646358	117
3	197	715644967	74.33	7	51	715646356	117.4
3	175	715643320	74.33	7	50	715646353	117.9
3	194	715644826	74.33	7	49*	715646352	119.1
3	173	715643230	74.33	7	48	715646351	120.1
3	160	715642476	74.33	7	47	715646350	120.6
3	172	715643115	74.33	7	65	715646494	122.1
3	170	715642960	74.33	7	66	715646495	124.2
3	114	715640717	74.63	7	67	715646496	124.6
3	115	715640719	74.73	7	68	715646498	125.2
3	184	715644057	75.13	7	12	715645681	132.2
3	107	715640482	75.13	7	19	715645696	137.6
3	169	715642952	75.13	7	18	715645695	137.9
3	138	715641329	75.93	7	17	715645691	138.2
3	126	715640990	76.23	7	16	715645689	139.2
3	73	715639882	76.53	7	15	715645687	141.6
3	56	715639640	77.13	7	14	715645686	141.9
3	55	715639639	77.43	7	13	715645683	143.4
3	177	715643408	77.43	7	126	715648396	144.6
3	81	715639949	77.53	7	127	715648397	145.6

3	110	715640550	77.63	7	123	715648384	146.4
3	108	715640483	77.63	7	125	715648392	146.5
3	84	715639996	77.93	7	124	715648389	146.6
3	159	715642437	78.03	7	128	715648401	146.9
3	87	715640070	78.03	8	183	715650659	0
3	79	715639918	78.03	8	182	715650658	0
3	86	715640069	78.03	8	56	715647404	0.3
3	77	715639902	78.03	8	55	715647403	0.3
3	76	715639901	78.13	8	58	715647407	0.4
3	157	715642125	78.23	8	57	715647406	0.7
3	78	715639904	78.23	8	54	715647400	1
3	155	715641974	78.23	8	52	715647389	1
3	99	715649258	78.23	8	53	715647396	1.1
3	104	715640407	78.23	8	10	715646109	17.13
3	98	715649257	78.23	8	9	715646105	18.13
3	57	715639671	78.23	8	8	715646104	18.73
3	164	715642683	78.33	8	7	715646101	18.83
3	158	715642413	78.33	8	6	715646090	20.23
3	105	715640422	78.33	8	20	715646504	24.93
3	59	715639673	78.33	8	21	715646508	25.73
3	58	715639672	78.33	8	22	715646512	26.83
3	191	715644676	78.43	8	23	715646513	27.43
3	186	715644146	78.43	8	25	715646536	28.23
3	182	715643901	78.43	8	24	715646531	29.63
3	168	715642913	78.43	8	94	715639954	32.13
3	165	715642788	78.43	8	93	715639953	32.23
3	162	715642574	78.43	8	91	715648562	32.33
3	117	715640761	78.43	8	92	715639952	32.73
3	161	715642513	78.43	8	78	715648180	34.13
3	154	715641936	78.43	8	31	715639362	34.73
3	139	715641403	78.43	8	77	715648178	34.83
3	119	715640765	78.43	8	30	715646654	34.93
3	181	715643850	78.73	8	29	715646650	35.03
3	187	715644353	78.73	8	32	715646655	38.63
3	156	715642031	78.73	8	187	715641850	38.63
3	185	715644095	78.73	8	186	715650696	38.93
3	152	715641925	78.73	8	157	715640889	38.93
3	174	715643261	78.73	8	82	715639821	39.33
3	135	715641270	78.73	8	155	715640888	39.33

3	140	715641467	78.73	8	156	715649975	39.43
3	134	715641269	78.73	8	146	715649704	39.73
3	94	715640206	78.73	8	188	715641857	39.83
3	150	715641883	78.73	8	189	715650700	39.93
3	95	715640211	78.73	8	190	715641858	39.93
3	143	715641619	79.13	8	139	715649567	40.03
3	142	715641618	79.13	8	140	715640594	40.03
3	120	715640768	79.13	8	204	715643239	40.13
3	121	715640772	79.13	8	141	715640595	40.23
3	96	715640309	79.13	8	147	715649705	40.63
3	91	715648912	79.43	8	230	715645081	41.63
3	14	715646292	80.23	8	229	715644995	42.63
3	13	715646290	80.33	8	51	715647250	43.83
3	12	715646286	80.83	8	12	715639299	44.13
3	113	715649741	80.83	8	11*	715639297	44.73
3	127	715650135	80.93	8	13*	715639302	45.03
3	128	715650136	80.93	8	14	715639303	45.13
3	11	715646281	81.03	8	207	715643673	45.53
3	112	715649740	81.13	8	133	715640567	45.63
3	44	715647446	81.53	8	98	715640026	45.93
3	43	715647438	81.63	8	134	715649541	46.03
3	63	715647934	82.03	8	104	715648892	46.03
3	62	715647932	82.53	8	96	715648688	47.93
3	149	715650713	83.53	8	86*	715639872	48.23
3	151	715650755	83.83	8	184	715641802	48.63
3	85	715640005	84.13	8	167	715641417	48.73
3	53	715647684	85.13	8	214	715644007	49.73
3	52	715639629	86.33	8	66	715647892	50.03
3	82	715639971	86.63	8	67	715647897	50.03
3	83	715648618	87.23	8	26	715646562	50.33
3	131	715641086	87.83	8	162	715650322	50.93
3	166	715642800	88.43	8	210	715643790	51.53
3	193	715644715	88.43	8	112	715640218	51.63
3	66	715648032	89.23	8	126	715640429	51.63
3	196	715644947	89.63	8	199	715642502	51.63
3	24	715646546	90.03	8	205	715643483	51.63
3	25	715639337	90.63	8	232	715645134	51.63
3	123	715640856	91.23	8	125	715640428	51.73
3	133	715641226	91.33	8	218	715644418	51.83

3	97	715649212	92.33	8	127	715640430	51.83
3	33	715647200	92.43	8	196	715642230	51.83
3	46	715647636	94.33	8	79	715639814	52.13
3	48	715647638	94.33	8	176	715641667	52.13
3	49	715647639	94.33	8	178	715641725	52.13
3	64	715647965	94.33	8	192	715641864	52.13
3	47	715647637	94.43	8	195	715642117	52.13
3	45	715639604	94.73	8	193	715641886	52.13
3	101	715649325	95.73	8	60	715639549	52.13
3	106	715640477	96.33	8	166	715641303	52.13
3	69	715648301	96.63	8	203	715643226	52.13
3	28	715646879	103.13	8	95	715640014	52.13
3	17	715646394	104.93	8	174	715641648	52.13
3	16	715646393	105.03	8	181	715641740	52.13
3	15	715646392	105.03	8	194	715642116	52.13
4	204	715649777	0	8	197	715642382	52.13
4	200	715649772	0.1	8	201	715642676	52.13
4	199	715649771	0.1	8	206	715643651	52.13
4	202	715649774	0.1	8	211	715643802	52.13
4	145	715640024	0.1	8	215	715644092	52.13
4	203	715649776	0.2	8	164	715641252	52.13
4	201	715649773	0.2	8	208	715643678	52.13
4	147	715640025	0.2	8	219	715644485	52.23
4	144	715648681	0.2	8	216	715644311	52.23
4	197	715649768	0.3	8	225	715644789	52.23
4	65	715646904	1.8	8	76	715639800	52.33
4	63	715639414	1.8	8	227	715644873	52.43
4	149	715648684	2.4	8	222	715644637	52.43
4	206	715649779	3	8	123	715640398	52.93
4	205	715649778	3	8	154	715640831	53.03
4	198	715649770	3.1	8	132	715640558	53.03
4	146	715648682	3.1	8	172	715641492	53.03
4	143	715648680	3.2	8	111	715640203	53.03
4	141	715648679	3.3	8	131	715649513	53.33
4	150	715648686	3.3	8	135	715640574	53.63
4	142	715640023	3.3	8	136	715640577	53.93
4	175	715649432	3.6	8	200	715642675	54.03
4	173	715649425	3.6	8	16	715639311	54.33
4	148	715648683	3.7	8	116	715649167	55.13

4	177	715649434	4.3	8	59	715647428	55.53
4	176	715649433	4.3	8	118	715640318	55.83
4	174*	715649427	4.4	8	168	715641427	55.93
4	69	715646915	4.7	8	124	715649378	55.93
4	61	715646887	4.8	8	185	715641832	56.03
4	60	715646886	4.8	8	74	715648108	57.03
4	62	715646889	4.9	8	72	715648104	57.13
4	64	715646903	5.5	8	68	715647902	57.23
4	66	715646908	5.9	8	158	715649996	57.33
4	67	715646909	6.9	8	88	715648541	58.43
4	68	715646910	7.3	8	36	715646986	58.53
4	215	715649973	7.3	8	90	715648543	58.53
4	220	715650012	10.3	8	89	715648542	58.63
4	40	715646247	11.4	8	100	715648771	58.63
4	38	715646240	11.8	8	99	715640060	59.23
4	37	715646238	11.8	8	87	715648540	60.03
4	31	715646229	12.1	8	171	715650505	62.93
4	36	715646236	12.1	8	175	715650594	63.03
4	35	715646235	12.2	8	180	715650631	63.43
4	32	715646230	12.2	8	169	715650489	63.73
4	34	715646234	12.2	8	177	715650598	63.83
4	29	715646227	12.8	8	179	715650626	64.23
4	26	715646215	15.4	8	71	715648043	79.53
4	25	715646214	15.5	8	149	715640728	79.93
4	22	715646200	16.3	8	70	715639719	80.23
4	30	715646228	16.7	8	150	715649757	80.23
4	33	715646233	16.8	8	69	715647905	81.23
4	27	715646217	17.1	8	83	715648337	82.13
4	24	715646213	17.2	8	161	715650193	82.73
4	23	715646201	17.5	8	231	715645115	83.13
4	28	715646224	17.6	8	160	715641059	83.23
4	160	715649008	17.9	8	33	715639406	84.43
4	161	715649009	17.9	8	34	715639408	85.23
4	155	715649001	17.9	8	107	715648926	85.33
4	156	715649002	17.9	8	129	715649487	86.43
4	162	715649010	18	8	130	715649488	86.43
4	157	715649005	18.8	8	121	715649358	86.73
4	158	715649006	19.1	8	122	715649359	87.03
4	159	715649007	19.1	8	3	715645832	93.03

4	72	715647287	19.7	8	212	715651164	93.83
4	44	715646787	24.41	8	49	715647142	96.63
4	90	715647592	28.21	8	50	715647145	97.03
4	89	715647591	28.21	8	46	715647126	97.83
4	92	715647595	29.21	8	47	715647127	97.93
4	93	715647596	29.51	8	44	715647119	98.53
4	95	715647598	30.01	8	43	715647117	98.83
4	96	715639594	30.11	8	42	715647116	99.33
4	131	715648319	30.71	8	38	715647112	99.63
4	132	715648320	30.71	8	48	715647128	99.73
4	134	715648322	30.81	8	39	715647113	99.83
4	138	715648332	30.91	8	40	715647114	100.43
4	137	715648329	31.21	8	35	715646880	102.83
4	133	715648321	31.81	9	102	715646660	0
4	313	715645162	32.61	9	104	715646662	0.8
4	127	715648228	33.21	9	103	715639364	0.8
4	126	715648226	33.31	9	271	715644962	3.1
4	125	715648222	33.61	9	272	715644963	3.1
4	239	715641595	34.61	9	24	715639240	3.2
4	254	715641934	34.61	9	30	715645717	8.92
4	151	715640044	34.61	9	28	715645712	9.72
4	152	715640046	34.71	9	29	715645714	10.12
4	252	715641932	34.71	9	26	715645709	10.42
4	253	715641933	34.81	9	25	715645705	13.02
4	88	715639555	35.11	9	27	715645711	14.12
4	305	715644605	35.21	9	202	715648610	14.42
4	163	715649153	37.41	9	60	715646072	14.82
4	298	715644065	38.81	9	62	715639267	15.42
4	169	715649296	42.11	9	63	715639268	15.72
4	135	715648324	42.71	9	61	715646073	15.72
4	136	715648326	42.71	9	59	715639265	16.72
4	168	715649294	60.08	9	58*	715646071	20.02
4	262	715650885	60.08	9	57	715646069	20.82
4	182	715640536	60.18	9	149	715647190	20.92
4	184	715640539	60.18	9	148	715647189	20.92
4	227	715641011	60.18	9	144	715647183	20.92
4	108	715639734	60.18	9	145	715647184	21.02
4	124	715639806	60.18	9	147	715647187	21.02
4	183	715640538	60.18	9	142	715647180	21.42

4	109	715639735	60.18	9	141	715647179	21.72
4	110	715639736	60.18	9	140	715647178	22.12
4	111	715639737	60.18	9	139	715647177	22.12
4	112	715639738	60.18	9	136	715647174	22.72
4	123	715639804	60.18	9	135	715647173	23.02
4	179	715640465	60.18	9	138	715647176	23.12
4	178	715640464	60.18	9	134	715639494	23.52
4	196	715640733	60.18	9	132	715647168	23.92
4	195	715649766	60.18	9	131	715647165	24.02
4	213	715649949	60.18	9	130	715647163	24.42
4	216	715640899	60.18	9	152	715647196	24.72
4	240	715650593	60.18	9	146	715647186	24.82
4	229	715650238	60.18	9	220	715649397	24.82
4	243	715641680	60.18	9	143	715647181	24.82
4	230	715650239	60.18	9	150	715647193	24.82
4	226	715640974	60.18	9	151	715647195	24.82
4	181	715649493	60.18	9	137	715647175	24.82
4	236	715641495	60.18	9	221	715649399	24.92
4	242	715641662	60.18	9	154	715647198	24.92
4	250	715641891	60.18	9	222	715649401	25.22
4	225	715650031	60.18	9	8	715645613	25.72
4	235	715650486	60.18	9	3	715645607	26.02
4	248	715641792	60.18	9	5	715645609	26.02
4	255	715650761	60.18	9	6	715645610	26.02
4	281	715642753	60.18	9	7	715645611	26.12
4	289	715643110	60.18	9	153	715647197	27.32
4	260	715650859	60.18	9	9	715645615	28.42
4	238	715641527	60.18	9	1	715645602	30.62
4	241	715641661	60.28	9	23	715645655	31.62
4	302	715644369	60.38	9	22	715645650	32.42
4	301	715644279	60.38	9	20	715645643	32.72
4	237	715650511	60.38	9	21	715645646	32.72
4	251	715641892	60.38	9	19	715645641	32.82
4	246	715650671	60.38	9	18	715639226	32.82
4	247	715641785	60.38	9	17	715645636	32.92
4	273	715642565	60.38	9	15	715645634	33.02
4	275	715642660	60.38	9	16	715645635	33.42
4	265	715642229	60.38	9	14	715645626	34.52
4	290	715643132	60.38	9	12	715645622	34.62

4	268	715642248	60.38	9	232	715649929	34.92
4	292	715643436	60.38	9	233	715649931	34.92
4	282	715642849	60.38	9	262	715643697	34.92
4	293	715643550	60.38	9	167	715647621	35.02
4	299	715644183	60.38	9	169	715639596	35.02
4	297	715643913	60.38	9	171	715647625	35.02
4	312	715645133	60.38	9	173	715647627	35.02
4	308	715644857	60.48	9	2	715645606	35.62
4	41	715639347	60.78	9	4	715645608	35.72
4	194	715640726	60.78	9	10	715645616	35.72
4	209	715649807	60.78	9	273	715645097	36.02
4	211	715640822	60.78	9	11	715645621	36.32
4	212	715640824	60.78	9	96*	715646554	43.92
4	86	715639525	60.78	9	82	715646360	45.32
4	87	715639527	60.78	9	83	715646364	45.62
4	233	715641297	60.78	9	84	715646365	45.62
4	85	715639522	60.78	9	174	715647628	45.62
4	245	715641759	60.78	9	176	715647630	45.72
4	244	715650640	60.78	9	175	715647629	45.82
4	258	715642009	60.78	9	227	715649635	46.42
4	249	715641823	60.78	9	165	715647616	46.72
4	257	715641953	60.78	9	228	715649637	47.02
4	261	715650884	60.78	9	166	715647619	47.02
4	193	715640724	60.78	9	260	715642971	71.92
4	208	715640744	60.78	9	76	715639277	79.37
4	256	715641945	60.78	9	77	715639278	79.47
4	234	715650449	60.78	9	235	715641018	79.77
4	264	715642136	60.78	9	159	715647273	79.87
4	259	715642035	60.78	9	158	715647271	80.17
4	279	715642737	60.78	9	75	715639276	81.17
4	272	715642473	60.78	9	265	715644493	83.27
4	276	715642719	60.78	9	70	715646187	84.77
4	277	715642720	60.78	9	255	715650811	86.37
4	263	715642135	60.78	9	39*	715645749	88.37
4	266	715642242	60.78	9	276	715645176	88.97
4	286	715643068	60.78	9	36	715645741	89.37
4	267	715642243	60.78	9	37	715645742	89.37
4	270	715642408	60.78	9	261	715643256	92.87
4	271	715642440	60.78	9	264	715644186	92.97

4	294	715643649	60.78	9	268	715644807	92.97
4	278	715642723	60.78	9	188	715639831	95.57
4	295	715643777	60.78	9	246	715650427	96.97
4	296	715643900	60.78	9	247	715650429	96.97
4	300	715644271	60.78	10	154	715649822	0
4	304	715644432	60.88	10	66	715647383	0.8
4	303	715644377	60.88	10	1	715645496	14.75
4	306	715644628	60.88	10	16	715645532	22.5
4	280	715642740	60.88	10	15	715645530	22.5
4	307	715644837	60.98	10	17	715645533	22.6
4	231	715650318	62.48	10	18	715645534	22.7
4	128	715648246	65.08	10	14*	715645529	23
4	192	715640694	65.18	10	11	715645516	24.4
4	129	715639824	65.28	10	88	715647918	26.5
4	130	715639827	65.68	10	208	715641982	57.56
4	287	715643084	66.18	10	236	715643118	58.66
4	8	715639219	66.48	10	123	715640116	61.96
4	7	715639218	67.28	10	106	715639888	68.7
4	6	715639217	67.78	10	72	715639704	68.8
4	5	715639216	68.28	10	148	715649653	69.2
4	4	715639215	68.78	10	143	715640510	69.2
4	291	715643289	72.18	10	73	715647865	69.6
4	140	715640004	72.98	10	232	715643007	70.8
4	310	715644925	73.08	11	227	715650383	0
4	309	715644924	73.08	11	228	715641318	0.1
4	311	715644971	73.48	11	4*	715645482	3.3
4	283	715651054	74.98	11	1	715645474	8.92
4	285	715651056	74.98	11	333	715645010	9.72
4	284	715651055	74.98	11	3	715639211	10.92
4	166	715649204	75.58	11	151	715649044	12.02
4	122	715648139	77.08	11	150	715649043	12.52
4	21	715646135	79.58	11	14	715645562	17.52
4	17	715646130	80.38	11	16	715645565	18.62
4	16	715646128	83.58	11	15	715645564	21.72
4	164	715649179	85.18	11	17	715645566	22.12
4	153	715648778	85.98	11	9	715645540	26.22
4	13	715645811	91.4	11	10	715645555	29.32
4	12	715645808	93.82	11	11	715645558	29.62
4	11	715645803	94.42	11	13	715645560	30.42

4	9	715645801	95.52	11	157	715649142	32.02
4	10	715645802	95.62	11	156	715649141	32.32
4	81	715647354	100.84	11	155	715649139	32.42
4	78	715647349	102.04	11	158	715649143	32.72
4	75	715647345	102.44	11	25	715646672	35.32
4	76	715647346	102.74	11	279	715642797	41.32
4	83	715647360	103.54	11	312	715644136	41.72
5	236	715640449	0	11	268	715650945	47.95
5	331	715643586	0.3	11	27	715647103	54.49
5	237	715649413	0.9	11	23	715639348	55.09
5	238	715640451	1	11	22	715639328	55.19
5	134	715647826	3.1	11	55	715639657	55.29
5	135	715647827	3.5	11	296	715643348	55.39
5	136	715647830	3.8	11	223	715650297	55.49
5	137	715639686	3.9	11	233	715641443	55.79
5	138	715639687	4.3	11	198	715649759	56.09
5	131	715647645	6.1	11	243	715650698	56.49
5	174	715648461	9.6	11	319	715644364	56.49
5	173	715648460	12.2	11	240	715641793	56.79
5	175	715648462	12.5	11	303	715643753	57.19
5	247	715649583	12.6	11	272	715642592	57.49
5	246	715649582	12.9	11	317	715644314	57.79
5	78	715646173	14	11	334	715645044	57.79
5	76	715646170	15.1	11	262	715642200	57.89
5	325	715643332	19	11	260	715642122	58.19
5	270	715650116	20	11	161	715640317	58.29
5	164	715648245	20.4	11	138	715640121	58.39
5	161	715648236	20.4	11	218	715641051	58.69
5	162	715648238	20.7	11	235	715641446	59.09
5	163	715648242	21	11	280	715651023	59.09
5	244	715649504	22.1	11	51	715647606	60.99
5	168	715648340	23.5	11	52	715647612	61.39
5	268	715650069	23.9	11	69	715647871	61.99
5	269	715650070	23.9	11	162	715649229	62.49
5	245	715649545	24.9	11	98	715648198	62.89
5	209	715640177	25.8	11	250	715650778	63.29
5	208	715640176	25.9	11	251	715650779	63.29
5	142	715647938	27.8	11	83	715648016	63.39
5	160	715648076	28.1	11	82	715648015	63.39

5	253	715649793	28.1	11	81	715648010	63.39
5	152	715648062	28.1	11	78	715648006	63.39
5	159	715648075	28.9	11	77	715648005	63.39
5	156	715648067	29.5	11	199	715649818	63.49
5	155	715648065	29.6	11	101	715648207	63.59
5	154	715648064	29.6	11	204	715649895	63.69
5	153	715648063	29.6	11	203	715640807	63.69
5	157	715648068	29.7	11	212	715650163	63.79
5	158	715648070	30.1	11	215	715650167	64.19
5	320	715643155	30.2	11	106	715648254	64.99
5	319	715651069	30.2	11	105	715648253	65.09
5	217	715649118	30.5	11	107	715648255	65.09
5	215	715649114	30.6	11	104	715648252	65.09
5	218	715649120	30.7	11	111	715648268	65.19
5	214	715649113	30.7	11	103	715648248	65.29
5	213	715649111	30.8	11	270	715650948	65.39
5	277	715650412	30.9	11	109	715648265	65.39
5	216	715649115	30.9	11	110	715648266	65.39
5	191	715648734	31.3	11	269	715650947	65.49
5	188	715648673	31.3	11	146	715648952	65.59
5	190	715648675	31.3	11	147	715648956	65.59
5	148	715648053	31.3	11	145	715648950	65.59
5	192	715648740	31.4	11	142	715648944	65.59
5	140	715647879	31.8	11	141	715640153	65.59
5	189	715648674	32.1	11	124	715648809	65.59
5	149	715648057	32.4	11	271	715650949	65.69
5	150	715648058	32.4	11	144	715648947	65.69
5	255	715649849	32.7	11	143	715648946	65.69
5	259	715649855	32.8	11	123	715648808	65.69
5	257	715649853	32.8	11	301	715651146	65.99
5	256	715649851	32.8	11	126	715648813	65.99
5	258	715649854	32.9	11	48	715647554	65.99
5	254	715649846	33	11	43	715647547	65.99
5	151	715648059	33	11	45	715647550	65.99
5	194	715648750	33.1	11	47	715647552	66.29
5	193	715648748	33.1	11	41	715647544	66.29
5	186	715648669	33.1	11	38	715647535	66.29
5	185	715648668	33.1	11	46	715647551	66.39
5	184	715648666	33.2	11	40	715647542	66.39

5	145	715648048	33.3	11	44	715647549	66.39
5	187	715648672	33.3	11	125	715648812	66.49
5	144	715648046	33.3	11	50	715647558	66.89
5	146	715648051	33.3	11	32	715647459	67.19
5	195	715648751	33.4	11	33	715647460	67.19
5	147	715639762	33.4	11	49	715647555	67.19
5	141	715647881	33.7	11	34	715647462	67.19
5	139	715639709	33.7	11	42	715647546	67.29
5	132	715639624	33.8	11	36	715647467	67.29
5	127	715639543	34.1	11	37	715647468	67.59
5	128	715647421	34.5	11	35	715647463	68.39
5	120	715647319	35.1	11	170	715649352	69.19
5	121	715647322	35.1	11	245	715650720	69.19
5	119	715647318	35.5	11	244	715650717	69.29
5	118	715647316	35.8	11	239	715650600	70.09
5	115	715647313	35.9	11	129	715648857	70.89
5	116	715647314	36	11	128	715648856	71.29
5	117	715647315	36	11	148	715640170	71.59
5	114	715647312	36.1	11	92	715648090	73.39
5	196	715648900	37.2	11	87	715648079	73.79
5	198	715640122	37.6	11	88	715648080	73.89
5	197	715648902	37.7	11	89	715648082	74.19
5	123	715647325	38.1	11	90	715648083	74.29
5	122	715647323	38.1	11	196	715649719	77.09
5	124	715647326	38.4	11	152	715649064	77.09
5	126	715647332	38.5	11	95	715648098	77.19
5	125	715647330	38.5	11	197	715649721	77.29
5	179	715648583	39.3	11	163	715649249	77.39
5	178	715648582	39.4	11	165	715649251	77.49
5	311	715642710	39.8	11	167	715649254	77.59
5	279	715641556	40.1	11	166	715649253	77.69
5	317	715643140	40.2	11	61	715647768	78.29
5	282	715650679	40.3	11	178	715649459	81.09
5	266	715650039	40.3	11	177	715649457	82.19
5	267	715650040	40.6	11	112	715648343	82.49
5	227	715649287	40.7	11	113	715648346	82.59
5	278	715641555	40.7	11	114	715648349	82.69
5	228	715640336	40.7	11	115	715648350	82.69
5	226	715649286	40.7	11	247	715641910	83.49

5	169	715639884	41	11	67	715639694	83.79
5	330	715651124	41.3	11	254	715650816	83.89
5	210	715649033	41.3	11	174	715649383	84.19
5	343	715644386	41.4	11	175	715649384	84.29
5	281	715641778	41.4	11	211	715641015	84.39
5	314	715651049	41.4	11	172	715640405	84.69
5	323	715643265	41.5	11	248	715641911	85.49
5	315	715643035	41.5	11	246	715650748	85.79
5	110	715639461	41.6	11	173	715640406	87.59
5	326	715643341	42	11	224	715650328	87.89
5	353	715645111	42.3	11	160	715640315	88.49
5	348	715644982	42.3	11	182	715649519	88.79
5	337	715643869	42.3	11	201	715640757	89.19
5	300	715642428	42.3	11	200	715640756	89.29
5	288	715642068	42.4	11	189	715640612	89.39
5	284	715641929	42.4	11	202	715640758	89.49
5	341	715644173	42.5	11	191	715649591	89.79
5	332	715643605	42.5	11	192	715649592	90.19
5	285	715650769	42.5	11	190	715640613	90.79
5	260	715649860	42.5	End			

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CHAPTER II

Genome wide association study and identification of candidate genes for morpho-agronomic traits in the common bean RIL population Rudá x AND 277

Abstract

Morpho-agronomic traits have been the main targets of common bean breeding programs worldwide. To meet the demands of modern agriculture, breeders are developing beans with upright architecture, high and stable seed yield, and adequate seed size for the consumer market. This study aimed to use genome wide association study (GWAS) in the common bean recombinant inbred line (RIL) population derived from the cross Rudá × AND 277 to identify quantitative trait loci (QTL) related to seven morpho-agronomic traits. This analysis was made with 3,060 single-nucleotide polymorphisms (SNPs) and 376 common bean RIL, evaluated in field condition for the traits number of days to flowering (DF), number of days to maturity (DM), plant architecture (ARC), seed yield (YLD), degree of seed flatness (SF), seed shape (SS), and 100-seed weight (SW). To gain insights on the biological function of these QTLs, we studied the region surrounding significant SNPs to find candidate genes that explain each trait. In all *Phaseolus vulgaris* chromosomes (Pv), apart from Pv06 and Pv11, we identified 112 SNPs/QTLs associated with these traits. Seventeen of them were colocalized GWAS peaks related to the traits DF, DM, ARQ and YLD. Thirty-three positional candidate genes that explained the SNP-trait associations were described. These include the candidate genes *Phvul.001G168000* on Pv11, associated to a delay in maturation and stem lignification, and *Phvul.008G245300* on Pv08, associated to seed yield. This study allowed us to dissect the genetic architecture of these seven morpho-agronomic traits and the SNPs are potential to be used in marker-assisted breeding, and is a relevant step in the development of tools for molecular breeding of common bean in Brazil.

Keywords: Recombinant inbred line; Single nucleotide polymorphism; Quantitative trait loci; Gene annotation.

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is cultivated and consumed worldwide, mainly in the developing countries of Latin America, Africa and Asia. It is an important source of proteins, fibers, minerals and vitamins especially for the neediest population (Gepts et al. 2008). Several factors limit the productivity and production of beans in Brazil. Among them, the diseases that affect the crop and the lack of cultivars with suitable plant architecture that allow the machine harvesting with lower losses stand out. These traits, in addition to grain yield, have been the major targets of bean breeding programs (Tar'an et al. 2002; Kamwfa et al. 2015a).

An auxiliary approach to plant breeding is marker-assisted selection (MAS). The abundance of DNA markers in the common bean genome indicates genome-wide association studies (GWAS) as a potential tool for marker-assisted breeding (Perseguine et al. 2014; Cichy et al. 2015; Zuiderveen et al. 2016). These studies are based on the fact that a large number of markers distributed along the genome increases the probability that the QTLs of interest are in strong linkage disequilibrium with the markers and thus detected (Rafalski et al. 2010).

With the genome sequencing of common bean (Schmutz et al. 2014), each QTL detected can be physically located in the genome. The QTL region may be within or close to a candidate gene. A candidate gene is a sequenced gene that has a biological function known to be directly or indirectly involved in phenotypic expression (Cichy et al. 2015; Hoyos-Villegas et al. 2015; Paes et al. 2016; Pantalião et al. 2016).

In this context, Moghaddam et al. (2016) identified a QTL for plant architecture within the genomic region of the candidate gene *Phvul.007G246700*, which has a homolog in *Arabidopsis thaliana* (*AtPME4I*) that encodes an enzyme involved in altering cell wall rigidity, and may therefore be used to develop more upright plants. Some candidate genes related to phenological traits have also been reported in common bean. The *Phvul.001G221100* gene on chromosome 11, related to the QTL for number of days to flowering, is homologous to the genes *phyA* (*Phytochrome A*) and *GmPhyA3*, involved, respectively, in photoperiod sensitivity and flowering in *Arabidopsis* and soybean (*Glycine max* L.) (Kamfwa et al. 2015a). The gene *Phvul.011G158300*, related to a QTL for number of days to harvest, is homologous to the *SHL* gene in *Arabidopsis*, involved in flowering and senescence (Moghaddam et al. 2016).

Other QTLs close to or within candidate gene regions have been reported for common bean. These include candidates involved in drought tolerance mechanisms (Hoyos-Villegas et al. 2015), cooking time (Cichy et al. 2015), seed weight (Moghaddam et al. 2016), symbiotic nitrogen fixation (Kamfwa et al. 2015b), and resistance to pathogens causing anthracnose and angular leaf spot (Perseguine et al. 2014; Zuiderveen et al. 2016).

The different RIL populations developed for QTL mapping in common bean generally consist of less than 200 lines and are considered small, apart from one population described by Oblessuc et al. (2012), consisting of 346 RILs. Small populations compromise the accuracy of recombination estimates between loci, and the detection and estimation of QTL effects (Collard et al. 2005; Casañas et al. 2013).

In this context, the Common Bean Breeding Program of the Universidade Federal de Viçosa (UFV) (Viçosa, MG, Brazil) developed a population of 393 RILs, derived from the inter-genepool cross between Rudá (Mesoamerican) and AND 277 (Andean), called RA RILs (Sanglard et al. 2013; Silva et al. 2016). In common bean, this is the largest RIL population developed so far for genetic mapping and QTL detection. We performed a GWAS in this population to detect QTLs related to traits of phenology, plant architecture, seed yield, and seed size, and to identify new candidate genes related to these QTLs. The GWAS, associated to candidate genes, is a potential tool to dissect the genetic architecture of important economic traits in this Mesoamerican x Andean population.

2. Material and methods

2.1 Plant material

A cross between cultivar Rudá and landrace AND 277 was performed and the F_1 hybrid plants were identified by the flower color, which is white (recessive phenotype) in the female parent Rudá and pink (dominant phenotype) in the male parent AND 277. The F_1 seeds were sown in a greenhouse, and F_2 plants were advanced to the F_{10} generation by the single-seed descent method (Sanglard et al. 2013).

The cultivar Rudá (Landrace A285) of Mesoamerican origin was developed at CIAT (International Center for Tropical Agriculture, Cali, Colombia) from the cross Carioca × Rio Tibagi. It was introduced in Brazil by Embrapa Arroz e Feijão (Santo Antônio de Goiás, GO, Brazil) in 1995 (Embrapa 2014). Rudá belongs to the carioca grain class, which is the most widely consumed bean type in Brazil (market share of

70%). Landrace AND 277 was also developed at CIAT by crossing [(Cargabello × (Pompadour Checa × Linea 17)) × (Linea 17 × Red Cloud)]. It is a source of the gene of resistance to angular leaf spot (gene *Phg-1*) and an Andean red-mottled bush bean (Aggarwal et al. 2004). The cross of Rudá × AND 277 involved parents from two different gene pools and two genetically divergent parents in terms of agronomic traits (Silva et al. 2016) and molecular characterization (Grisi et al. 2007; Souza et al. 2012).

2.2. Morpho-agronomic characterization

The RA RILs was field-tested at an experimental station of the Department of Plant Science of the Universidade Federal de Viçosa, in Coimbra, Minas Gerais, Brazil (latitude 20°50'30" South, longitude 42°48'30" West, 720 m asl) in the winter of 2012. On a total of 395 plots, 393 RILs and the parents Rudá and AND 277 were tested in a randomized block design with additional controls with three replications. In each experimental plot, 30 plants were grown, distributed in two 1.0-m rows spaced 0.5 m apart, with 15 plants per row.

The following morpho-agronomic traits were evaluated: number of days to flowering (DF); number of days to maturity (DM) or crop cycle; and seed yield (YLD), in kg ha^{-1}, of plants at physiological maturity (when 90% of the pods were yellow-green to brown); 100-seed weight (SW) in gram, randomly chosen per plot; degree of seed flatness (SF), given by the ratio between the seed thickness and width; and seed shape (SS), given by the ratio between the seed length and width of five randomly chosen seeds per plot. The plant architecture (ARC) of each plot was evaluated at physiological maturity, based on a scale proposed by Collicchio et al. (1997), scoring more upright plants with lower grades. The data of days to flowering, days to maturity, seed yield, and plant architecture were based on evaluations of all plants per plot.

2.3. Genotyping and alignment of SNP markers

Plants of RA RILs and their parents were grown in a greenhouse. Only 376 of all 393 RILs could be genotyped. The DNA was extracted from bulk samples consisting of the leaf tissue of 10 plants per RIL and parents. The commercial Invisorb® Spin Plant Mini Kit was used for DNA extraction and purification, according to the manufacturer's instructions. The plants were genotyped in the Soybean Genomics and Improvement Laboratory, USDA- ARS/BARC-W (Beltsville, MD, USA), using the

BARBean6K_3 Illumina BeadChip, resulting in 5,398 SNPs. The procedures of genotyping with the Illumina Infinium® HD Assay Ultra protocol were applied as described by Song et al. (2015). The SNP allele for each genotype was called with software Genome Studio v2011.1 (Illumina, San Diego, CA, USA). To obtain pre-information about the chromosome to which each SNP marker was linked, the sequences containing informative SNPs were aligned against the common bean reference genome (genotype G19833) (Schmutz et al. 2014), available at Phytozome (http://www.phytozome.net/commonbean.php) by version 1.0 BlastN of the CLC Genomics Workbench version 5.5.

2.4. Phenotypic data analyses

Analysis of variance was carried out for each trait in a randomized block design with additional controls, i.e., the RILs and two controls (parents Rudá and AND 277), based on the model:

$$Y_{ij} = \mu + t_i + b_j + e_{ij}$$

where: Y_{ij} is the observation (such as days to flowering) of treatment *i* in block *j*; μ is the overall mean; t_i the effect of the i^{th} treatment, considered as random effect; b_j the effect of block *j*, where bj ~ NID (0, σ^2_b); and e_{ij} is the random error, where e_{ij} ~ NID (0, σ^2). The population distributions were evaluated for normality, and the mean values of each trait were analyzed by Pearson's correlation analysis, using software Genes (Cruz 2016).

2.5. Marker-trait association analysis

In the genotyping quality control, only the SNPs with allele calling quality higher than 0.91, minor allele frequency (MAF) higher than 0.05 and not monomorphic between the parents were used for association analysis. Genome association studies were performed using the following mixed model (Zhang et al. 2010; Kang et al. 2010):

$$Y = Zu + Ma + e$$

where: Y represents the vector of the pre-corrected phenotypic observation for each plant; u is the vector of random polygenic effect for each plant, $u \sim N(0, G\sigma^2_u)$, where G is the additive genomic relationship matrix; a represents the fixed additive SNP effect, and e the residual vector, $e \sim N(0, I\sigma^2_e)$. The matrices Z and M represent the incidence matrices for u and a, respectively. The polygenic effect was used to identify a possible sub-population structure (e.g. family). The presented model was fitted using the GWAS function of the rrBLUP package of R software (R Core Team 2015).

To draw conclusions on the statistical significance of each SNP, the reported model was compared with the null model (no SNP effects) by using the likelihood ratio test (LRT) considering a general null hypothesis (H_0: no marker effect). For these LRT values, chi-square ($\chi 2$)-distribution is assumed with D degrees of freedom, where D is the difference between the numbers of parameters of the two compared models. The FDR (False Discovery Rate) method was used to adjust the P-values for multiple tests, since each SNP was tested separately in the model. Alternatively, a less stringent cut-off threshold based on the logarithm of detection (LOD) value of three was established, since LOD and LRT are related (LOD = 0.2171 x LRT = - $\log_{10}$ (P-value)). By the LOD value of three, the probability of the reported model is 1,000 times greater than the probability of the null model (no SNP effects). Final Manhattan plots were created using the mhtplot () function from R package gap (Zhao 2007). The GWAS peak was ranked from the most to the least significant p-value for each trait and identified according to the abbreviation of the trait (e.g., DF); followed by the serial number (e.g., DF1 and DF2) (Supplementary material - Table S1).

We exploited the functions of the candidate genes underlying the significant SNP markers identified by GWAS. The primary focus was on SNPs falling in the P-value obtained by the LRT, and secondly on SNPs falling in the less stringent cut-off threshold (LOD = 3). The SNP sequences in FASTA format were obtained from Song et al. (2015) and used to perform a BLAST search (Zhang et al. 2000) against the *Phaseolus vulgaris* 2.1 reference genome using Jbrowse on Phytozome 12.0 (Goodstein et al. 2012).

To identify candidate genes, we searched within 50 Kb upstream and downstream of each significant SNP region in the genome browser. The position of the candidate gene and the distance from the SNP were calculated as well as the genome data version 1.0 available on “Bulk data” from Phytozome 12.0

(https://phytozome.jgi.doe.gov/pz/portal.html#!bulk?org=Org_Pvulgaris), once the SNPs were aligned against this version (1.0) in the SNP alignment. In the "Bulk data", the correspondent homolog of the candidate gene in *Arabidopsis thaliana* is available. This homolog was used to search a possible role of the candidate gene in the control of a trait associated to SNP, by researching this homolog on The Arabidopsis Information Resource (TAIR) (Rhee et al. 2003).

3. Results and discussion

3.1. Phenotypic data analyses

All traits measured were normally distributed, suggesting that all of them were inherited in a quantitative manner (Fig. 1). In the analysis of variance, the experimental variation coefficients (CV%) were low (Table 1), ranging from 2.22 for DM to 17.58% for ARQ, indicating high experimental precision. The significant effect ($P < 0.01$) of the RILs for all studied traits identified genetic variability, important to detect marker-trait associations. Although no significant effect of the source of variation of the parents was observed for DM and YLD, it can be concluded that the parents are also divergent for these traits, as indicated by their significant source of variation in the RILs. The contrast of RILs versus parents was significant ($P < 0.01$) for the traits DF, DM, YLD, and SW, indicating additive x additive epistatic interaction. High narrow-sense heritability was observed for all traits, from 82.81 for ARC to 97.09% for SW (Table 1). The means of 393 RILs for the seven traits under study were compared to the means of the parents (Rudá and AND 277) by Dunnett's test at 5% probability, and grouped in mean classes statistically equal to or different from those of the parents (Table 2). Only for trait SW, no RIL was found that exceeded the limits of the parents statistically.

The phenological traits (DF and DM) were significantly correlated with the others, with exception of SS (Table 3). A highly significant positive correlation ($r = 0.76$; $P < 0.001$) was observed between DF and DM, indicating that, as expected, RILs with delayed flowering also had delayed maturation. Both traits were positively correlated with ARC, indicating that RILs with a delay in reaching the reproductive and senescence stages tend to have a non-erect architecture, characterized by a high score in ARC. The negative correlation between YLD with DF and with DM can be attributed to RILs that significantly delay flowering but do not delay maturation in the same proportion, resulting in a short seed-filling period.

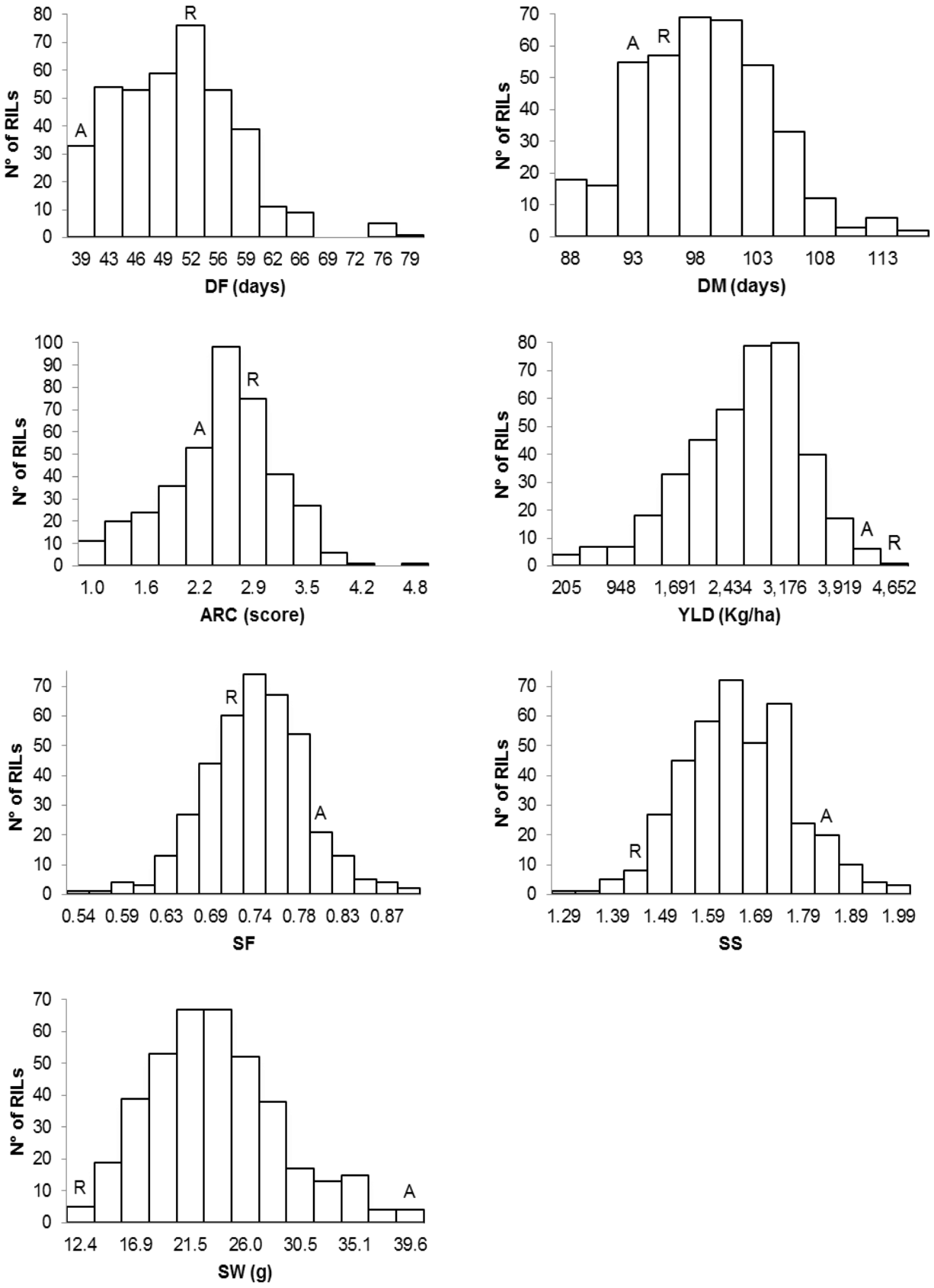


Fig. 1 Population distributions for the seven morpho-agronomic traits in the RA RIL population. Letters 'A' and 'R' indicate the phenotypic means of parents AND 277 and Rudá, respectively

Table 1 Mean square among blocks and RILs, estimated means (μ), heritability based on the mean of the RILs (H^2), and maximum (Max) and minimum (Min) range of the RA RILs

SV	df	Mean squares[a]						
		DF	DM	ARQ	YLD	SF	SS	SW
Blocks	2	164.57	45.23	7.17	12,313,995.90	0.0067	0.019	70.67
RILs	392	156.47**	82.16**	1.32**	1,935,032.23**	0.0089**	0.041**	89.21**
Parents (Pa)	1	240.67**	2.67^{ns}	1.04*	37,040.82^{ns}	0.0136**	0.235**	2003.27**
RILs *vs.* Pa	1	86.89**	88.48**	0.012^{ns}	18,122,690.18**	0.0021^{ns}	0.0002^{ns}	689.06**
Error	788	6.39	4.99	0.23	210,563.99	0.0011	0.0032	2.59
CV %		4.79	2.22	17.58	16.16	4.52	3.34	6.50
μ Parents		49	96.67	2.75	4,573.81	0.77	1.68	35.46
μ RILs		52.82	100.52	2.70	2,831.45	0.75	1.69	24.72
H^2 %		95.91	93.93	82.81	89.12	87.27	92.23	97.09

**, *: significant at 1 and 5% probability, respectively, by the F test

^{ns}: not significant

[a] DF: number of days to flowering; DM: number of days to maturity; ARC: plant architecture; YLD: seed yield (kg/ha); SF: degree of seed flatness; SS: seed shape; SW: seed weight (gram)

Table 2 Grouping of RILs in different mean classes in relation to the parent mean, by the Dunnett test for each of the seven morpho-agronomic traits in the morpho-agronomic characterization of the RA RILs

	Number of RILs							
	Equal[b]			Different[c]			Parent mean	
Traits[a]	A	B	AB	Greater	Smaller	Between A-B	AND 277 (A)	Rudá (B)
DF	127	221	0	37 > (B)	0 < (A)	8	42.67	55.33
DM	7	44	196	134 > (B)	12 < (A)	0	96.00	97.33
ARQ	63	53	267	2 > (B)	8 < (A)	0	2.33	3.17
YLD	32	0	63	0 > (B)	298 < (A)	0	4,495.24	4,652.38
SF	55	140	186	3 > (A)	9 < (B)	0	0.81	0.72
SS	127	105	0	1 > (A)	1 < (B)	159	1.88	1.48
SW	0	100	0	0 > (A)	0 < (B)	293	53.73	17.19

[a] DF: number of days to flowering; DM: number of days to maturity; ARC: plant architecture; YLD: seed yield (kg/ha); SF: degree of seed flatness; SS: seed shape; SW: seed weight (gram)
[b] Equal: number of lines with means statistically equal to the parents AND 277 (A), Rudá (B) and both (AB) (Dunnett, P <5%)
[c] Different: number of lines with higher mean than the parent with highest mean (Higher); number of lines with lower mean than the parent with lowest mean (Lower); and number of lines with mean between the means of the two parents (Between A-B) (Dunnett, P < 5%)

Table 3 Pearson correlation coefficients among seven morpho-agronomic traits measured on RA RILs

Traits[a]	DM	ARQ	YLD	SF	SS	SW
DF	0.76***	0.30***	-0.44***	-0.10*	-0.04 [ns]	-0.48***
DM		0.11*	-0.48***	0.02[ns]	0.01 [ns]	-0.35***
ARQ			0.21**	-0.21**	0.09 [ns]	0.12*
YLD				-0.01 [ns]	0.09 [ns]	0.45***
SF					0.00 [ns]	-0.12*
SS						0.37***

[a] DF: number of days to flowering; DM: number of days to maturity; ARC: plant architecture; YLD: seed yield (kg/ha); SF: degree of seed flatness; SS: seed shape; SW: seed weight (gram)
***, **, *: significant at 0.1, 1 and 5% probability level, respectively, by the t test
[ns]: not significant by the t test

This assumption can be validated by the fact that SW was negatively correlated with both DF (r = -0.48) and DM (r = -0.35), but positively correlated with YLD (r = 0.45). According to Kamfwa et al. (2015a), a similar correlation observed between phenological traits and yield was attributed to the presence of photoperiod-sensitive and late maturing genotypes in the population studied.

3.2. SNP selection and marker-trait associations

From a total of 5,398 SNP markers used in the genotyping of the RA RILs, 60.91% (3,288) had a minor allele frequency (MAF) > 0.05 and were informative, for being polymorphic in the mapping population and due to their high allele calling quality (> 0.91). Of these, 228 SNPs (6.93%) were eliminated because they were found to be monomorphic in the parents, resulting in 3,060 aligned SNPs distributed across the 11 common bean chromosomes (Table 4). The number of aligned polymorphic SNPs varied from 172 (Pv06) to 353 (Pv05) with a mean of 278 SNPs per chromosome. A mean of 5.9 SNPs/Mbp was found, ranging from a minimum of 3.81 SNPs/Mbp on Pv03 to a maximum of 8.65 SNPs/Mbp on Pv05 (Table 4).

The Manhattan plots (Fig. 2) show each point as a SNP distributed on the common bean chromosomes from left to right, and the heights correspond to the strength of association to morpho-agronomics traits. The association of each significant SNP, or GWAS peak, to a morpho-agronomic trait was considered a QTL. A total of 112 (64 + 48) significant SNPs (QTLs) were detected for the seven traits, at all Pvs, with exception of Pvs 06 and 11 (Table 4 and Fig. 2). 64 of the SNPs were above the FDR significant threshold (dotted line) and 48 only above LOD significant threshold (continuous line). The FDR threshold was established for all traits, with exception to SW in which the threshold was established only based in LOD=3 (Table 4). The SNP peaks ranged from seven (SW) to 22 (SF), and their distribution at chromosomes ranged from two (Pv 09) to 37 SNPs (Pv 01). This study generated a high density of SNP markers, as well as a high number of SNPs after filtering by polymorphism, MAF, and allele calling quality, enough to cover the entire common bean genome, thus laying the basis for a GWAS analysis.

A set of many significant SNPs were found for a same trait on a same chromosome, e.g., for DF (11+3 SNPs) on Pv01; SS (9+7 SNPs) on Pv02; SF (14+2 SNPs) on Pv05; and YLD (5+5 SNPs) on Pv08 (Table 4). However, a few significant SNPs were found

for other traits, e.g., for YLD (0+1) on Pv03, ARC (0+1) on Pv08, and DM (2+0) on Pv09. The different peaks for the seven traits in the RA RILs may be regions associated to both the Mesoamerican and the Andean gene pool. In general, these peaks suggest a combined expression, implying in a highly relevant physical region that should be studied. Thus, hypotheses about candidate genes associated with the seven traits in these regions were proposed

In the Q-Q plots (Fig. 2), the deviations from the identity line suggest that the sample contains values arising by a true association for each trait. In general, the Q-Q plot is a simple way to validate the results reported in the Manhattan plots. Thus, in the present results, it is possible to infer that this validation was successfully performed for both mentioned conditions. Furthermore, there is no inflation of observed statistics at the beginning of the trajectory, indicating that the inclusion of polygenic effect in the model used was able to adjust the GWAS for possible population structure effects.

Table 4 Number of aligned SNPs per chromosome and associated to each one of morpho-agronomic traits

Chromosome	Lengh (Mpb)	N° of aligned SNPs	SNPs/Mb	N° of significant SNP associated to trait[a]							
				DF	DM	ARC	YLD	SF	SS	SW	Total
Pv01	52.21	327	6.26	11+3[b]	5+3	10+1	-	-	-	0+4	26+11
Pv02	49.04	321	6.55	-	-	-	-	-	9+7	-	9+7
Pv03	52.28	199	3.81	1+5	5+4	-	0+1	-	-	-	6+10
Pv04	45.96	313	6.81	-	-	-	0+3	-	-	-	0+3
Pv05	40.82	353	8.65	-	-	-	-	14+2	-	-	14+2
Pv06	31.98	172	5.38	-	-	-	-	-	-	-	0+0
Pv07	51.76	249	4.81	-	-	0+2	-	-	-	0+3	0+5
Pv08	59.66	232	3.89	-	-	0+1	5+5	-	-	-	5+6
Pv09	37.47	276	7.37	-	2+0	-	-	-	-	-	2+0
Pv10	43.28	283	6.54	-	-	-	-	2+4	-	-	2+4
Pv11	50.37	335	6.65	-	-	-	-	-	-	-	0+0
Total	514.83	3,060	5.94	12+8	12+7	10+4	5+9	16+6	9+7	0+7	64+48

[a] DF: number of days to flowering; DM: number of days to maturity; ARC: plant architecture; YLD: seed yield (kg/ha); SF: degree of seed flatness; SS: seed shape; SW: seed weight (gram)

[b] In the sum in each column, the N° before and after the plus sign correspond to the number of significant SNPs by FDR and by LOD = 3 significant thresholds, respectively

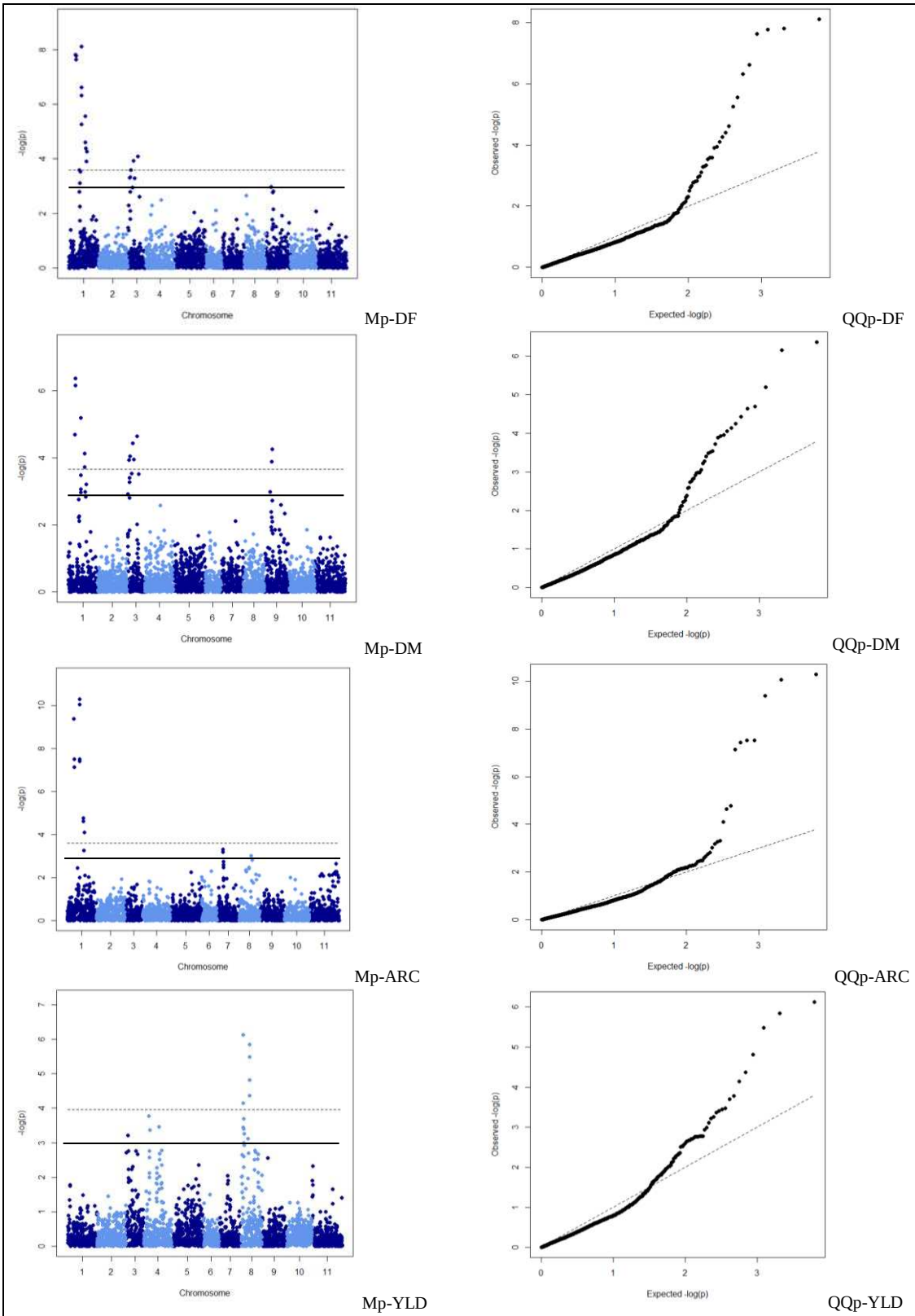
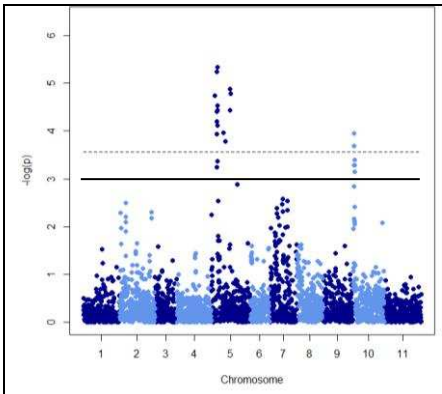
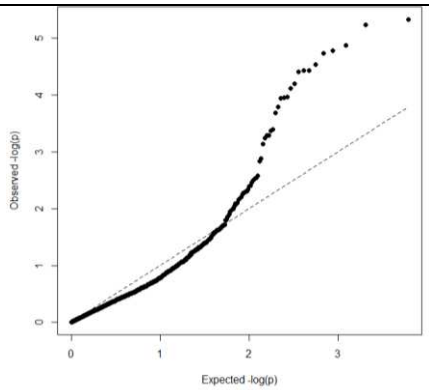


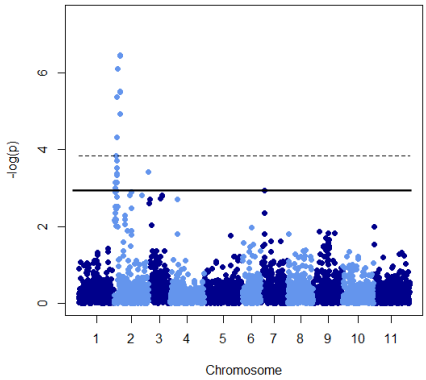
Fig. 2 Manhattan plots (Mp) and QQ-plots (QQp) from GWAS for DF: number of days to flowering; DM: number of days to maturity; ARC: plant architecture; YLD: seed yield (kg/ha); SF: degree of seed flatness; SS: seed shape; SW: seed weight (gram) in RA RIL population. In Manhattan plots (Mp), the dotted and continuous lines correspond to the threshold obtained from FDR and LOD=3, respectively.



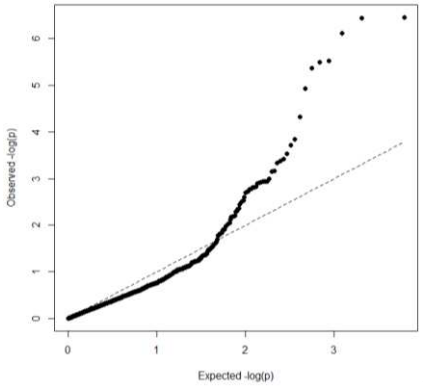
Mp-SF



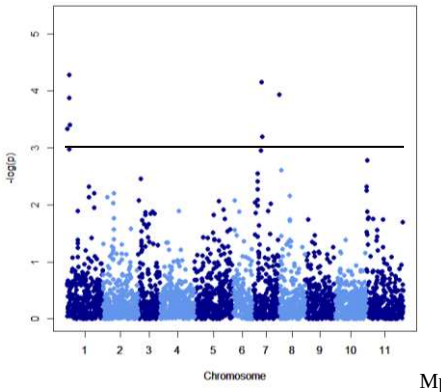
QQp-SF



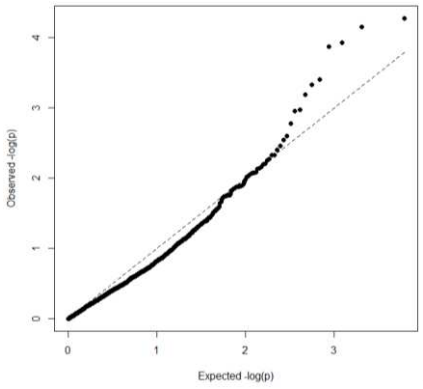
Mp-SS



QQp-SS



Mp-SW



QQp-SW

Fig. 2 Continued

Phenological traits (DF and DM)

For DF, GWAS peaks were detected across Pv01 (42.6 - 46.6 Mb) and at Pv03 (36.6 - 39.9 Mb). The four most significant ($P < 1.0 \times 10^{-7}$) peaks occurred at Pv01 (45 Mb). Several days to flowering QTLs were also found in previous work on Pv01, Pv04, Pv05, Pv06, Pv07, Pv08, Pv09, and Pv11 (Blair et al. 2006a; Pérez-Vega et al. 2010; Blair et al. 2012; Kamfwa et al. 2015a; Moghadam et al. 2016). Kanfwa et al. (2015a) also detected a GWAS peak upstream on Pv01 region (48.3 Mb) using the current genotyping chip.

For DM, significant SNPs were noted at Pv01 (42.6 - 45.8 Mb), Pv03 (36.6 - 39.7 Mb), and Pv09 (29.9 Mb). The two most significant ($P < 1 \times 10^{-6}$) SNPs were detected at Pv01 (45.2 Mb). Kamfwa et al. (2015a) identified only one significant SNP for DM on Pv01, in the 48.3 Mb region. Previous studies reported many QTLs for DM on Pv 02, Pv04, Pv05, Pv06, Pv07, Pv08, and Pv11 (Beattie et al. 2003; Blair et al. 2006a; Perez-Vega et al. 2010; Moghaddam et al. 2016) and one at Pv03 (Johnson and Gepts 2002).

Plant architecture (ARC)

A very strong Pv01 (42.9 - 46.0 Mb; $P < 1 \times 10^{-9}$) peak and weak Pv07 (45.9 - 48.6 Mb; $P < 1 \times 10^{-5}$) and Pv08 (40.6 Mb; $P < 1 \times 10^{-5}$) peaks were observed for ARC. The most significant ($P < 1.05 \times 10^{-9}$) peaks among traits were found for ARC at Pv01 (45 Mb). Previous studies reported QTLs and candidate genes related to plant architecture phenotypes in common bean detected by GWAS on Pv02 and Pv07 (Hoyos-Villegas et al. 2015; Moghaddam et al. 2016), but not on Pv01 and Pv08. The significant SNPs related to plant architecture reported by Moghaddam et al. (2016) and Hoyos-Villegas et al. (2015) were at 46.0 and 46.1 Mb on Pv07, respectively, surrounding the significant region of the RA RILs.

Seed yield (YLD)

The significant GWAS peaks for YLD were at Pv03 (39.5 Mb), Pv04 (0.59 - 1.0 Mb), and Pv08 (54.9 - 57.0 Mb). The Pv08 (55.9 - 56.7 Mb) had the three most significant ($P < 1 \times 10^{-5}$) GWAS peaks for YLD. Previous studies also identified QTL for YLD on Pv03, Pv04 and Pv08 (Blair et al. 2006a; Blair et al. 2012). Moreover, a

significant peak was reported at 38.2 Mb on PV03 (Kamfwa et al. 2015a), downstream of the significant peak in RA RILs on the same chromosome at 39.5 Mb.

Seed size traits (SF, SS, and SW)

Twenty-two GWAS signals were noted for SF at Pv05 (34.7 - 38.27 Mb) and Pv10 (41.0 Mb). Among them, the two most significant ($P < 1 \times 10^{-5}$) were at Pv05 (38.0 Mb). The peaks for SS were detected at Pv02 (46.1 - 48.1 Mb) only and the two strongest signals had a probability of less than 1.0×10^{-6}. Several QTLs related to seed length, width and thickness, aside from the ratio of some of these measures, were detected through linkage analysis on all chromosomes (Park et al. 2000; Blair et al. 2010; Pérez-Vega et al. 2010a; Yuste-Lisbona et al. 2014). Our study appears to be the first to report a set of marker-seed dimension measurements association on Pv05, including candidate genes that explain the trait, as will be detailed.

The GWAS peaks for SW were on Pv01 (51.3 Mb) and Pv07 (54.1 - 56.0 Mb). Previous studies reported QTLs detected on this same Pvs by linkage analysis (Blair et al. 2006a; Yuste-Lisbona et al. 2014). Other significant GWAS peaks for SW were related to Pv09 (Hoyos-Villegas et al. 2015), Pv03, Pv08, and Pv10 (Moghaddam et al. 2016). The mixed model selected a significant cutoff threshold based on LRT for all traits except SW.

3.3. Colocalization of GWAS peaks among traits

Twelve peaks on Pv01, spanning from 42.6 to 46.0 Mb, were shared by the traits DF, DM, and ARC. According to Koinange et al. (1996), plant height is associated with the indeterminacy gene (*fin*) on linkage group B01 (Pv01) and the linkage of the indeterminacy gene with loci for flowering time and photoperiod response. On Pv 03 (36.6 - 38.2 Mb), four peaks for DF were in common with those for DM, and one peak, in the 39.5-Mb region, was the same for DF, DM and YLD. In addition, Tar'an et al (2002) reported that a flowering time QTL was associated with a yield QTL, though with no significant correlation, on linkage group B09 (Pv09), in a cultivated x cultivated Mesoamerican recombinant inbred line population.

The colocalization of GWAS peaks among traits indicated that SNP is associated with genes that have a pleiotropic effect on these traits, or that this SNP may be in linkage disequilibrium with linked genes controlling them, since correlation

analysis detected correlations among the traits (Table 3) (Aastveit and Aastveit 1993). Working with the same BARCBean6K_3 BeadChip, Kamfwa et al. (2015) also detected significant SNP for DF and DM on PV01, but in the 48.3-Mb region, and a significant correlation between them. A significant correlation and colocalized GWAS signal in the Pv 07 (46.0 Mb) region were also observed by Moghaddam et al. (2016) for the traits lodging, canopy height and growth habit, all related to plant architecture.

3.4. Candidate genes associated with significant SNPs

Once 112 GWAS peaks had been detected, we looked for candidate genes focusing only on the peaks established by the FDR threshold and on that above the LOD threshold when colocalized to the first. The Supplementary material - Table S2 shows each GWAS peak associated to candidate genes, as detailed below.

Plant architecture, days to flowering and to maturity

These three traits exhibited 17 colocalized GWAS peaks, so we tried to explore the role of candidate genes that explain these traits, including seed yield in one colocalized peak. Furthermore, the traits were significantly correlated with each other (Table 3). Genes involved in vegetative to reproductive phase transition, embryo formation, biosynthesis of hormones related to photoperiod and plant growth, and cell wall lignification were considered to explain these traits.

A Pv01 (42.6 Mb) GWAS peak is shared between DF12 and DM19 and is near the candidate gene *Phvul.001G165800*, homolog of *Arabidopsis thaliana ATP-BINDING CASSETTE G40* (*AtABCG40*). *ABCG* is a transporter family involved in abscisic acid (ABA) transport from the endosperm to the embryo and in the control of seed germination. The ABA level in seeds is important once mutant seeds unable to synthesize ABA have no dormancy, i.e., they germinate precociously and may even germinate while still attached to the mother plant. This phenomenon, termed vivipary, causes economic losses in commercial crops (Robertson 1955; Kang et al. 2015).

Another colocalized (DF10; ARC14) peak on PV01 (42.9 Mb) was mapped near the *YUCCA8* homolog (*Phvul.001G168000*). The *YUCCA* family of flavin monooxygenase is involved in the auxin biosynthetic process. The overexpression of *YUCCA 8* led to a number of auxin-related phenotypes, such as elongated hypocotyls and petioles and an increase in stem diameter, attributed to pronounced cell expansion

growth, followed by an increase in the degree of lignification (Hentrich et al. 2013). Plants overexpressing a member of the *YUCCA* family also had delayed leaf senescence after flowering, mainly due to the continuous production of new lateral shoots during the reproduction phase (Kim et al. 2011).

Phvul.001G174200, a homolog of *REVEILLE8* (*RVE8*), is a candidate on the Pv01 (43.6 Mb) colocalized (DF14; ARC10) peak. *RVE8* encodes a *MYB*-like transcription factor involved in the regulation of circadian clock. The phenotype of *Arabidopsis* mutants for this gene (*rve*) was compared with the wild type, with longer hypocotyls, more aerial biomass and more rosette leaves that lead to a slightly delayed flowering (Gray et al. 2017).

A colocalized signal on Pv01 (44.3 Mb) for DF9, DM8, and ARC9, was mapped next to the *PHOSPHO-GLYCOPROTEIN1* (*PGP1*) homolog (*Phvul.001G179300*). A mutation in this gene was associated to a delayed phototropic response of the stem, affecting seedling hypocotyl elongation (Sato et al. 2014; Sato et al. 2015).

Two colocalized peaks 40 Kb apart, one (DF3; DM1; ARC5) downstream, and the other (DF4; DM2; ARC7) upstream of the homolog *PETAL LOSS* (*PTL*) (*Phvul.001G187000*) were observed on PV01 (45.2 Mb). *PTL* encodes a trihelix transcription factor (*GT* factor) involved in light responses and other plant developmental processes. An *Arabidopsis* mutant of a *GT-2* factor gene (*ptl-D*) resulted in pleiotropic phenotypes including dwarfism, curly leaves, stunted growth, and male sterility (Li et al. 2008).

Phvul.001G191600 and *Phvul.001G192000*, homologs of *CELL WALL INVERTASE4* (*CWINV4*), and *NAC domain-containing protein 90* (*NAC90*), respectively, are candidates within and upstream from the Pv01 (45.7 Mb) colocalized (DF2; DM4; ARC3) peak, respectively. *CWINV4* appears to function as a cell wall-localized invertase that can catalyze the hydrolysis of sucrose into fructose and glucose, based on the phenotype of *cwinv4* mutants. A specific invertase of cotton (*Gossypium hirsutum* L.), named *GhCWIN*, may play an important role in generating glycose signals to stimulate the endosperm nuclear division and embryonic provascular formation (Wang and Ruan 2012). The natural *CWIN* mutant of maize (*Zea mays*), named Miniature-1 (*Mn1*), shows an interruption of the transport of photoassimilates into the developing kernel, resulting in a miniature seed phenotype (Miller and Chourey 1992). *NAC*-domain genes codify a class of transcription factors known to control multiple

processes in plants, including flowering time, leaf senescence, and plant architecture. One of these *NAC*-domain genes called *Long Vegetative Phase 1* (*LOV1*), functions as a flowering repressor by negatively regulating the expression *of Constans* (*CO*), a floral promoter that acts in the photoperiod pathway. *Arabidopsis* with a mutated *LOV1* domain overexpressing the *NAC*-domain protein gene had a late-flowering phenotype (Yoo et al. 2007). Xu et al. (2012) obtained transgenic switchgrass (*Panicum virgatum* L.) expressing constitutively *LOV1* from Arabidopsis. Overexpression of *AtLOV1* in switchgrass induced a smaller leaf angle in plants by changing the morphology and organization of epidermal cells in the leaf collar region, altering the lignin content and monolignol composition of cell walls, and causing delayed flowering. *NAP* is another gene encoding a *NAC* family transcription factor associated with the senescence process of *Arabidopsis* rosette leaves. Two T-DNA insertion lines of this gene displayed a significantly delayed leaf senescence phenotype, given the lower expression or absence of *NAP* transcript in these lines. In contrast, inducible overexpression of *NAP* tends to cause early senescence. The regulation of leaf senescence by homologs of *NAP* in common bean (*PvNAP*) and rice (*OsNAP*; *Oryza sativa*) is the same as in *Arabidopsis* (*AtNAP*) (Guo et al. 2006).

The common bean homolog of *SPINDLY* (*SPY*), *Phvul.001G192300*, is mapped near the Pv01 (45.8 Mb) colocalized (DF5; DM16; ARC1) GWAS peak. *SPY* acts as both a repressor of gibberellin (GA) responses and as a positive regulator of cytokinin signaling, playing a key role in regulating plant development, cell differentiation, cytokinin-activated signaling pathway, flower development, and rhythmic *or* circadian processes (Steiner et al. 2016).

Phvul.001G193400, a homolog of *SHORT-CHAIN DEHYDROGENASE/REDUCTASE 2* (*SDR2*), is a candidate on the Pv01 (45.9 Mb) colocalized (DF1; DM3; ARC2) peak. Members of the *SDR* superfamily are involved in ABA biosynthesis and function as a molecular link between nutrient signaling and plant hormone biosynthesis. According to Cheng et al. (2002), endogenous ABA also plays crucial roles as growth-promoting hormone in fertility control, transition from vegetative to reproductive growth and in determining organ size.

Phvul.001G194000 and *Phvul.001G194400*, homologs of *OVATE FAMILY PROTEIN 13* (*OFP13*), and *LONELY GUY 7* (*LOG7*), respectively, are candidates surrounding the Pv01 (46.0 Mb) colocalized (DF8; ARC6) peak. Plants overexpressing

OFP in *Arabidopsis* were dwarf, with reduced length of the hypocotyl, leaf petiole, and inflorescence stems. Probably, *OFP* regulates genes encoding key enzymes in gibberellin biosynthesis negatively and, consequently, reduces cell elongation once gibberellins are plant hormones known to promote cell elongation in various organs (Wang et al. 2007). The *LOG* proteins are involved in the direct activation pathway of cytokinins, a phytohormone that plays key roles in the cell activity of plants, including shoot and root growth, plant height, inflorescence growth, leaf senescence, and seed size. A null *Arabidopsis* mutant for a group of *LOG* (*log3 log4 log7*) showed a delay in inflorescence growth, resulting in decreased plant height, and the formation of fewer flower buds and flowers than the wild type (*Log3 Log4 Log7*). On the other hand, transgenic plants overexpressing *LOG* increased chlorophyll content and, consequently, delayed leaf senescence. These results suggest that *LOG* activity results in pleiotropic phenotypes in *Arabidopsis* (Kuroha et al. 2009).

The colocalized (DF6; ARC4) GWAS peak on Pv01 (46.0 Mb) includes the homologs *LATE EMBRYOGENESIS ABUNDANT* (*LEA*) (*Phvul.001G194900*), and *HYDROXYPROLINE-RICH GLYCOPROTEIN FAMILY* (*HRGP*) (*Phvul.001G195100*), downstream and upstream of the peak, respectively. The *LEA* gene family encode a large and diverse family of proteins (Hundertmark and Hincha 2008) expressing late embryo maturation and extended seed dehydration period. In mutant seeds of *Arabidopsis* for group one of *LEA* family (*ATEM1* and *ATEM6*), premature seed dehydration and maturation at the distal silique ends were observed, demonstrating that this protein is required for normal seed development (Manfre et al. 2005). The plant cell wall structural protein *HRGP* wasalready described in many species, including soybean and common bean. It is involved in cell wall maturation, especially root and hypocotyl, in cell wall specialization, and in cell wall strengthening (Bradley et al. 1992; Ahn et al. 1996).

SUCROSE PHOSPHATE SYNTHASE (SPS) is a homolog of the candidate *Phvul.003G170100*, near the colocalized (DF15; DM9) peak on Pv03 (38.0 Mb). SPS is a multigenic family important for both vegetative and reproductive growth by sucrose biosynthesis (Bahaji et al. 2015). In transgenic tobacco (*Nicotiana tabacum* cv. Xanthi) plants overexpressing maize *SPS* senescence is delayed, in response to the increase in *SPS* activity in the leaves (Baxter et al. 2003).

The colocalized (DF11; DM5) GWAS peak on PV03 (38.1 Mb) was mapped near the *PURPLE ACID PHOSPHATASE 26* (*PAP26*) homolog (*Phvul.003G170500*). *PAP* helps to recycle phosphor (P) from organic P sources to young tissues and seeds during leaf senescence of *Arabidopsis* cultivated in inorganic orthophosphate (Pi)-deficient soils. The T-DNA mutants (*atpap26*) reduce acid phosphatase activity followed by decreased P remobilization and delayed senescence (Robinson et al. 2012). *Phvul.003G171500*, a homolog of *AVP1*, is a candidate on the Pv03 (43.6 Mb) colocalized (DF20; DM10) peak. The *Arabidopsis AVP1* gene encodes the vacuolar pyrophosphatase protein involved in assimilate partitioning between the source and sink sites during transition from the vegetative to the reproductive phase. In transgenic *Arabidopsis* plants overexpressing *AVP1*, improved photosynthesis, biomass accumulation, and transport to sink organs were observed (Khadilkar et al. 2016).

The single colocalized peak involving YLD13, with DF19 and DM11, is mapped on PV03 (39.5 Mb). The peak coincided with the candidate gene *Phvul.003G183100* whose homolog, *GIANT EMBRYO (GE)*, encodes a *CYP78A* subfamily P450 monooxygenase that controls rice embryo development and cell proliferation and improves grain yield. Transgenic *Arabidopsis* plants overexpressing *AtCYP78A10*, a *GE* homolog, also produced larger seeds with higher seed weight (Yang et al. 2013).

The Pv07 (48.6 Mb) GWAS peak (ARC11), mapped close to candidate *Phvul.007G246700*, a homolog of *PECTIN METHYLESTERASE 41* (*PME41*), is a gene that encodes a pectin methylesterase that modifies the degree of pectin methylesterification, leading to cell wall loosening or strengthening (Michelli et al. 2001; Pelloux et al. 2007).

Seed yield (YLD)

Five candidate genes for yield are discussed in this study, ranging from 55.9 to 56.7 Mb on Pv08. Homologous genes involved in several biological processes such as hormone response, plant architecture, disease resistance, and seed cell proliferation were used to describe the marker-candidate association, since YLD is a complex trait associated to these processes (Geffroy et al. 1998; Li et al. 2002; Schilmiller et al. 2009). Furthermore, a positive correlation between ARC and YLD was observed (Table 3).

Two seed yield candidate genes on PV08, *Phvul.008G245300* (55.9 Mb peak; YLD2) and *Phvul.008G245900* (56.0 Mb peak; YLD3), are homologs of the *Leucine-Rich Repeat* (*LRR*) class of *Receptor-Like Kinase* (*RLK*). Two proteins of this class, named *BRI1* and *BAK1*, interact to modulate brassinosteroid signaling in *Arabidopsis*. Overexpression of *BAK1* (*serine/threonine protein kinase*) resulted in elongated organ phenotypes, while a null allele of *BAK1* displayed a semi-dwarf phenotype with reduced brassinosteroid sensitivity (Li et al. 2002). The effect of accumulation of this plant hormone by gene overexpression has been associated to an increase in both vegetative growth and seed yield in *Arabidopsis* (Choe et al. 2001). Several *LRR* classes of *RLK* proteins have been associated to increases in rice grain yield (Zha et al. 2009), and to disease resistance in common bean (Geffroy et al. 1998) and soybean (Hayes et al. 2004).

Two close GWAS peaks (YLD4, and YLD5), about 2 Kb apart on Pv08 (56.1 Mb), were mapped downstream of the candidate *Phvul.008G247400*, homolog to *Cinnamic Acid 4-Hydroxylase* (*C4H*)/*Reduced Epidermal Fluorescence 3* (*REF3*), involved in lignin metabolism. A mutation in this gene in *Arabidopsis* (*ref3*) results in collapsed stem vasculature and reduced lignin content, causing alterations in plant architecture such as dwarfism, loss of apical dominance, male sterility, and enlarged branch junctions (Schilmiller et al. 2009). A gene similar to *C4H* and responsible for lignin synthesis was also reported in French bean (*Phaseolus vulgaris* L.) (Haddson and Northeote 1976; Nedelkina et al. 1999).

The *A*- and *B*-type of *Cyclin-Dependent Kinase* (*CDK*) are the main drivers of the plant cell cycle and are regulated by *Cyclin-Dependent Kinase Inhibitors* (*ICK*). *Phvul.008G253500*, a homolog of *CDKB1*, is a candidate gene on Pv08 (56.7 Mb) peak (YLD1). Increases in *CDK* activity in mutants without *ICK* stimulate endosperm and leaf cell proliferation, increasing both seed size and grain yield (Mizutani et al. 2009; Cheng et al. 2013).

Seed size traits (SF, SS, and SW)

The final size of the seed seems to depend on cell differentiation/proliferation, hormone signaling/biosynthesis, embryo development, and nutrient biosynthesis/remobilization (Li et al. 2014; Kubo et al. 2010; Fuji et al. 2015). Thus, all

these aspects were considered to search the nine candidate genes associated with significant SNPs for seed size traits.

Four GWAS peaks for seed flatness (SF10, SF21, SF2, and SF1) on PV05 (37.9 to 38.0 Mb), were mapped surrounding the candidate *Phvul.005G153100*, homolog to *CRINKLY4* (*CR4*) that encodes a membrane-localized protein similar to receptor kinases involved in epidermal cell differentiation in many organs. Mutants for *CRINKLY4* (*cr4*) show abnormal shape and texture of developing seeds, which were rounded and rough in appearance, instead of elliptical and smooth, as in the wild type (*CR4*) (Gifford et al. 2003).

The candidate gene *Phvul.005G155900* on PV05 (38.2 Mb), surrounded by two GWAS peaks (SF11 and SF18), is homolog to the *PENTATRICOPEPTIDE REPEAT* (*PPR*) superfamily protein. The maize genes *Empty Pericarp 5* (*Emp5*) and *Small kernel 1* (*Smk1*) encode *PPRs* that plays a role in mitochondrial transcript editing and seed development. The *emp5* and *smk1* mutations block the normal development of both embryo and endosperm, resulting in small, white and wrinkled kernels (Liu et al. 2013; Li at el. 2014).

For seed shape (SS), a Pv02 (46.4 Mb) GWAS peak (SS6) was mapped near the candidate gene *Phvul.002G302100*, homolog to *CYTOCHROME P450* (*CYP*). The gene *D11* of rice encodes a member of *CYP* family enzymes that play a role in brassinosteroid biosynthesis. Rice dwarf mutants (*d11*) have several phenotypic characteristics, such as leaf erection in mature stages, inability to elongate the second internode, and fruit set of small round seeds. Seed length is shorter in *d11* than in wild-type plants (*D11*), while the width is the same (Tanabe et al. 2005).

The common bean homolog of *ARABIDOPSIS THALIANA ISOAMYLASE 1* (*AtISA1*), *Phvul.001G192300* maps near the Pv02 (46.6 Mb) GWAS peak (SS7). *ISA1* encodes an isoamylase-type debranching enzyme involved in starch synthesis. It was postulated that *ISA1* interacts with *ISA2* to form the *Iso1* complex. In common bean, the isoamylase genes *PvISA1/2* and *PvISA3* / were characterized (Takashima et al. 2007). Mutations in *ISA1* cause a loss in detectable isoamylase activity and the disruption of normal starch structure and content in developing maize endosperm, leading to an alteration of the kernel shape (Kubo et al. 2010).

Phvul.002G304400, a homolog of the *MYB-Like Transcription Factor Family* protein *(MYB)*, is a candidate on Pv05 (46.6 Mb) peak (SS5). Due to loss-of-function or

knock-down of *MYB56* in *Arabidopsis*, the contracted endothelial cells were smaller and the cell number in the outer integument layer of the seed coat lower, resulting in smaller seeds than of the wild type. Conversely, overexpression of *MYB56* expanded endothelial cells and increased cell number in the outer integument layer of the seed coat, producing larger seeds (Zhang et al. 2013). According to Haughn and Chaudhury (2005), the final establishment of seed size in *Arabidopsis* depends on the interaction between the developing endosperm and seed coat growth.

Two GWAS peaks (SS2 and SS1) downstream of the homolog *ADAPTOR PROTEIN COMPLEX 4S* (*AP4S*) (*Phvul.002G306700*) were observed on PV02 (46.8 Mb). *Adaptor Protein* (*AP*) is a complex that encodes five proteins (*AP-1* to *AP-5*) in plants and is involved in vacuolar sorting of seed storage proteins (Fuji et al. 2015).

For seed weight (SW), a Pv01 (51.35 Mb) GWAS peak (SW1), mapped near the candidate gene *Phvul.001G258000*, homolog to *ARABIDOPSIS RESPONSE REGULATOR 11 (ARR11)*. *ARR11* encodes a protein that acts in concert with other *type-B ARRs* in the cytokinin-signaling pathway. Mutants of *Arabidopsis* for a group of these genes have larger embryos and seeds than those of the wild type (Ishida et al. 2008). In the triple mutant for *AUTHENTIC HISTIDINE KINASE* (*AHK*), another gene involved in cytokinin response with *ARR*, the seed volume was up to ∼250% higher than in the wild-type, indicating a cytokinin-dependent endospermal and/or maternal control of embryo and seed size (Riefler et al. 2005).

Phvul.001G262600 and *Phvul.001G263400*, homologs of *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 2* (*SPL2*), and *MADS-BOX TRANSCRIPTION FACTOR FAMILY PROTEIN*, respectively, are candidate genes surrounding the Pv01 (51.7 Mb) peak (SW6). The *SPL* gene family is involved in determining organ size (Wang et al. 2008). A specific member of this family, *OsSPL16*, encodes a protein that is a positive regulator of cell proliferation and controls rice grain size, shape, quality, and yield (Wang at al. 2012; Wang et al. 2015). The *MADS* box is formed by a family of genes, including *AGAMOUS-LIKE* (*AGL*) and *SEPALLATA* (*SEP*), which encode transcription factors involved in many steps of seed development. In Arabidopsis, *AGL80* and *AGL61* play an important role as gene transcription factors required for central cell specification and differentiation in the female gametophyte after fertilization to produce the endosperm (Portereiko et al. 2006) and to regulate the central cell size (Steffen et al. 2008).

A high number of significant SNPs was detected, demonstrating the utility of genome-wide association studies to detect QTLs related to the seven morpho-agronomic traits. The candidate genes in the regions surrounding these significant SNPs explained each trait, by comparisons to homologous genes of other species related to similar biological function. The results led to the conclusion that these QTLs could be used to develop marker-assisted breeding strategies in common bean. In this way, this study is of particular importance for providing more tools for molecular breeding of common bean in Brazil.

4. Additional file

Supplementary Table S1 Significant GWAS peak for each trait ranked from the most to the less significant p-value in the Rudá x AND 277 RIL. GWAS peak for each trait; SNP ID obtained from Song et al (2015); NCBI submitted SNP ID number; Chromosome (Pv) and SNP position according to the version 1.0 of *P. vulgaris* assembly; and p-value associated to each GWAS significant peak. In bold, are the GWAS peaks significant by the threshold, and the others by LOD = 3.

GWAS peak	SNP ID obtained from Song et al (2015)	NCBI ssID	Pv	SNP position	P-value
DFL1	**BARCPV_1.0_Chr01_45894030_A_G**	**ss715647368**	**1**	**45894030**	**1.64E-08**
DFL2	**BARCPV_1.0_Chr01_45746595_A_C**	**ss715639271**	**1**	**45746595**	**3.38E-08**
DFL3	**BARCPV_1.0_Chr01_45246824_C_T**	**ss715639272**	**1**	**45246824**	**3.51E-08**
DFL4	**BARCPV_1.0_Chr01_45286536_G_A**	**ss715646075**	**1**	**45286536**	**4.90E-08**
DFL5	**BARCPV_1.0_Chr01_45855284_A_C**	**ss715647370**	**1**	**45855284**	**4.74E-07**
DFL6	**BARCPV_1.0_Chr01_46081668_G_A**	**ss715647366**	**1**	**46081668**	**9.57E-07**
DFL7	**BARCPV_1.0_Chr01_44557247_G_A**	**ss715639957**	**1**	**44557247**	**5.26E-06**
DFL8	**BARCPV_1.0_Chr01_46027600_A_C**	**ss715639536**	**1**	**46027600**	**1.01E-05**
DFL9	**BARCPV_1.0_Chr01_44336644_G_A**	**ss715639956**	**1**	**44336644**	**4.36E-05**
DFL10	**BARCPV_1.0_Chr01_42994016_C_T**	**ss715640009**	**1**	**42994016**	**7.54E-05**
DFL11	**BARCPV_1.0_Chr03_38185459_T_C**	**ss715649522**	**3**	**38185459**	**9.94E-05**
DFL12	**BARCPV_1.0_Chr01_42672556_T_G**	**ss715640152**	**1**	**42672556**	**1.04E-04**
DFL13	BARCPV_1.0_Chr03_36635373_A_C	ss715648025	3	36635373	1.43E-04
DFL14	BARCPV_1.0_Chr01_43699409_C_T	ss715648889	1	43699409	2.19E-04
DFL15	BARCPV_1.0_Chr03_38087648_T_C	ss715639501	3	38087648	3.13E-04
DFL16	BARCPV_1.0_Chr01_43150606_C_T	ss715646869	1	43150606	4.62E-04
DFL17	BARCPV_1.0_Chr03_39939960_C_T	ss715639426	3	39939960	4.65E-04
DFL18	BARCPV_1.0_Chr01_46631601_G_A	ss715647097	1	46631601	4.90E-04
DFL19	BARCPV_1.0_Chr03_39501748_A_G	ss715646619	3	39501748	5.99E-04
DFL20	BARCPV_1.0_Chr03_38268628_G_A	ss715648538	3	38268628	6.14E-04
DM1	**BARCPV_1.0_Chr01_45246824_C_T**	**ss715639272**	**1**	**45246824**	**5.24E-07**
DM2	**BARCPV_1.0_Chr01_45286536_G_A**	**ss715646075**	**1**	**45286536**	**8.72E-07**
DM3	**BARCPV_1.0_Chr01_45894030_A_G**	**ss715647368**	**1**	**45894030**	**8.16E-06**
DM4	**BARCPV_1.0_Chr01_45746595_A_C**	**ss715639271**	**1**	**45746595**	**2.73E-05**
DM5	**BARCPV_1.0_Chr03_38185459_T_C**	**ss715649522**	**3**	**38185459**	**2.90E-05**
DM6	**BARCPV_1.0_Chr03_36635373_A_C**	**ss715648025**	**3**	**36635373**	**4.59E-05**
DM7	**BARCPV_1.0_Chr09_29813231_G_A**	**ss715639305**	**9**	**29813231**	**6.67E-05**
DM8	**BARCPV_1.0_Chr01_44336644_G_A**	**ss715639956**	**1**	**44336644**	**9.08E-05**
DM9	**BARCPV_1.0_Chr03_38087648_T_C**	**ss715639501**	**3**	**38087648**	**1.09E-04**
DM10	**BARCPV_1.0_Chr03_38268628_G_A**	**ss715648538**	**3**	**38268628**	**1.40E-04**
DM11	**BARCPV_1.0_Chr03_39501748_A_G**	**ss715646619**	**3**	**39501748**	**1.43E-04**
DM12	**BARCPV_1.0_Chr09_29840085_C_T**	**ss715639304**	**9**	**29840085**	**1.57E-04**
DM13	BARCPV_1.0_Chr01_44557247_G_A	ss715639957	1	44557247	2.41E-04
DM14	BARCPV_1.0_Chr03_39765257_T_C	ss715650182	3	39765257	3.66E-04
DM15	BARCPV_1.0_Chr03_38799776_G_A	ss715647689	3	38799776	3.69E-04
DM16	BARCPV_1.0_Chr01_45855284_A_C	ss715647370	1	45855284	4.22E-04
DM17	BARCPV_1.0_Chr03_37740758_G_A	ss715647091	3	37740758	4.94E-04
DM18	BARCPV_1.0_Chr03_39662131_A_G	ss715646624	3	39662131	6.58E-04
DM19	BARCPV_1.0_Chr01_42672556_T_G	ss715640152	1	42672556	7.44E-04

Table S1 (Continued)

GWAS peak	SNP ID obtained from Song et al (2015)	NCBI ssID	Pv	SNP position	P-value
ARQ1	**BARCPV_1.0_Chr01_45855284_A_C**	**ss715647370**	**1**	**45855284**	**1.05E-10**
ARQ2	**BARCPV_1.0_Chr01_45894030_A_G**	**ss715647368**	**1**	**45894030**	**1.83E-10**
ARQ3	**BARCPV_1.0_Chr01_45746595_A_C**	**ss715639271**	**1**	**45746595**	**8.74E-10**
ARQ4	**BARCPV_1.0_Chr01_46081668_G_A**	**ss715647366**	**1**	**46081668**	**5.48E-08**
ARQ5	**BARCPV_1.0_Chr01_45246824_C_T**	**ss715639272**	**1**	**45246824**	**5.78E-08**
ARQ6	**BARCPV_1.0_Chr01_46027600_A_C**	**ss715639536**	**1**	**46027600**	**6.71E-08**
ARQ7	**BARCPV_1.0_Chr01_45286536_G_A**	**ss715646075**	**1**	**45286536**	**1.37E-07**
ARQ8	**BARCPV_1.0_Chr01_44557247_G_A**	**ss715639957**	**1**	**44557247**	**2.82E-05**
ARQ9	**BARCPV_1.0_Chr01_44336644_G_A**	**ss715639956**	**1**	**44336644**	**3.96E-05**
ARQ10	**BARCPV_1.0_Chr01_43699409_C_T**	**ss715648889**	**1**	**43699409**	**1.44E-04**
ARQ11	BARCPV_1.0_Chr07_48635019_C_T	ss715646609	7	48635019	2.89E-04
ARQ12	BARCPV_1.0_Chr07_45923787_C_T	ss715646526	7	45923787	4.19E-04
ARQ13	BARCPV_1.0_Chr08_40625195_G_A	ss715649555	8	40625195	7.68E-04
ARQ14	BARCPV_1.0_Chr01_42994016_C_T	ss715640009	1	42994016	9.43E-04
YLD1	**BARCPV_1.0_Chr08_56747675_T_C**	**ss715646531**	**8**	**56747675**	**1.20E-06**
YLD2	**BARCPV_1.0_Chr08_55974271_C_A**	**ss715639952**	**8**	**55974271**	**2.41E-06**
YLD3	**BARCPV_1.0_Chr08_56047197_G_A**	**ss715648562**	**8**	**56047197**	**5.72E-06**
YLD4	**BARCPV_1.0_Chr08_56161894_G_T**	**ss715639954**	**8**	**56161894**	**2.58E-05**
YLD5	**BARCPV_1.0_Chr08_56163233_A_G**	**ss715639953**	**8**	**56163233**	**6.96E-05**
YLD6	BARCPV_1.0_Chr08_57027345_T_C	ss715646512	8	57027345	1.01E-04
YLD7	BARCPV_1.0_Chr04_1099511_T_C	ss715646886	4	1099511	2.00E-04
YLD8	BARCPV_1.0_Chr08_56911339_A_G	ss715646536	8	56911339	2.93E-04
YLD9	BARCPV_1.0_Chr04_593836_G_A	ss715649427	4	593836	4.06E-04
YLD10	BARCPV_1.0_Chr08_57013959_G_T	ss715646513	8	57013959	5.04E-04
YLD11	BARCPV_1.0_Chr04_1058238_C_T	ss715646915	4	1058238	5.19E-04
YLD12	BARCPV_1.0_Chr08_55560522_T_C	ss715646654	8	55560522	6.22E-04
YLD13	BARCPV_1.0_Chr03_39501748_A_G	ss715646619	3	39501748	6.55E-04
YLD14	BARCPV_1.0_Chr08_54945818_G_A	ss715646655	8	54945818	8.11E-04
SF1	**BARCPV_1.0_Chr05_38045825_A_G**	**ss715645406**	**5**	**38045825**	**6.77E-06**
SF2	**BARCPV_1.0_Chr05_38037907_A_G**	**ss715645405**	**5**	**38037907**	**8.38E-06**
SF3	**BARCPV_1.0_Chr05_37803021_C_A**	**ss715648404**	**5**	**37803021**	**1.93E-05**
SF4	**BARCPV_1.0_Chr05_37784720_A_G**	**ss715648405**	**5**	**37784720**	**2.36E-05**
SF5	**BARCPV_1.0_Chr05_37888316_G_T**	**ss715645373**	**5**	**37888316**	**2.60E-05**
SF6	**BARCPV_1.0_Chr05_38115217_A_G**	**ss715645412**	**5**	**38115217**	**4.27E-05**
SF7	**BARCPV_1.0_Chr05_37818800_A_C**	**ss715648403**	**5**	**37818800**	**5.13E-05**
SF8	**BARCPV_1.0_Chr05_38270393_C_T**	**ss715645426**	**5**	**38270393**	**5.36E-05**
SF9	**BARCPV_1.0_Chr05_37956078_C_T**	**ss715645396**	**5**	**37956078**	**5.49E-05**
SF10	**BARCPV_1.0_Chr05_37973626_T_C**	**ss715645398**	**5**	**37973626**	**8.83E-05**
SF11	**BARCPV_1.0_Chr05_38168089_C_A**	**ss715645416**	**5**	**38168089**	**1.09E-04**
SF12	**BARCPV_1.0_Chr10_41102305_A_G**	**ss715645516**	**10**	**41102305**	**1.31E-04**
SF13	**BARCPV_1.0_Chr05_35473109_A_C**	**ss715646996**	**5**	**35473109**	**1.45E-04**
SF14	**BARCPV_1.0_Chr05_37950624_G_T**	**ss715645395**	**5**	**37950624**	**1.56E-04**
SF15	**BARCPV_1.0_Chr05_34786660_C_A**	**ss715647500**	**5**	**34786660**	**2.23E-04**
SF16	**BARCPV_1.0_Chr10_41097019_C_T**	**ss715645515**	**10**	**41097019**	**2.46E-04**
SF17	BARCPV_1.0_Chr10_41177925_A_C	ss715645522	10	41177925	4.79E-04
SF18	BARCPV_1.0_Chr05_38215713_G_A	ss715645421	5	38215713	5.97E-04
SF19	BARCPV_1.0_Chr10_41362559_G_A	ss715645530	10	41362559	5.99E-04
SF20	BARCPV_1.0_Chr10_41132114_A_C	ss715645518	10	41132114	6.15E-04
SF21	BARCPV_1.0_Chr05_37986682_A_G	ss715645400	5	37986682	7.61E-04

Table S1 (Continued)

GWAS peak	SNP ID obtained from Song et al (2015)	NCBI ssID	Pv	SNP position	P-value
SF22	BARCPV_1.0_Chr10_41404964_C_T	ss715645533	10	41404964	8.28E-04
SS1	**BARCPV_1.0_Chr02_46891082_T_C**	**ss715646919**	**2**	**46891082**	**5.02E-07**
SS2	**BARCPV_1.0_Chr02_46884229_G_T**	**ss715646920**	**2**	**46884229**	**5.42E-07**
SS3	**BARCPV_1.0_Chr02_46519870_T_C**	**ss715646161**	**2**	**46519870**	**1.10E-06**
SS4	**BARCPV_1.0_Chr02_46814418_G_A**	**ss715646923**	**2**	**46814418**	**4.42E-06**
SS5	**BARCPV_1.0_Chr02_46685108_T_C**	**ss715646929**	**2**	**46685108**	**4.46E-06**
SS6	**BARCPV_1.0_Chr02_46464813_T_G**	**ss715646157**	**2**	**46464813**	**6.15E-06**
SS7	**BARCPV_1.0_Chr02_46640265_G_A**	**ss715646932**	**2**	**46640265**	**1.66E-05**
SS8	**BARCPV_1.0_Chr02_46240208_G_T**	**ss715646146**	**2**	**46240208**	**6.66E-05**
SS9	**BARCPV_1.0_Chr02_47948594_A_G**	**ss715645989**	**2**	**47948594**	**1.90E-04**
SS10	BARCPV_1.0_Chr02_46250506_T_G	ss715646147	2	46250506	2.58E-04
SS11	BARCPV_1.0_Chr02_46336206_G_A	ss715646153	2	46336206	4.14E-04
SS12	BARCPV_1.0_Chr02_46359501_G_A	ss715645186	2	46359501	5.24E-04
SS13	BARCPV_1.0_Chr02_46370770_C_T	ss715646154	2	46370770	5.78E-04
SS14	BARCPV_1.0_Chr02_46110065_C_T	ss715646142	2	46110065	6.44E-04
SS15	BARCPV_1.0_Chr02_47865017_C_T	ss715645986	2	47865017	9.04E-04
SS16	BARCPV_1.0_Chr02_48160048_G_T	ss715646006	2	48160048	9.15E-04
SW1	BARCPV_1.0_Chr01_51353193_C_T	ss715645299	1	51353193	6.25E-05
SW2	BARCPV_1.0_Chr07_5483860_C_T	ss715639546	7	5483860	9.20E-05
SW3	BARCPV_1.0_Chr01_51819821_T_G	ss715645301	1	51819821	1.52E-04
SW4	BARCPV_1.0_Chr07_5600859_C_T	ss715644972	7	5600859	1.52E-04
SW5	BARCPV_1.0_Chr01_51795359_T_C	ss715645303	1	51795359	4.50E-04
SW6	BARCPV_1.0_Chr01_51726047_A_C	ss715645250	1	51726047	5.23E-04
SW7	BARCPV_1.0_Chr07_5419733_T_C	ss715639547	7	5419733	8.24E-04

Supplementary Table S2 Candidate genes associated surrounding the significant SNPs in GWAS peaks. In the marker distance (bp) from the candidate genes, the negative sign indicates that the marker is downstream of the candidate gene and no sign indicates the marker is upstream of the candidate. In bold, are the GWAS peaks significant by the FDR significant threshold, and the others by LOD = 3.

GWAS peak	**Pv**	**SNP position**	**Candidate gene associated to the SNP**	**Marker distance from candidate gene (bp)**	***Arabidopsis* Locus**	***Arabidopsis* annotation**	***Arabidopsis* gene symbol(s)**
DF12; DM19	1	42,672,556	Phvul.001G165800	8,578	AT1G15520	Pleiotropic drug resistance 12	*ABCG40, ATABCG40, ATPDR12, PDR12*
DF10; ARC14	1	42,994,016	Phvul.001G168000	4,104	AT4G28720	Flavin-binding monooxygenase family protein	*YUC8, YUCCA 8*
DF14; **ARC10**	1	43,699,409	Phvul.001G174200	-5,250	AT3G09600	Homeodomain-like superfamily protein	*RVE8*
DF9; DM8; ARC9	1	44,336,644	Phvul.001G179300	-35,402	AT2G36910	ATP binding cassette subfamily B1	*ABCB1, ATPGP1, PGP1*
DF3; DM1; ARC5	1	45,246,824	Phvul.001G187000	-11,259	AT5G03680	Duplicated homeodomain-like superfamily protein	*PTL*
DF4; DM2; ARC7	1	45,286,536	Phvul.001G187000	24,816	AT5G03680	Duplicated homeodomain-like superfamily protein	*PTL*
DF2; DM4; ARC3	1	45,746,595	Phvul.001G191600	0	AT2G36190	Cell wall invertase 4	*AtcwINV4, cwINV4*
			Phvul.001G192000	-39,184	AT5G22380	NAC domain containing protein 90	*ANAC090, NAC090*

Supplementary Table S2 (continued)

GWAS peak	Pv	SNP position	Candidate gene associated to the SNP	Marker distance from candidate gene (bp)	Arabidopsis Locus	Arabidopsis annotation	Arabidopsis gene symbol(s)
DF5; DM16; **ARC1**	1	45,855,284	Phvul.001G192300	17,972	AT3G11540	Tetratricopeptide repeat (TPR)-like superfamily protein	*SPY*
DF1; DM3; ARC2	1	45,894,030	Phvul.001G193400	-22,855	AT3G51680	NAD(P)-binding Rossmann-fold superfamily protein	*SDR2*
DF8; ARC6	1	46,027,600	Phvul.001G194000	28,649	AT5G04820	Ovate family protein	*ATOFP13, OFP13*
			Phvul.001G194400	-10,296	AT5G06300	Putative lysine decarboxylase family protein	*LOG7*
DF6; ARC4	1	46,081,668	Phvul.001G194900	4,112	AT2G35980	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	*ATNHL10, NHL10, YLS9*
			Phvul.001G195100	-16,199	AT3G52460	Hydroxyproline-rich glycoprotein family protein	*HRGP*
DF15; **DM9**	3	38,087,648	Phvul.003G170100	17,061	AT5G20280	Sucrose phosphate synthase 1F	*ATSPS1F, SPS1F*
DF11; DM5	3	38,185,459	Phvul.003G170500	31,996	AT5G34850	Purple acid phosphatase 26	*ATPAP26, PAP26*

Supplementary Table S2 (continued)

GWAS peak	**Pv**	**SNP position**	**Candidate gene associated to the SNP**	**Marker distance from candidate gene (bp)**	***Arabidopsis* Locus**	***Arabidopsis* annotation**	***Arabidopsis* gene symbol(s)**
DF20; **DM10**	3	38,268,628	Phvul.003G171500	15,247	AT1G15690	Inorganic H pyrophosphatase family protein	*ATAVP3, AtVHP1, AVP-3, AVP1*
DF19; **DM11**; YLD13	3	39,501,748	Phvul.003G183100	0	AT1G74110	Cytochrome P450, family 78, subfamily A, polypeptide 10	*CYP78A10*
ARC11	7	48,635,019	Phvul.007G246700	3,965	AT4G02330	Plant invertase/pectin methylesterase inhibitor superfamily	*ATPMEPCRB*
YLD2	8	55,974,271	Phvul.008G245300	862	AT1G06840	Leucine-rich repeat protein kinase family protein	*LRR-RLK*
YLD3	8	56,047,197	Phvul.008G245900	-13,782	AT5G58300	Leucine-rich repeat protein kinase family protein	*LRR-RLK*
YLD4	8	56,161,894	Phvul.008G247400	-58,152	AT2G30490	Cinnamate-4-hydroxylase	*ATC4H, C4H, CYP73A5, REF3*
YLD5	8	56,163,233		-56,813			
YLD1	8	56,747,675	Phvul.008G253500	-2,988	AT2G38620	Cyclin-dependent kinase B1;2	*CDKB1;2*

Supplementary Table S2 (continued)

GWAS peak	Pv	SNP position	Candidate gene associated to the SNP	Marker distance from candidate gene (bp)	Arabidopsis Locus	Arabidopsis annotation	Arabidopsis gene symbol(s)
SF10	5	37,973,626	Phvul.005G153100	-27,594	AT3G59420	Crinkly4	*ACR4, CR4*
SF21		37,986,682		-14,538			
SF2		38,037,907		33,267			
SF1		38,045,825		41,185			
SF11	5	38,168,089	Phvul.005G155900	-36,687	AT2G13600	Pentatricopeptide repeat (PPR) superfamily protein	-
SF18		38,215,713		9,354			
SS6	2	46,464,813	Phvul.002G302100	7,303	AT5G36110	Cytochrome P450, family 716, subfamily A, polypeptide 1	*CYP716A1*
SS7	2	46,640,265	Phvul.002G303600	37,170	AT2G39930	Isoamylase 1	*ATISA1, ISA1*
SS5	2	46,685,108	Phvul.002G304400	-3,628	AT5G56840	MYB-like transcription factor family protein	-
SS2	2	46,884,229	Phvul.002G306700	-9,192	AT2G19790	SNARE-like superfamily protein	*AP4S*
SS1		46,891,082		-2,339			
SW1	1	51,353,193	Phvul.001G258000	-38,006	AT1G67710	Response regulator 11	*ARR11*
SW6	1	51,726,047	Phvul.001G262600	34,144	AT5G43270	Squamosa promoter binding protein-like 2	*SPL2*
			Phvul.001G263400	-9,604	AT1G24260	K-box region and MADS-box transcription factor family protein	AGL9, SEP3

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