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Abstract:	Next Generation Sequencing (NGS) technologies have increased markedly the throughput of genetic studies, allowing the identification of several thousands of SNPs within a single experiment. Even though sequencing cost is rapidly decreasing, the price for whole genome re-sequencing of a large number of individuals is still costly, especially in plants with a large and highly redundant genome. In recent years, several reduced representation library (RRL) approaches has been developed for reducing the sequencing cost per individual. Among them, Genotyping-By-Sequencing (GBS) represents a simple, cost-effective, and highly multiplexed alternative for species with or without an available reference genome. However, this technology requires specific optimization for each species, especially for the restriction enzyme (RE) used. Here we report on the application of GBS in a test experiment with 18 genotypes of wild and domesticated Phaseolus vulgaris. After an in silico digestion with different RE of the P. vulgaris genome reference sequence, we selected CviAII as the most suitable RE for GBS in common bean based on the high frequency and even distribution of restriction sites. A total of 44,875 SNPs, 1,940 deletions and 1,693 insertions were identified, with 50% of the variants located in genic sequences and tagging 11,027 genes. SNPs and InDels distribution was positively correlated with gene density across the genome. In addition, we were able to also identify putative copy number variations (CNVs) of genomic segments between different genotypes. In conclusion, GBS with the CviAII enzyme results in thousands of evenly spaced markers and provides a reliable, high-throughput and cost-effective approach for genotyping both wild and domesticated common beans.
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Genome-wide identification of SNPs and Copy Number Variation in Common Bean (*Phaseolus vulgaris* L.) using Genotyping-By-Sequencing (GBS)

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Abstract

Next Generation Sequencing (NGS) technologies have increased markedly the throughput of genetic studies, allowing the identification of several thousands of SNPs within a single experiment. Even though sequencing cost is rapidly decreasing, the price for whole genome re-sequencing of a large number of individuals is still costly, especially in plants with a large and highly redundant genome. In recent years, several reduced representation library (RRL) approaches has been developed for reducing the sequencing cost per individual. Among them, Genotyping-By-Sequencing (GBS) represents a simple, cost-effective, and highly multiplexed alternative for species with or without an available reference genome. However, this technology requires specific optimization for each species, especially for the restriction enzyme (RE) used. Here we report on the application of GBS in a test experiment with 18 genotypes of wild and domesticated *Phaseolus vulgaris*. After an *in silico* digestion with different RE of the *P. vulgaris* genome reference sequence, we selected *Cvi*AII as the most suitable RE for GBS in common bean based on the high frequency and even distribution of restriction sites. A total of 44,875 SNPs, 1,940 deletions and 1,693 insertions were identified, with 50% of the variants located in genic sequences and tagging 11,027 genes. SNPs and InDels distribution was positively correlated with gene density across the genome. In addition, we were able to also identify putative copy number variations (CNVs) of genomic segments between different genotypes. In conclusion, GBS with the *Cvi*AII enzyme results in thousands of evenly spaced markers and provides a reliable, high-throughput and cost-effective approach for genotyping both wild and domesticated common beans.

Keywords: Common Bean, Copy Number Variation (CNV), Genome-wide SNPs calling, Genotyping-by-Sequencing (GBS), Next-generation Sequencing

Introduction

Common bean (*Phaseolus vulgaris* L.) is an important legume crop for human nutrition, being an important source of protein, complex carbohydrates, fiber, and beneficial minerals for millions of individuals worldwide (Broughton et al. 2003; Gepts et al. 2008). The species belongs to a large and diverse genus that comprises 70-80 species, five of which have been domesticated (Freytag and Debouck 2002). Among these domesticated species, common bean is the one with the broadest geographic distribution and the highest agronomic, nutritional and economic value (Gepts 2014). It is a diploid species with a haploid complement of 11 chromosomes and a genome size of ~587 Mb (Schmutz et al., 2014).

Repeated experimental evidence highlights the existence of two different and genetically divergent wild gene pools in common bean, called Mesoamerican and Andean gene pools, which underwent domestication independently (Bitocchi et al. 2013; Gepts 1998; Kwak and Gepts 2009; Schmutz et al. 2014) and diversified into distinct eco-geographic races (Singh et al. 1991; Chacón et al., 2007). Indeed, the Andean gene pool is generally adapted to relatively higher altitudes and lower temperature, while the Mesoamerican gene pool is adapted to lower altitudes and higher temperatures (Beebe et al. 2011). A range of molecular markers have been developed and employed in beans for the analysis of genetic diversity (domestication, gene pool divergence, and population structure), linkage mapping and association studies, and maker-assisted selection (MAS) in breeding programs (Blair et al. 2009; Kwak and Gepts 2009; Miklas et al. 2006; Talukder et al. 2010). However, marker development and use remain relatively expensive and the coverage of available markers in the genome is still modest (Varshney et al. 2014).

Next Generation Sequencing (NGS) technologies are revolutionizing genetic studies and molecular markers development by exponentially increasing the number of genetic variants that can be discovered in a single experiment (Stapley et al. 2010). With these technologies, single nucleotide polymorphism (SNP) and insertion-deletion (InDel) detection and genotyping have become feasible on a whole-genome scale and are widely applied to diversity and association studies in plants (Thudi et al. 2012; Varshney et al. 2014). Nevertheless, in spite of the reduced cost of sequencing technologies and the increased throughput and multiplexing, the cost of sequencing and genotyping large numbers of individuals is still prohibitive in plants with complex and repetitive genomes (Davey et al. 2011; Deschamps and Campbell 2010).

Several complexity reduction approaches that couple restriction enzyme (RE) genome digestion with NGS and SNP calling have been developed in the last years for high-throughput molecular marker discovery in different organisms (Davey et al. 2011). These approaches include reduced-representation libraries (RRLs) (Altshuler et al. 2000), restriction-site-associated DNA sequencing (RAD-Seq) (Baird et al. 2008), restriction enzyme sequence comparative analysis (RESCAN) (Monson-Miller et al. 2012), and GBS (Elshire et al. 2011).

GBS is a robust, high-throughput, cost-effective, and simple technique for obtaining thousands of markers from large numbers of individuals. It has been applied in genetic diversity studies to both plants and animal species (De Donato et al. 2013; Elshire et al., 2011; Glaubitz et al. 2014). In addition, in spite of the high percentage of missing data (Glaubitz et al. 2014; Beissinger et al. 2013), GBS technology has demonstrated its usefulness in the identification of quantitative trait loci (QTLs) in several crops like barley, soybean, chickpea, wheat, and common bean (Hart and Griffiths 2015; Iquira et al. 2015; Li et al. 2015; Liu et al. 2014; Jaganathan et al. 2015). Despite its several advantages, GBS requires a species-specific optimization regarding the RE used to avoid repetitive regions of the genome and to determine marker number, distribution, and depth (Beissinger et al. 2013). For example, Hart and Griffiths (2015) found good SNP coverage in common bean using *Ape*KI, but there was uneven density distribution, probably because *Ape*KI is a methylation-sensitive enzyme. On the other hand, Zou et al. (2014) employed a methylation-insensitive enzyme (*Hae*III) in common bean, but detected a high proportion of the SNPs (~73%) in repetitive regions. In the research reported here, an *in silico* analysis of different RE was performed to identify suitable enzymes for GBS in common beans, based on the availability of a *P. vulgaris* reference genome sequence (Schmutz et al. 2014). We then tested the GBS method with a panel of 18 wild and domesticated *P. vulgaris* accessions. Results are considered in light of read mapability among genotypes, marker distribution, and sequence depth. We evaluate also the possibility of using GBS with *Cvi*AII for identifying copy number variations (CNVs) across different genotypes. The information reported here will be useful for planning other GBS experiments in common bean using a larger number of genotypes, for both diversity and association studies.

Materials and Methods

In silico digestion, library preparation and sequencing

Thanks to the availability of the *P. vulgaris* whole-genome sequence (Schmutz et al. 2014), a survey of different restriction enzymes (RE) and their relative cutting sites could be performed. Using the Biopython suite (Cock et al. 2009), we selected enzymes that create a 'sticky' end after cleaving, cut only once for each recognition site, and do not recreate the restriction site after digestion. Elshire *et al.* (2011) suggested a methylation-sensitive enzyme to avoid repetitive elements of the genome when using GBS with maize, a plant with a large genome composed mainly of transposable elements (Schnable et al. 2009). In contrast, common bean has a relative small genome, with only 50% of the genome belonging to pericentromeric regions, which contain 26% of the genes (Schmutz et al. 2014). In addition, because of possible genotype-dependent differences in DNA methylation (Grativol et al. 2012), which could bias genotyping, we followed another approach. For each selected enzyme, we counted the number of recognition sites in the masked (where all the repetitive sequences are converted into string of Ns) and unmasked genome sequences, and kept those enzymes that preferentially cut in the non-repetitive part of the genome, based on a binomial test. In this sub-set of enzymes, we selected *Cvi*AII (recognition site C'ATG), because this enzyme showed the higher restriction site count and displayed a preferential localization in the non-repetitive part of the genome. Since *Ape*KI has been recently applied in common bean (Hart and Griffiths 2015), we also compared the *in silico* distribution of digested fragments suitable for sequencing (50 to 350 bp length) between *Ape*KI and *Cvi*AII across the genome (Supplementary File S1).

In order to check the applicability of the GBS protocol using *Cvi*AII, a test experiment was performed with 17 wild and domesticated *P. vulgaris* genotypes belonging to both Andean and Mesoamerican gene pools. In addition, a representative of the wild ancestral gene pool from northern Peru, G21245, was also included (Supplementary File S2). As internal control for our analysis, we included also the common bean genotype used for generating the genome reference sequence (G19833; Schmutz et al. 2014). Specific barcodes and adapters for *Cvi*AII were designed with the GBS barcoded adapter generator (http://www.deenabio.com/services/gbs-adapters) (Supplementary File S2).

DNA was extracted from freeze-dried bean leaves of greenhouse-grown plants using a modified protocol of Pallotta et al. (2003) with an extra step consisting in re-suspension with 4 μl of RNAse and incubation for 30 minutes at 37^0C. DNA quality was checked with NanoDrop Lite (Thermo Fisher Scientific) and by 1% agarose gel electrophoresis. DNA with an absorbance ratio (A260/A280) > 1.7 and with no visible degradation on agarose gel was used for subsequent library preparation. Genomic DNA and library adapters were quantified with QUBIT dsDNA HS assay kit (Thermo Fisher Scientific/Invitrogen, Grand Island, NY). GBS libraries and adapters were prepared following the protocol of Elshire *et al.* (2011), using *Cvi*AII (New England Biolabs, Ipswitch, MA) for DNA digestion and a 1:4 dilution of adapter mix (common and barcoded adapter) at a final concentration of 4.5 ng per reaction. In the ligation step, we reduced the ligation buffer concentration to 0.6x per reaction, instead of the suggested 1x. During the fragment enrichment step, four separate PCR amplifications were performed and the different reactions were then pooled for PCR purification. The presence of adapter dimers in the sequencing libraries was checked with the Experion DNA analysis kit (Biorad, Berkeley, CA). Genomic libraries were sequenced in a single lane of Illumina HiSeq2000 flowcell, using the 50bp cycle protocol, in the QB3 Vincent J. Coates Genomics Sequencing Laboratory at the University of California, Berkeley, CA. The raw sequencing reads have been deposited in the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) under the accession number SRX1308469.

Sequencing pre-processing, alignment and SNP calling

Recently, TASSEL-GBS (Glaubitz et al. 2014), a specific algorithm for analysis and SNP-calling of GBS datasets, was released. The software was specifically implemented for calling the maximum number of SNPs in low coverage and highly multiplexed datasets, favoring allelic redundancy over quality score (Glaubitz et al. 2014). Since our dataset contained few lines at high coverage, we preferred to follow a different, more robust, and accepted pipeline for bioinformatic analysis (Altmann et al 2012). In particular, we used SAMtools for SNP calling since different studies indicate that it is more conservative in variant calling compared to other algorithms, also in datasets obtained from reduced representation libraries (Altmann et al 2012, Greminger et al. 2014).

Reads were quality trimmed at the 3'-end using sickle (https://github.com/najoshi/sickle), keeping only reads with no more than 2 Ns and a minimum length after trimming of 30bp. Then, the reads that recreated the *Cvi*AII cutting site (possible chimeras, partial digestion or sequencing errors) or that contained the common adapter sequence (short fragments) were trimmed and only those reads longer than 30bp after this second trimming step were retained. The last filtering step kept only the reads that contained, after the barcode sequence, the overhang sequence of *Cvi*AII digestion (i.e., ATG). The resulting reads were then demultiplexed using sabre (https://github.com/najoshi/sabre) allowing one mismatch for each barcode.

Read alignment was performed on the *P. vulgaris* unmasked genome sequence (http://www.phytozome.net/commonbean) using BWA (Li and Durbin 2009). After the alignment, only the reads with a minimum mapping quality of 10 were used for downstream application. Base call recalibration was performed with the R package (www.r-project.org) ReQON (Cabanski et al. 2012). After quality score recalibration, variants were called with SAMtools considering only loci covered by more than 30% of the lines analyzed (6 lines). The resulting variants were filtered with VCFtools (Danacek et al. 2011); only those with a Minor Allele Frequency (MAF) higher than 0.05, a minimum quality more than 10, and a mean read depth, across all lines, from 5 to 1000 (--maf 0.05 --minQ 10 --min-meanDP 5 --max-meanDP 1000) were considered for downstream analysis. SNP and InDel statistics were performed with VCFtools; SNP density and transition to transversion ratio (Ts/Tv) were calculated for non-overlapping bins of 1Mb.

Identification of repetitive regions and phylogenetic analysis

SNPs located in repeated regions were removed with VCFtools using the annotation of *P. vulgaris* repeats available in Phytozome (Goodstein et al. 2012).

For phylogenetic analysis, only the variants located in annotated coding DNA sequences (CDS) were used, since these regions are generally subjected to higher evolutionary pressure than non-coding DNA sequences. A FASTA multiple alignment file was created for subsequent phylogenetic analysis by concatenating the extracted variants at each position for each genotype analyzed. During the creation of the multiple alignment file, individual genotypes with a quality below 10 or missing genotypes were treated as missing data. Due to the self-pollinating nature of *P. vulgaris*, the heterozygous calls were also treated as missing data, since they could be sequencing or SNP calling errors. The resulting multiple alignment file was then analyzed using the seaview toolkit (Gouy et al. 2010). A phylogenetic tree was built using the Neighbor-Joining (NJ) clustering approach, with the Kimura two-parameter (Kimura, 1980) nucleotide substitution model and 1000 bootstrap replicates using the seaview toolkit (Gouy et al. 2010).

CNV identification and annotation

CNVs were identified using the reference genotype G19833 as baseline for identifying coverage shifts, as a proxy of CNV, in the other sequenced genotypes. First, we calculated the number of reads in 100Kb non-overlapping genomic bins in each genotype. Then, we normalized the read counts in each bin by dividing the count by the total number of reads mapped in each genotype, and calculating the relative read coverage (RRC) as a ratio between the normalized read counts of the genotype of interest and the reference genotype (G19833). The RRC should be normally distributed with a mean ~1. For this analysis, we removed the genomic bins without mapped reads in the G19833 genotype. We selected as putative CNV the genomic bins with a RRC < 0.1 or > 1.9; the genes located in these genomic bins were then subjected to Gene Ontology (GO) enrichment analysis using the Blast2Go tool (Conesa et al. 2005).

Results and Discussion

In silico genome digestion and analysis of high-throughput sequencing raw data

Comparison of *in sili*co genome digestion between *Cvi*AII and *Ape*KI showed that *Cvi*AII would produce more fragments suitable for sequencing but that it will require a higher sequencing coverage than *Ape*KI. On the other hand, by usin*g C*viAII, we would be able to tag 97% of the genes present in *P. vulgari*s genome, 30% more than when usin*g A*pe*K*I (Supplementary File S1).

Sequencing on a HiSeq2000 (Illumina, San Diego, CA) generated 137,026,622 50bp single-end reads of which 127,384,853 (93%) passed the initial sickle quality trimming. Among these ~127M reads, 3,002,729 (2.4%) were removed because they were shorter than 30bp after the trimming of reads containing the RE recognition site or adapter contaminants, or because they did not contain the overhang RE sequence after the barcode sequence. As expected from the library preparation strategy, there was a high level of duplicated reads, with only 13,278,501 unique reads in the dataset, suggesting a mean 10x redundancy for each read tag. Nevertheless, these data suggest that the overall library quality was high and consistent with the experimental approach.

After de-multiplexing, alignment, and filtering of the low-quality aligned reads, the number of reads was almost equally distributed among the different genotypes, with > 90% of annotated genes (~ 25,000) being tagged by at least one read (Table 1). In particular, almost 50% of the reads in each line could be aligned to the reference genome; and 50% of the aligned reads tagged gene sequences. The total number of reads per gene in each line ranged from 36 to 84, with a mean of 52 reads per gene in each line. These results are consistent with the *in silico* digestion of *P. vulgaris* genome, and showed a homogeneous read mapping rate among wild and domesticated races belonging to different gene pools (Table 1).

Analysis of identified SNPs and InDels

A total of 77,595 SNPs and InDels were identified after keeping variants with a Minor Allele Frequency (MAF) higher than 0.05 (--maf 0.05), a minimum calling quality higher than 10 (-minQ 10) and a mean read depth per sites between 5 and 1000 (--min-meanDP 5, --max-meanDP 1000). Among the variants identified, 73,656 (95%) were SNPs, 2,088 (3%) were deletions and 1,851 (2%) were insertions. The InDels ranged from 1 to 8 bp, with the majority of them being mononucleotide insertions and deletions. Due to the repetitive nature of most plant genomes and the resulting miscalls of SNPs and InDels in repetitive regions, all the variants that were located in these regions were removed. The remaining number of variants were 47,838 (61%), divided between 44,875

(94%) SNPs, 1,940 (3%) deletions and 1,693 (3%) insertions. This ratio is similar to the occurrence of *Cvi*AII recognition sites in non-repetitive vs. repetitive regions of the genome, highlighting the reliability of *in silico* digestion-based approaches. In addition, the percentage of variants located in non-repetitive regions was three times higher than the variants identified by Zou et al. (2014) in common bean. For further analysis, only these non-repetitive SNPs were considered.

The SNPs and InDels distributions were significantly highly correlated with chromosome length (r=0.79, *p*=0.004) (Supplementary File S3), with a mean of ~4,328 and a median of 4,312 variants per chromosome, and a median of 79 variants per Mb. These results exceeded markedly the ones, obtained after *Ape*KI digestion, of Hart and Griffiths (2015). In particular, they found a correlation of 0.45 between SNPs density and chromosome length using the *ApeKI* restriction enzyme in common bean. The highest number of variants were observed on chromosome 2 (5,311) and the lowest on chromosome 10 (3,314). On the other hand, no significant correlation was found between mean SNP density (in 1Mb non-overlapping bins) and chromosome length (r=-0.35, *p*= 0.28) (Supplementary File S3). The variant mean read depth for each line ranged from 5 to 12 reads per site, with a mean and median of ~8 reads for SNPs. The variant coverage, averaged across all the lines, ranged from 5 to 439, with a mean and median of 8 and 7, respectively. A plot of variant density in 1Mb non-overlapping bins closely resembled the density of annotated genes in the *P. vulgaris* chromosomes (Fig. 1), with a Pearson's correlation coefficient (r) of 0.89 (p < $2{,}2e^{-16}$).

SNPs were classified into transitions (Ts) and transversions (Tv), based on the type of nucleotide substitution, using VCFtools (Supplementary File S4). The number of C/T and A/G transitions was similar (~13,000); the A/C and G/T transversions had a similar frequency, while A/T and C/G transversions were slightly higher or lower, respectively, compared to A/C and G/T transversions. The Ts/Tv ratio in our dataset was 1.56 for the SNPs localized in non-repetitive regions, slightly higher than previously reported in common beans using a RRLs approach (Zou et al. 2014).

Characterization of SNP and InDel distribution and phylogenetic analysis

The total number of SNPs and InDels per line ranged from 3,512 to 21,415, with the lower number of SNPs and InDels identified in genotypes G19833 (3,512), UC0801 (5,354), CAL143 (5,479), and Midas (9,033) (Table 2). All these genotypes were domesticated beans belonging to the Andean gene pool, as does the genotype used for the reference sequence (G19833), which was also the one with the fewest SNPs in our analysis. SNPs and InDels in Mesoamerican entries ranged from 17,308 (accession PI417653) to 19,664 (PI311859 or G35101). PI311589 is a domesticated bean with black, shiny seed (seed weight of 28 g/100 seed), which could potentially have been subjected to introgression from *P. dumosus* or *P. coccineus*. However, further research is needed to clarify the status of this accession. The genotype with the highest number of variant sites was G21245, a wild bean from the ancestral gene pool originating in northern Peru (Kami et al. 1995), with 21,416 variants detected.

Of the 47,838 SNPs and InDels identified, 23,273 (49%) were located in genic sequences, with 11,163 in CDS, 2,285 in untranslated regions (UTRs), and 9,825 in introns (Table 2). For all the genotypes analyzed, 45-49% of the SNPs and InDels were located in genic sequences; among them ~50% were located in CDS, ~40% in introns, and ~10% in UTRs. The 23,273 SNPs and InDels located in genic sequences identified 11,027 different genes (or 40% of genes identified in the whole-genome reference sequence), with an average of 2 variants per gene.

The phylogenetic analysis based on the identified SNPs and InDels was clearly consistent with the division in different gene pools and domesticated/wild lines, and was also significantly supported by high bootstrapping values (Fig. 2). The Andean and Mesoamerican gene pools were clearly divided with a bootstrap support > 95. In particular, both domesticated groups of Andean and Mesoamerican genotypes were strongly supported by a bootstrap value of 100, confirming the major bottleneck that occurred during each of the two independent domestications of common bean (Bitocchi et al. 2013; Gepts 1998; Schmutz et al. 2014). In addition, the phylogenetic tree automatically was rooted with the ancestral genotype G21245 from northern Peru (Kami et al. 1995). Overall, the phylogenetic analysis of the variants identified using GBS with *Cvi*AII correctly identified genetic relationships among the accessions included in this study, and the level of genetic diversity of the respective gene pools based on previous information about this species (Bitocchi et al. 2013; Gepts 1998; Kwak and Gepts 2009; Schmutz et al. 2014).

CNV identification and annotation

*Cvi*AII, having a 4bp recognition sites, is a frequent-cutting enzyme and shows a diffuse read coverage across the genome (Supplementary File S5). Thus, this enzyme could be suitable for identifying CNVs across different genotypes with GBS, and could also represent a cost-effective approach for identifying this kind of variation in different bean genotypes. Indeed, CNVs are extremely important in plant genome evolution, but also affect plant phenotypes and resistance to both biotic and abiotic stresses (Żmieńko et al. 2014). The approach used in our study showed a RRC normally distributed, with a mean approximately equal to 1 (Supplementary File S6), suggestive of the reliability of this approach for the identification of CNV in common bean. Analysis of RRC showed 162 genomic bins, containing 343 genes, which could contain potential CNVs in the genotypes analyzed, with some of them shared across different genotypes (Supplementary File S7). GO enrichment analysis of these genes highlight a significant enrichment in genes involved in the apoptotic process, innate immune response, transmembrane signaling receptor activity, signal transduction, ATP binding and protein binding (Fig. 3). A large number of these genes are annotated as Leucine-rich repeat proteins and transmembrane kinases, NB-ARC domain-containing disease resistance protein, TIR-NBS-LRR class proteins, and cysteine-rich receptor-like kinases (Supplementary File S8). These observations suggest that the majority of putative CNVs segments identified in these genotypes contain genes involved in biotic stress response. This result is in agreement with previous studies in several plants that identify regions harboring CNVs as enriched in biotic stress-response genes (Cook et al. 2012; deBolt 2010; McHale et al. 2012; Żmieńko et al. 2014), further highlighting the feasibility of CNVs identification using GBS with a frequent-cutting enzyme.

Conclusions

GBS is a simple, cost-effective, and highly multiplexed protocol for plant genotyping using NGS technologies. Using this protocol, we were able to identify 47,838 variants in 18 wild and domesticated bean genotypes. Even though the use of a frequent-cutting, methylation-insensitive enzyme will require a higher genome sequencing coverage, the small genome size of common bean and the results presented in this study clearly show the advantages of using *Cvi*AII for GBS in common bean. We identified thousands of evenly spaced markers across the entire common bean genome, with a high density that closely resembles genes distribution. This high density could help in narrowing QTL regions in mapping experiments, and facilitating a more precise location of recombination events. In addition, 50% of the variants identified lay in genic sequences, while the others were situated in the non-coding part of the genome. The variants in genic sequences reliably identified known phylogenetic subdivisions in common bean. They could also be useful in Genome Wide Association Studies (GWAS) for identifying candidate genes responsible for traits of interest. On the other hand, the variants in the non-coding parts of the genome could be useful - as predominantly neutral markers - for ecological studies in this species, in particular for population modeling and for inferring demographic history in wild common bean. Our approach also allowed us to identify several putative CNVs that could be involved in pathogen response and resistance in different common bean genotypes. Last but not least, the increased throughput and reduced cost of sequencing technology will soon leverage the cost and depth of sequencing required when using GBS with different restriction enzymes such as 4bp-recognizing, methylation-insensitive enzymes, especially for plants with small genomes like common bean.

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Table 1 Distribution of de-multiplexed reads among different individuals.

Genotype	Total reads	Aligned reads*	Aligned reads (%)	Reads aligned to gene sequences	Tagged genes
G21245	8,742,974	4,244,092	48.54	1,813,606	25,299
CAL143	9,421,387	4,829,345	51.26	1,834,224	25,625
G19833	4,905,688	2,570,710	52.40	951,376	25,357
UC0801	7,642,501	3,886,102	50.89	1,467,374	25,419
Midas	5,096,265	2,469,232	48.45	953,307	25,114
PI417653	4,791,423	2,402,640	50.14	995,149	25,147
PI319441	4,423,056	2,172,861	49.13	926,544	25,113
PI343950	8,545,592	4,022,666	47.07	1,693,329	25,494
G12873	5,577,279	2,505,178	44.92	1,040,117	25,010
SEA5	8,044,529	3,724,263	46.29	1,500,884	25,255
Pinto San Rafael	8,533,643	4,053,508	47.50	1,631,999	25,380
Flor de Mayo	5,748,661	2,621,742	45.61	1,063,173	25,077
SER118	6,108,084	2,834,653	46.41	1,123,882	25,199
Matterhorn	4,938,106	2,397,027	48.54	939,353	25,047
UCD9634	11,235,426	5,389,721	47.97	2,141,599	25,434
L88-63	7,657,785	3,633,907	47.45	1,466,989	25,360
Victor	5,591,787	2,624,902	46.94	1,050,803	25,087
PI311859	7,212,192	3,399,587	47.17	1,396,867	25,266

*Only reads with a mapping quality (Q) higher than 10.

Table 2 SNPs and InDels distributions among different genotypes and genomic features.

Genotype	Total SNPs	Genic*	Tagged genes**	CDSs	Introns	UTRs
G21245	21,416	10,327	6,574	4,899	4,404	1,024
CAL143	5,479	2,618	1,769	1,477	897	244
G19833	3,512	1,578	1,308	836	604	138
UC0801	5,354	2,464	1,744	1,300	928	236
Midas	9,033	4,196	2,860	2,167	1,618	411
PI417653	17,308	8,515	5,516	4,128	3,542	845
PI319441	17,741	8,737	5,706	4,240	3,677	820
PI343950	18,955	9,251	5,932	4,455	3,912	884
G12873	18,799	9,102	5,928	4,400	3,796	906
SEA5	18,532	8,929	5,660	4,354	3,693	882
Pinto San Rafael	18,586	8,924	5,638	4,371	3,706	847
Flor de Mayo	18,782	9,029	5,733	4,414	3,728	887
SER118	18,047	8,835	5,579	4,277	3,690	868
Matterhorn	17,525	8,553	5,532	4,165	3,566	822
UCD9634	18,570	9,025	5,718	4,424	3,721	880
L88-63	18,550	8,946	5,698	4,361	3,689	896
Victor	18,712	9,021	5,763	4,382	3,762	877
PI311859	19,664	9,531	5,941	4,603	3,980	948
All Genotypes	47,838	23,273	11,027	11,163	9,825	2,285

*SNPs and InDels located in genic loci. ** Genes identified by at least one SNPs or InDels

Figure Legends

Fig. 1 Distribution of variants and genes with the relative density in 1Mb non-overlapping bins in the 11 *P. vulgaris* chromosomes.

Fig. 2 Neighbor-Joining (NJ) phylogenetic tree based on variants located in genic sequences of the different bean lines. Bootstrap values and gene pools of the different lines are shown. PhI: Ancestral wild; DA: Domesticated Andean; WM: Wild Mesoamerican; DM: Domesticated Mesoamerican.

Fig. 3 Significant GO terms (FDR < 0.05) enriched in the genes located in putative CNVs. Test Set is the set of the up-regulated genes, Reference Set is the background of the *P. vulgaris* GO terms mapping.

Supplementary material

Supplementary File S1 Comparison *P. vulgaris* genome *in silico* digestion and distribution of fragment suitable for sequencing between *Cvi*AII and *Ape*KI. The number of genes tagged by the fragments produced by the two restriction enzymes is shown.

Supplementary File S2 Bean genotypes analyzed in this study with the barcode used for multiplexed sequencing

Supplementary File S3 Correlation between SNP distribution (Total SNPs) and density on a 1Mb non-overlapping bin (SNPs/Mb) with chromosome length. Regression lines and Pearson regression coefficient (r) are shown.

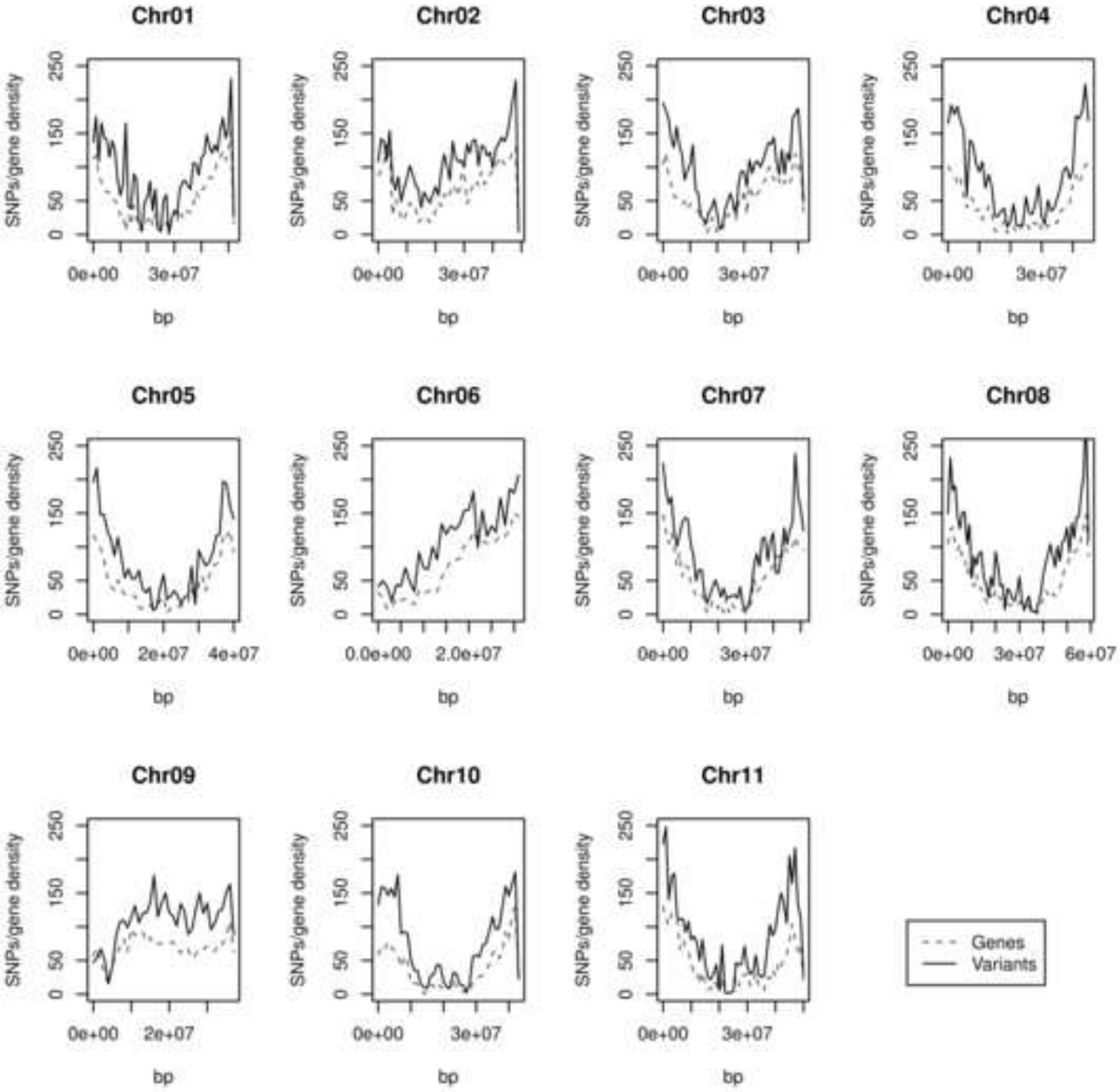
Supplementary File S4 Transition and Transversion counts for the identified SNPs

Supplementary File S5 Read coverage in 1Mb non-overlapping bins across the 11 chromosomes for the G19833 reference genotype.

Supplementary File S6 RRC in the analyzed genotypes.

Supplementary File S7 Regions harboring putative CNVs in the different genotypes. The coordinates of the genomic bins in the different chromosomes are reported in BED format.

Supplementary File S8 Annotation, together with the best *Arabidopsis* hit, of the genes located in putative CNVs. When available the best *Arabidopsis* hit common name is used.


Chr01
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Chr10
Chr11
SNPs/gene density
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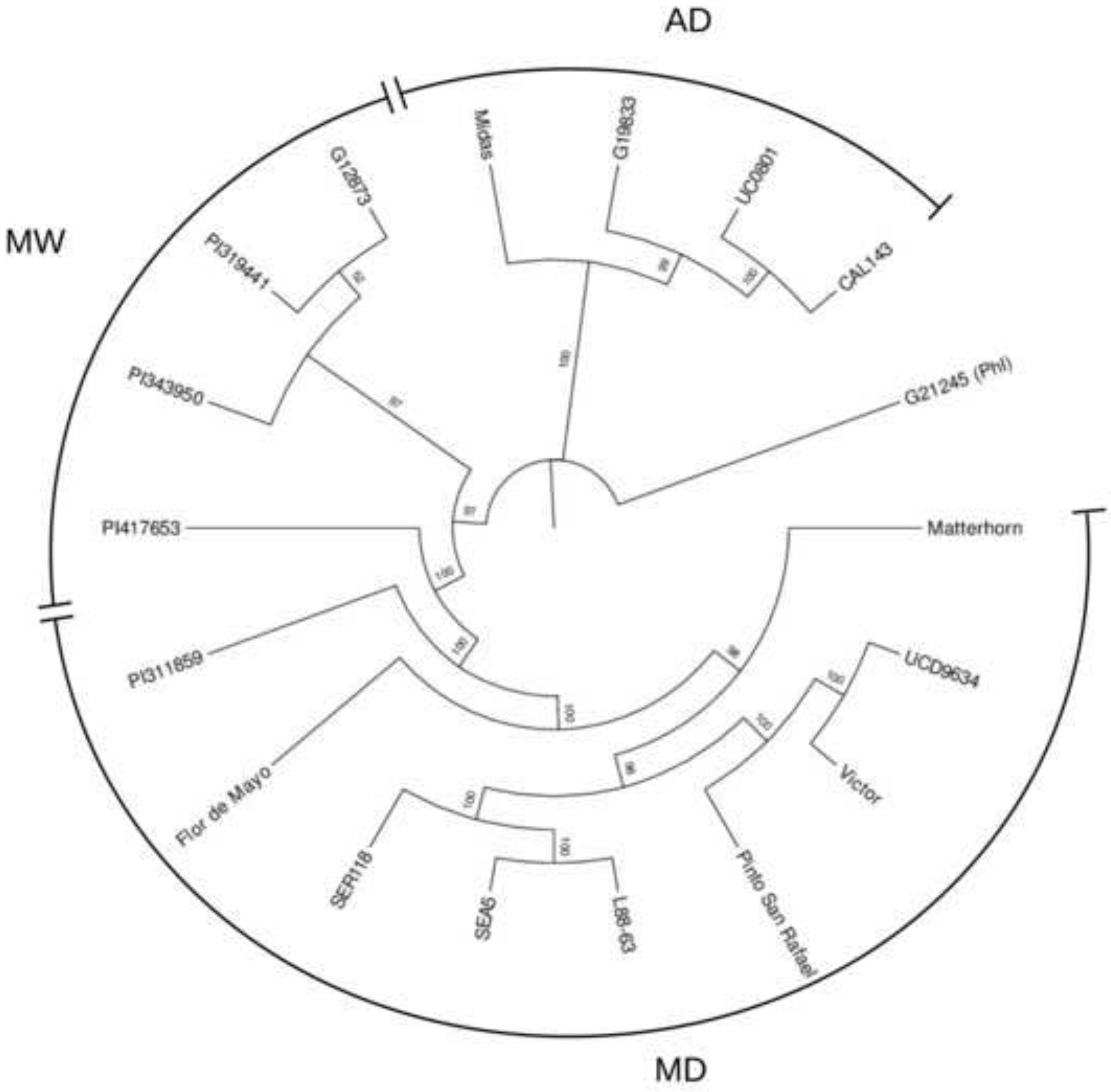
50
150
250
bp
0e+00
3e+07
2e+07
4e+07
6e+07
0.0e+00
2.0e+07
Genes
Variants


AD
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MD
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UC0801
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G12873
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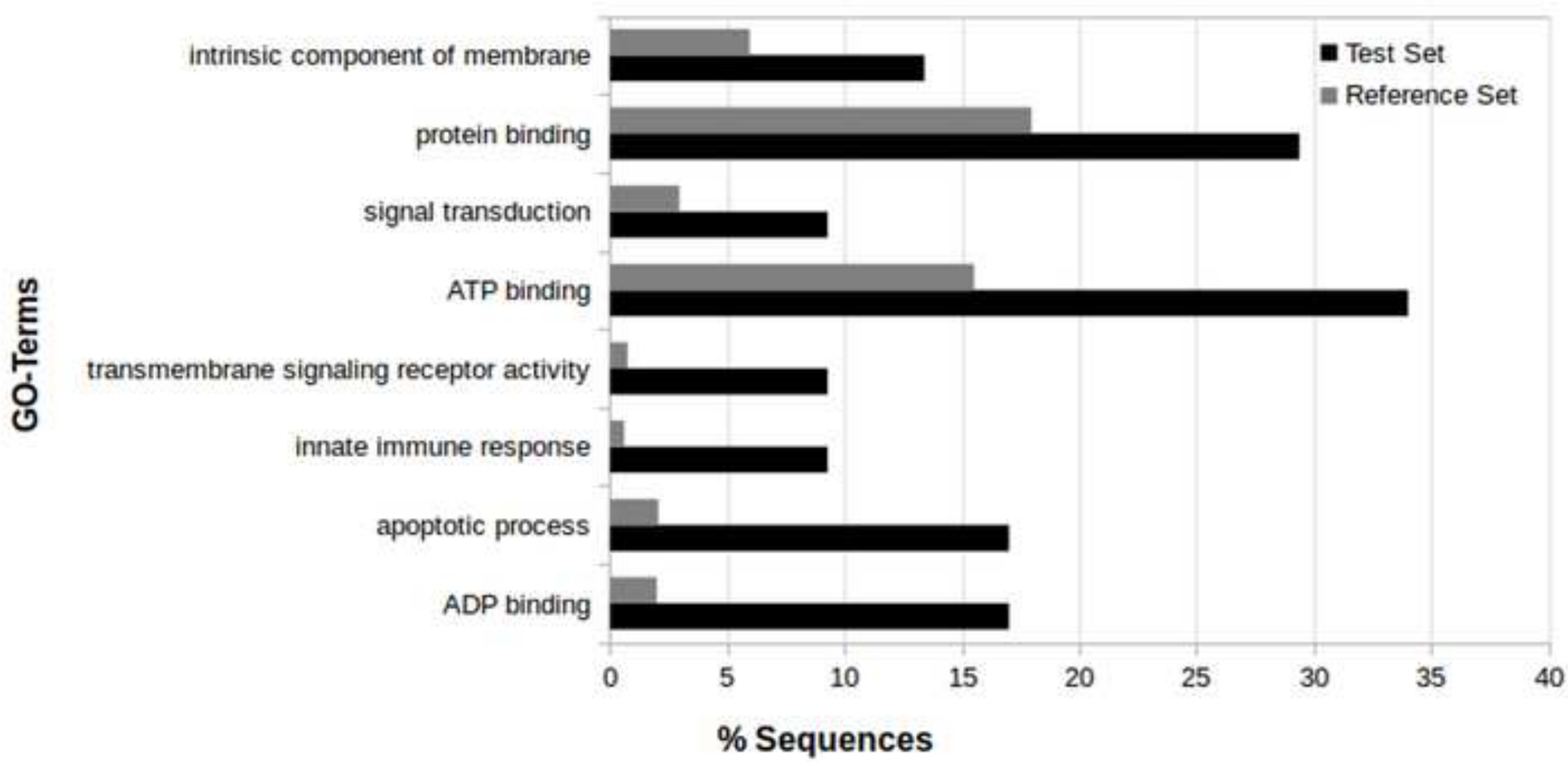
Flor de Mayo
SER118
SEA5
L88-63
Pinto San Rafael
Victor
UCD9634
Matterhorn


intrinsic component of membrane
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transmembrane signaling receptor activity
innate immune response
apoptotic process
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Supplementary Material

Supplementary Material