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Microsatellite markers in common bean (*Phaseolus vulgaris* L.)

Mikrosatelitni biljezi kod graha (*Phaseolus vulgaris* L.)

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Abstract

Common bean is one of the most cultivated and consumed grain legumes worldwide, showing a high level of genetic diversity. Here is presented a detailed review of development and mapping of simple sequence repeats (SSRs, microsatellite markers) in the common bean. In the last 25 years, common bean has been the subject of numerous genetic studies, in which the identification and use of SSRs were conducted, and lead to the development of genetic maps. First genetic maps of common bean have been developed in the 1990s and were based on different molecular markers, and included domestication genes and important agronomic traits. Later, SSRs allowed the genetic mapping of more narrow crosses that are often of interest in plant breeding. Most genetic maps have been correlated with the core map established in the recombinant inbred population BAT93 x Jalo EEP558, and includes different markers, RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), and SSRs in particular. More than 2,000 SSR markers are available for the common bean and they are an important tool to evaluate the genetic diversity of common bean landraces. SSRs are also useful to evaluate intra-specific diversity within the genus *Phaseolus*.

Keywords: common bean, genetic diversity, genetic maps, microsatellite markers

Sažetak

Grah je jedna od najviše uzgajanih i najviše korištenih zrnatih mahunarki u svijetu koja ima visoku razinu genetske raznolikosti. U ovom radu je prikazan detaljan pregled razvoja i kartiranja mikrosatelitnih biljega (SSR, ponavljajuće jednostavne sekvence) kod graha. U posljednjih 25 godina, grah je bio predmetom brojnih genetskih istraživanja u kojima je provedena identifikacija i upotreba SSR-ova što je dovelo do razvoja genetskih karata. Prve genetske karte graha su razvijene u 1990-ima i bile su bazirane na različitim molekularnim biljezima, a uključivale su gene koji su povezani uz udomaćenje i važna agronomska svojstva. Kasnije su SSR-ovi omogućili genetsko kartiranje križanaca koji su često od interesa u oplemenjivanju bilja. Većina genetskih karata povezana je s osnovnom genetskom kartom uspostavljenom na rekombinantnoj inbred liniji BAT93 x Jalo EEP558, koja uključuje različite biljege kao što su RFLP (polimorfizam dužine restrikcijskih ulomaka), RAPD (nasumično umnožena polimorfna DNA), AFLP (polimorfizam dužine umnoženih ulomaka) te SSR-ove. Više od 2 000 SSR biljega je dostupno za grah i oni su važan alat za procjenu genetske raznolikosti tradicijskih kultivara graha. SSR-ovi su također korisni za procjenu unutarvrsne raznolikosti unutar roda *Phaseolus*.

Ključne riječi: grah, genetska raznolikost, genetske karte, mikrosatelitni biljezi

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most agriculturally and economically important crops in human consumption with very good nutritional content (Fetahu et al., 2014). It is a morphologically diverse crop distributed worldwide and cultivated in the tropical, subtropical and temperate zones (Gaitán-Solís et al., 2002). From a genomic perspective, common bean has a small genome (580 Mbp/haploid genome) that is distributed among 22 chromosomes (n=11) (Gepts et al., 2008), and a low index of genome duplication (most loci are single copy making common bean a suitable experimental organism) (Müller et al., 2014).

Mendel, Sax, and Johannsen used common bean in 19th and 20th centuries to derive important principles in genetics (Gepts, 2001). Moreover, genetic and archaeological studies have shown two independent centres of domestication: Mesoamerican (distributed from northern Mexico to Colombia) and Andean (distributed from southern Peru to north-western Argentina) (Gepts et al., 1986). Evidence supporting common bean organization into two major domesticated gene pools were based on morphological and agronomical traits (Singh, 1989; Gepts and Debouck, 1991; Singh et al., 1991), phaseolin type (Gepts et al., 1986; Gepts and Bliss, 1988; Koenig et al., 1990), allozymes (Koenig and Gepts, 1989), a number of molecular markers (Becerra Velasquez and Gepts, 1994; Freyre et al., 1996; Papa and Gepts, 2003; Díaz and Blair, 2006; Blair et al., 2006b, 2007; Kwak and Gepts, 2009; Angioi et al., 2010), and DNA sequences (Bitocchi et al., 2013). Other secondary centres of diversity are found in Africa, Asia, Europe and parts of South America (Gepts and Debouck, 1991).

In the last 25 years, significant progress has been made to understand the common bean genome. Common bean has been subject of multiple genomic studies, beginning with development of genetic maps by integrating different classes of molecular markers, identification of multiple quantitative traits loci (QTL), and identification and use of molecular markers that cosegregate with genes conferring resistance to pests and diseases that affect the crop (Kole, 2011).

Molecular markers and genetic mapping

Molecular markers are particularly useful because they can reveal differences between individuals of the same or different species and have been used to tag genes of economic importance in many crop species and as a valuable source of information about the genetic structure. Based on the method of their detection, DNA markers are divided into three classes: (1) hybridization-based; (2) polymerase chain reaction (PCR)-based and (3) DNA sequence-based (Collard et al., 2005). In agricultural research, one of the main uses of DNA markers was in construction of linkage maps (Collard et al., 2005).

In Europe, many landraces of common bean, together with old and modern cultivars are preserved in gene banks and they have been evaluated in many studies using different molecular markers (Maras et al., 2013). Molecular markers are an important tool to describe and determine genetic diversity among common beans (Müller et al., 2014). Among the first molecular markers that were used in genetic maps for common bean were isozymes and seed proteins (Pathania et al., 2014) then, allozymes, RFLPs (restriction fragment length polymorphism), RAPDs (random amplified polymorphic DNA), AFLPs (amplified fragment length polymorphism) (McClean et al., 2004). Later, development of microsatellite markers simplifies the gaining of a large quantity of genetic information of relevance for genotype identification and therefore provides an opportunity to characterize germplasm collections. Nowadays, the most abundant sources of DNA variations suitable for marker development are simple sequence repeats (SSRs, microsatellite markers) and single nucleotide polymorphism (SNP); they require little DNA and can be automated for high-throughput analysis (Hamblin et al., 2007).

SSRs are repeating DNA sequences composed of 1 - 6 nucleotides that occur in all prokaryotic and eukaryotic genomes (Field and Willis, 1996; Tóth et al., 2000). They are characterized by their codominant transmission (the heterozygotes can be distinguished from homozygotes), wide genomic distribution, high polymorphism, hypervariable, and informative nature, providing considerable pattern, relative abundance with uniform genome coverage, higher mutation rate than standard, and easy sample preparation (Abdul-Muneer, 2014; Senan et al., 2014). Di-, tri- and tetranucleotide repeats are the most frequently used in molecular genetic studies (Selkoe and Toonen, 2006). Based on their location in the genome, microsatellites can be classified as nuclear (nuSSR), mitochondrial (mtSSR) or chloroplastic SSRs (cpSSR) (Kalia et al., 2011).

Usually, SSR loci were isolated from partial genomic libraries of the species of interest. According to Zane et al. (2002), although this method is relatively simple, especially for SSR rich genomes, it can turn out to be extremely inefficient for

species with low microsatellite frequencies. With the advancement in genomics, availability of new molecular tools and sequencing platforms for exploring genomic information, several alternative strategies were devised to reduce the time invested in microsatellite isolation and to significantly increase the yield of microsatellite loci. Microsatellites can be developed through enriched/non-enriched small insert genomic library construction, by utilizing the products generated by other molecular markers or by the application of next-generation sequencing systems (gSSRs), from EST sequences (expressed sequence tag) (genic or EST–SSRs), through interspecific or intergeneric transferability (Kalia et al., 2011; Senan et al., 2014).

A growing number of SSR markers are available for the common bean making it possible to implement studies like assessment genetic diversity and population structure in the cultivated and wild germplasm as well as genetic mapping experiments with an emphasis on the identification of QTLs (Müller et al., 2014). SSRs have also been used to evaluate intra-specific diversity within the *Phaseolus* genus (Gaitán-Solís et al., 2002).

Genetic mapping of common bean before 1999

Genetic maps are important for common bean because a gene related to an economically important trait or diseases like powdery mildew (Pérez-Vega et al., 2013) or anthracnose (Rodríguez-Suárez et al., 2007, 2008; Campa et al., 2009) can be located on the map, which leads to finding the precise chromosomal location.

For genetic mapping in common bean, a large number of markers are in use (Gepts et al., 2008). Genetic mapping of common bean started in the 1990s and included domestication genes and important agronomic traits, and was based on different molecular marker classes (Grisi et al., 2007). Vallejos et al. (1992) were among the first who developed a molecular linkage map of common bean based mostly on RFLP markers. Linkage map was constructed using a backcross progeny between Mesoamerican breeding line XR-235-1-1 and Andean cultivar ‘Calima’. They developed backcross (BC_1) population (Florida map) (Miklas and Singh, 2007). Later, Nodari et al. (1993a, b) developed a low-density genetic map (Davis map) which included RFLPs, RAPDs, isozymes, and morphoagronomic traits, including one virus resistance gene. This map was developed based on F2 mapping population derived from the wide cross between BAT93 of the Mesoamerican and Jalo EEP558 of Andean gene pool, nominated as BJ population. Adam-Blondon et al. (1994) established genetic map (Paris map) by using a backcross population (BC_1) derived from a cross between two European bean genotypes: Ms8E02 and Corel, also based on RFLP and RAPD markers. Freyre et al. (1998) constructed an integrated linkage map to align Davis, Florida and Paris maps. A core linkage map was established in the recombinant inbred population BAT93 x Jalo EEP558 (BJ). The resulting integration of these maps enabled the approximate location of more than 1,070 markers.

Development and mapping of microsatellite markers in common bean after 1999

Use of markers, especially highly polymorphic such as SSRs, allows the genetic mapping of more narrow crosses that are often of interest in plant breeding (Gepts et al., 2008).

For the first time, Yu et al. (1999) identified bean SSRs from searches on sequence databases and they have shown that: the frequency of microsatellite sequences in bean genome is quite high, some common bean microsatellite loci are multi-allelic, and they segregate as Mendelian traits in a segregating population. In the year 2000, Yu et al. generated a set of 37 SSRs and integrated, for the first time, 15 SSRs onto framework map based on RAPD and RFLP markers (BJ population).

Métais et al. (2002) detected 81 alleles at the 15 microsatellite loci and a common bean genomic library enriched for SSR motifs $(ATA)_8$, $(CA)_{12}$, $(CAC)_{8,}$ and $(GA)_{12}$ was constructed. They developed the first ATA microsatellites. Gaitán-Solís et al. (2002) obtained the first set of 68 SSRs derived from the GA enriched genomic libraries. Authors concluded that SSRs can be valuable genetic markers for assessing genetic diversity in common bean and useful for mapping and molecular characterization.

Blair et al. (2003) have developed a set of genomic SSRs from enriched libraries of common bean DNA and have implemented gene-based SSRs from Yu et al. (1999, 2000), and from additional searches for SSRs in the Genbank sequence database. They integrated a total of 100 new microsatellite loci onto two genetic maps (78 on the DG population (DOR364 x G19833) and 22 on the BJ population) with AFLP, RAPD, and RFLP markers. These markers were used for evaluation of a broad range of diversity (Blair et al., 2006b) and for evaluating race structure (Díaz and Blair, 2006; Blair et al., 2007).

An additional series of 21 (GA)n SSRs using a highly microsatellite-enriched library which was described for the first time in common bean was isolated by Yaish and Pérez de la Vega (2003). Guerra-Sanz (2004) identified 20 sequences containing SSRs and designed primer pairs to amplify those SSRs to enlarge the number of SSR loci of common bean. These molecular markers have been used primarily to increase the density of current maps, especially the core linkage map established in the BJ population.

Additional SSRs were needed, to increase their density on the linkage map. Melotto et al. (2004) obtained the first sequence contig from a BAC (bacterial artificial chromosome) clone in the common bean. Since then, Caixeta et al. (2005) developed 21 SSRs based on four BAC common bean clones.

Melotto et al. (2005) identified potential 243 SSRs in common bean expressed sequence tags (ESTs) that can be readily tested for polymorphism and added to the bean map.

Ramírez et al. (2005) sequenced a total of 21,026 ESTs derived from 5 different cDNA libraries (4 from the Mesoamerican cultivar Negro Jamapa and 1 from the Andean cultivar G19833) from various plant organs. They will be used in future researches because EST sequencing can be used to discover genic microsatellites

and direct screening of cDNA libraries for repeat motifs can save on overall sequencing costs (Blair et al., 2009a).

According to Buso et al., in the year 2006, around 180 SSR markers were available for common bean and half of them obtained from gene sequences deposited in GenBank which were less informative than those obtained from enriched genomic libraries. They developed a total of 20 SSRs derived from genomic libraries, which were initially developed, and 10 were characterized using a panel of 85 representative accessions of the common bean gene bank. After that, Blair et al. (2006a) used 80 SSRs to create a genetic map for the advanced backcross population. Ochoa et al. (2006) developed a new genetic map for the G2333 x G19839 population which represents one of the densest microsatellite-based molecular marker maps containing a total of 79 SSR loci integrated with 70 additional RAPD, SCAR (sequence characterized amplified region), and STS (sequence tagged site) loci as well as the genes *Phs* (biochemical marker phaseolin) and *V* (morphological marker flower color). In the year 2007, Grisi et al. described a new set of 61 polymorphic SSRs for common bean and constructed the first linkage map for the BJ population based exclusively on SSRs.

Hanai et al. (2007) have developed a new set of SSRs based on EST and genomic sequences. A subset of 80 SSRs, 40 EST-SSRs, and 40 genomic-SSRs were evaluated for molecular polymorphism. The most frequent were AG, GAA, CAT, TGG motifs. Blair et al. (2008) developed 85 new SSRs, 71 were developed from an ATA-enriched library, 3 AT-rich SSRs from gene-based sequences and 11 were based on non-AT-rich sequences and were made for GA, CA or CAC motif loci. A total of 45 new loci could be mapped on the DG integrated map and this brings to over 160 mapped loci for common bean with the previous genetic mapping of SSRs conducted by Yu et al. (2000), Blair et al. (2003) and Grisi et al. (2007).

Through a cDNA library screening approach, the number of gene-based SSRs was increased by a large-scale library developed from multiple source tissues of an Andean genepool genotype (Blair et al., 2009a). They designed a total of 248 SSR primer pairs from the positive cDNA clones from the G19833 cDNA library of Ramírez et al. (2005). The new markers were named as part of the BMc series (Bean Microsatellites based on cDNA). In the same year, Blair et al. (2009b) created 7 non-enriched genomic libraries from common bean genomic DNA. Library quality was compared and three were selected for further analysis. They developed a total of 100 SSR primer pairs from the non-enriched, small-insert libraries and 80 were tested for polymorphism in a standard parental survey.

Córdoba et al. (2010) searched for SSRs in the 89,017 BAC-end sequences (BES) from the physical map and genetically mapped any polymorphic BES-SSRs onto the genetic map and they identified 875 SSRs. They integrated 99 new BMb markers into the genetic map with a high LOD score with the most common motifs AT/TA and ATA/TAT. The new genetic map had a total of 215 SSRs and included 116 previously mapped SSR loci from Blair et al. (2003, 2008). Hanai et al. (2010) developed a set of new 282 markers (50 EST-SSR, 32 RGA (resistance gene analogs), and 200 AFLP) and a new, expanded version of the core linkage map, using the BJ population. They placed markers with putative known function in some existing gaps including regions with QTL for resistance to anthracnose and rust.

In the year 2011, a total of 3,123 EST sequences were screened by Blair et al. (2011) from leaf and root from the G19833 cDNA library of Ramírez et al. (2005) and used for direct simple sequence repeat discovery. They found 184 SSRs with tri-nucleotide motifs in particular, many of which were GC-rich (ACC, AGC, and AGG) and provided a new set of 120 BMc markers which combined with the 248 previously developed by Blair et al. (2009a) brings the total in this series to 368 markers. The current total of gene-based markers from their laboratory surpasses 500 markers and they produced BM, BMa, BMb, BMc, BMd and BMe marker types.

Garcia et al. (2011) also evaluated the libraries from Ramírez et al. (2005) and other ESTs from GenBank and developed a set of 377 EST-SSR markers, out of which 302 showed good amplification quality and are available for genetic analysis of common bean. These EST-SSR markers were incorporated into BJ map. Authors concluded that these markers showed high rates of transferability, and thereby are suitable for use in other economically important legume species; that their genetic diversity was comparable with genomic SSRs, so they can be used in the genetic characterization of the common bean and related species.

Blair and Hurtado (2013) have evaluated the large set of ESTs sequenced by Ramírez et al. (2005) for EST-SSRs using three bioinformatics pipelines to determine the best of the search types. These authors designed primers for a 175 SSRs concentrating on class I SSRs identified with SSR locator. In the year 2013, according to Blair and Hurtado, common bean had a total of over 700 EST-SSRs.

Although a large number of SSR markers have been developed until the year 2014 for common bean, many have been based on gene sequences and still are not perfectly distributed. Chen et al. (2014) concluded that it is necessary to develop new genomic SSR markers to fill some gaps in the genetic map. Therefore, they created abundant bean DNA sequences through the genome sequencing of a standard Chinese landrace (Hong Yundou), and developed 90 genomic-SSRs (BMg marker type) from these sequences with a 454-FLX pyro sequencer. They integrated 85 primers into the common bean map. 454 pyrosequencing technique was used because it has the benefit of providing large amounts of sequence data at low cost, accurate results, high sensitivity, automated reads and no need for fluorescence-labeled primers compared to other sequencing technologies.

Considering that physical association with RGH (resistance-gene homologue) can identify SSRs clones on large-insert DNA clones such as BACs, Garzon and Blair (2014) identified RGH-SSR in a BAC library from the Andean genotype G19833. They developed a total of 629 RGH-SSRs named BMr (bean microsatellite RGH-associated markers), and integrated a total of 264 markers into a genetic map, among which were 80 RGH loci anchored to single-copy RFLP and SSR markers.

According to Müller et al. (2014), in the year 2014 more than 2000 SSR markers derived from genomic sequences and genes are available for the common bean.

Most of the analysed accessions of common bean share common ancestors so they have high rates of genetic similarity (Chiorato et al., 2007). Along with other advantages, SSRs are very useful because data from SSR analysis can be reproducible between laboratories and suitable for comparisons between studies and germplasm sets (Blair et al., 2012). In recent years, to characterize common bean genetic diversity, population structure and determination of evolutionary origin

numerous studies have been carried out based on SSRs (Blair et al., 2006b; Díaz and Blair, 2006; Asfaw et al., 2009; Blair et al. 2009c; Kwak and Gepts, 2009; Angioi et al., 2010; Lobo Burle et al., 2010; Cabral et al., 2011; Khaidizar et al., 2012; McClean et al., 2012; Gioia et al., 2013; Okii et al., 2014; Carović-Stanko et al., 2017; Leitão et al., 2017).

Conclusion

Molecular markers are an important tool to describe and determine genetic diversity among common beans. Some of their advantages are: (1) they can reveal differences between individuals of the same or different species; (2) are used to tag genes of economic importance; (3) are a valuable source of information about the genetic structure; and (4) are used in construction of linkage maps.

For genetic mapping in common bean, many different markers are in use, SSRs in particular. Genetic maps are important for common bean because a gene related to an economically important trait or diseases can be located on the map, which leads to finding the precise chromosomal location. Microsatellite analyses are useful to characterize genetic diversity, population structure and origin of common bean.

For common bean, SSR markers have been derived from GenBank sequences, through SSR-enriched libraries by various SSR capture techniques including bead- or membrane-bound oligonucleotides, through bacterial artificial chromosome (BAC) sequences, then screening of EST collections or gene sequences and then cDNA libraries.

EST-SSR markers have high rates of transferability so they are suitable for use in other economically important legume species. Also, their genetic diversity is comparable with genomic SSRs and they can be used in the genetic characterization of the common bean and related species.

References

Abdul-Muneer, P. M. (2014) Application of microsatellite markers in conservation genetics and fisheries management: recent advances in population structure analysis and conservation strategies. Genetics Research International, 2014, 691759. DOI: 10.1155/2014/691759

Adam-Blondon, A.-F., Sevignac, M., Dron, M., Bannerot, H. (1994) A genetic map of common bean to localize specific resistance genes against anthracnose. Genome, 37 (6), 915-924. DOI: 10.1139/g94-131

Angioi, S. A., Rau, D., Attene, G., Nanni, L., Bellucci, E., Logozzo, G., Negri, V., Spagnoletti Zeuli, P. L., Papa, R. (2010) Beans in Europe: origin and structure of the European landraces of *Phaseolus vulgaris* L. Theoretical and Applied Genetics, 121 (5), 829-843. DOI: 10.1007/s00122-010-1353-2

Asfaw, A., Blair, M. W., Almekinders, C. (2009) Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) landraces from the East African highlands. Theoretical and Applied Genetics, 120 (1), 1-12. DOI: 10.1007/s00122-009-1154-7

Becerra Velasquez, V. L., Gepts, P. (1994) RFLP diversity of common bean (*Phaseolus vulgaris*) in its centres of origin. Genome, 37 (2), 256-263. DOI: 10.1139/g94-036

Bitocchi, E., Bellucci, E., Giardini, A., Rau, D., Rodriguez, M., Biagetti, E., Santilocchi, R., Spagnoletti Zeuli, P., Gioia, T., Logozzo, G., Attene, G., Nanni, L., Papa, R. (2013) Molecular analysis of the parallel domestication of the common bean in Mesoamerica and the Andes. New Phytologist, 197 (1), 300-313. DOI: 10.1111/j.1469-8137.2012.04377.x

Blair, M. W., Pedraza, F., Buendia, H. F., Gaitán-Solís, E., Beebe, S. E., Gepts, P., Tohme, J. (2003) Development of a genome-wide anchored microsatellite map for common bean (*Phaseolus vulgaris* L.). Theoretical and Applied Genetics, 107 (8), 1362-1374. DOI: 10.1007/s00122-003-1398-6

Blair, M. W., Iriarte, G., Beebe, S. (2006a) QTL analysis of yield traits in an advanced backcross population derived from a cultivated Andean x wild common bean (*Phaseolus vulgaris* L.) cross. Theoretical and Applied Genetics, 112 (6), 1149-1163. DOI: 10.1007/s00122-006-0217-2

Blair, M. W., Giraldo, M. C., Buendía, H. F., Tovar, E., Duque, M. C., Beebe, S. E. (2006b) Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.). Theoretical and Applied Genetics, 113 (1), 100-109. DOI: 10.1007/s00122-006-0276-4

Blair, M. W., Díaz, J. M., Hidalgo, R., Díaz, L. M., Duque, M. C. (2007) Microsatellite characterization of Andean races of common bean (*Phaseolus vulgaris* L.). Theoretical and Applied Genetics, 116 (1), 29-43. DOI: 10.1007/s00122-007-0644-8

Blair, M. W., Buendía, H. F., Giraldo, M. C., Métais, I., Peltier, D. (2008) Characterization of AT-rich microsatellites in common bean (*Phaseolus vulgaris* L.). Theoretical and Applied Genetics, 118 (1), 91-103. DOI: 10.1007/s00122-008-0879-z

Blair, M. W., Muñoz Torres, M., Giraldo, M. C., Pedraza, F. (2009a) Development and diversity of Andean-derived, gene-based microsatellites for common bean (*Phaseolus vulgaris* L.). BMC Plant Biology, 9, 100. DOI: 10.1186/1471-2229-9-100

Blair, M. W., Muñoz Torres, M., Pedraza, F., Giraldo, M. C., Buendía, H. F., Hurtado, N. (2009b) Development of microsatellite markers for common bean (*Phaseolus vulgaris* L.) based on screening of non-enriched, small-insert genomic libraries. Genome, 52 (9), 772-782. DOI: 10.1139/g09-053

Blair, M. W., Díaz, L. M., Buendía, H. F., Duque, M. C. (2009c) Genetic diversity, seed size associations and population structure of a core collection of common beans (*Phaseolus vulgaris* L.). Theoretical and Applied Genetics, 119 (6), 955-972. DOI: 10.1007/s00122-009-1064-8

Blair, M. W., Hurtado, N., Chavarro, C. M., Muñoz Torres, M., Giraldo, M. C., Pedraza, F., Tomkins, J., Wing, R. (2011) Gene-based SSR markers for common bean (*Phaseolus vulgaris* L.) derived from root and leaf tissue ESTs: an integration of the BMc series. BMC Plant Biology, 11, 50. DOI: 10.1186/1471-2229-11-50

Blair, M. W., Soler, A., Cortés, A. J. (2012) Diversification and population structure in common beans (*Phaseolus vulgaris* L.). PLoS ONE, 7 (11), e49488. DOI: 10.1371/journal.pone.0049488

Blair, M. W., Hurtado, N. (2013) EST-SSR markers from five sequenced cDNA libraries of common bean (*Phaseolus vulgaris* L.) comparing three bioinformatic algorithms. Molecular Ecology Resources, 13 (4), 688-695. DOI: 10.1111/1755-0998.12099

Buso, G. S. C., Amaral, Z. P. S., Brondani, R. P. V., Ferreira M. E. (2006) Microsatellite markers for the common bean *Phaseolus vulgaris*. Molecular Ecology Resources, 6 (1), 252-254. DOI: 10.1111/j.1471-8286.2006.01210.x

Cabral, P. D. S., Soares, T. C. B., Lima, A. B. P., de Miranda, F. D., Souza, F. B., Gonçalves, L. S. A. (2011) Genetic diversity in local and commercial dry bean (*Phaseolus vulgaris*) accessions based on microsatellite markers. Genetics and Molecular Research, 10 (1), 140-149. DOI: 10.4238/vol10-1gmr993

Caixeta, E. T., Borém, A., Kelly, J. D. (2005) Development of microsatellite markers based on BAC common bean clones. Crop Breeding and Applied Biotechnology, 5, 125-133. DOI: 10.12702/1984-7033.v05n02a01

Campa, A., Giraldez, R., Ferreira, J. J. (2009) Genetic dissection of the resistance to nine anthracnose races in the common bean differential cultivars MDRK and TU. Theoretical and Applied Genetics, 119 (1), 1-11. DOI: 10.1007/s00122-009-1011-8

Carović-Stanko, K., Liber, Z., Vidak, M., Barešić, A., Grdiša, M., Lazarević, B., Šatović, Z. (2017) Genetic diversity of Croatian common bean landraces. Frontiers in Plant Science, 8, 604. DOI: 10.3389/fpls.2017.00604

Chen, M., Wu, J., Wang, L., Zhang, X., Blair, M. W., Jia, J., Wang, S. (2014) Development of mapped simple sequence repeat markers from common bean (*Phaseolus vulgaris* L.) based on genome sequences of a Chinese landrace and diversity evaluation. Molecular Breeding, 33 (2), 489-496. DOI: 10.1007/s11032-013-9949-2

Chiorato, A. F., Morais Carbonell, S. A., Benchimol, L. L., Chiavegato, M. B., dos Santos Dias, L. A., Colombo, C. A. (2007) Genetic diversity in common bean accessions evaluated by means of morpho-agronomical and RAPD data. Scientia Agricola, 64 (3), 256-262. DOI: 10.1590/S0103-90162007000300007

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., Pang, E. C. K. (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica, 142 (1-2), 169-196. DOI: 10.1007/s10681-005-1681-5

Córdoba, J. M., Chavarro, C., Schlueter, J. A., Jackson, S. A., Blair, M. W. (2010) Integration of physical and genetic maps of common bean through BAC-derived microsatellite markers. BMC Genomics, 11, 436. DOI: 10.1186/1471-2164-11-436

Díaz, L. M., Blair, M. W. (2006) Race structure within the Mesoamerican gene pool of common bean (*Phaseolus vulgaris* L.) as determined by microsatellite markers. Theoretical and Applied Genetics, 114 (1), 143-154. DOI: 10.1007/s00122-006-0417-9

Fetahu, S., Aliu, S., Rusinovci, I., Behluli, A., Kelmendi, B. (2014) Genetic diversity for micronutrients contents in some common bean landraces (*Phaseolus vulgaris* L.). In: Marić, S., Lončarić, Z., Proceedings of 49th Croatian and 9th International symposium on agriculture. Dubrovnik, Croatia, 16-21 February 2015, Osijek, Croatia: Faculty of Agriculture, University of Josip Juraj Strossmayer in Osijek. DOI: 10.13140/RG.2.1.1126.8568

Field, D., Wills, C. (1996) Long polymorphic microsatellites in simple organisms. Proceeding of the Royal Society of London, Series B: Biological Sciences, 263 (1367), 209-215. DOI: 10.1098/rspb.1996.0033

Freyre, R., Ríos, R., Guzmán, L., Debouck, D. G., Gepts, P. (1996) Ecogeographic distribution of *Phaseolus* spp. (*Fabaceae*) in Bolivia. Economic Botany, 50 (2), 195-215. DOI: 10.1007/BF02861451

Freyre, R., Skroch, P. W., Geffroy, V., Adam-Blondon, A.-F., Shirmohamadali, A., Johnson, W. C., Llaca, V., Nodari, R. O., Pereira, P. A., Tsai, S.-M., Tohme, J., Dron, M., Nienhuis, J., Vallejos, C. E., Gepts P. (1998) Towards an integrated linkage map of common bean. 4. Development of a core linkage map and alignment of RFLP maps. Theoretical and Applied Genetics, 97 (5-6), 847-856. DOI: 10.1007/s001220050964

Gaitán-Solís, E., Duque, M. C., Edwards, K. J., Tohme, J. (2002) Microsatellite repeats in common bean (*Phaseolus vulgaris*): isolation, characterization, and cross-species amplification in *Phaseolus* ssp. Crop Science, 42 (6), 2128-2136. DOI: 10.2135/cropsci2002.2128

Garcia, R. A. V., Rangel, P. N., Brondani, C., Martins, W. S., Melo, L. C., Carneiro, M. S., Borba, T. C. O., Brondani, R. P. V. (2011) The characterization of a new set of EST-derived simple sequence repeat (SSR) markers as a resource for the genetic analysis of *Phaseolus vulgaris*. BMC Genetics, 12, 41-54. DOI: 10.1186/1471-2156-12-41

Garzon, L. N., Blair, M. W. (2014) Development and mapping of SSR markers linked to resistance-gene homologue clusters in common bean. The Crop Journal, 2 (4), 183-194. DOI: 10.1016/j.cj.2014.04.002

Gepts, P., Osborn, T. C., Rashka, K., Bliss, F. A. (1986) Phaseolin protein variability in wild forms and landraces of common bean (*Phaseolus vulgaris* L.): evidence for multiple centers of domestication. Economic Botany, 40 (4), 451-468. DOI: 10.1007/BF02859659

Gepts, P., Bliss, F. A. (1988) Dissemination pathways of common bean (*Phaseolus vulgaris*, Fabaceae) deduced from phaseolin electrophoretic variability. II. Europe and Africa. Economic Botany, 42 (1), 86-104. DOI: 10.1007/BF02859038

Gepts, P., Debouck, D. G. (1991) Origin, domestication, and evolution of the common bean (*Phaseolus vulgaris* L.). In: A. van Schoonhoven, O. Voysest, eds. (1991) Common beans: research for crop improvement. Wallingfor, UK: CAB Publishing, 7-53.

Gepts, P. (2001) *Phaseolus vulgaris* (Beans). In: S. Brenner, J. H. Miller, eds. (2001) Encyclopedia of genetics. Massachusetts, USA: Academic Press, 1444-1445. DOI: 10.1006/rwgn.2001.1749

Gepts, P., Aragão, F. J. L., de Barros, E., Blair, M. W., Brondani, R., Broughton, W., Galasso, I., Hernández, G., Kami, J., Lariguet, P., McClean, P., Melotto, M., Miklas, P., Pauls, P., Pedrosa-Harand, A., Porch, T., Sánchez, F., Sparvoli, F., Yu, K. (2008) Genomics of *Phaseolus* beans, a major source of dietary protein and micronutrients in the tropics. In: P. H. Moore, R. Ming, eds. (2008) Genomics of tropical crop plants. New York, USA: Springer, 113-143. DOI: 10.1007/978-0-387-71219-2_5

Gioia, T., Logozzo, G., Attene, G., Bellucci, E., Benedettelli, S., Negri, V., Papa, R., Spagnoletti Zeuli, P. (2013). Evidence for introduction bottleneck and extensive inter- gene pool (Mesoamerica x Andes) hybridization in the European common bean (*Phaseolus vulgaris* L.) germplasm. PLoS ONE 8, e75974. DOI: 10.1371/journal.pone.0075974

Grisi, M. C. M., Blair, M. W., Gepts, P., Brondani, C., Pereira, P. A. A., Brondani R. P. V. (2007) Genetic mapping of a new set of microsatellite markers in a reference common bean (*Phaseolus vulgaris*) population BAT93 x Jalo EEP558. Genetics and Molecular Research, 6 (3), 691-706.

Guerra-Sanz, J. M. (2004) New SSR markers of *Phaseolus vulgaris* from sequence databases. Plant Breeding, 123 (1), 87-89. DOI: 10.1046/j.0179-9541.2003.00929.x

Hamblin, M. T., Warburton, M. L., Buckler, E. S. (2007) Empirical comparison of simple sequence repeats and single nucleotide polymorphisms in assessment of maize diversity and relatedness. PLoS ONE, 2 (12), e1367. DOI: 10.1371/journal.pone.0001367

Hanai, L. R., Campos, T., Camargo, L. E. A., Benchimol, L. L., Souza, A. P., Melotto, M., Carbonell, S. A. M., Chioratto, A. F., Consoli, L., Formighieri, E. F., Siqueira, M. V. B. M., Tsai, S. M., Vieira, M. L. C. (2007) Development, characterization, and comparative analysis of polymorphism at common bean SSR loci isolated from genic and genomic sources. Genome, 50 (3), 266-277. DOI: 10.1139/G07-007

Hanai, L. R., Santini, L., Camargo, L. E. A., Fungaro, M. H. P., Gepts, P., Tsai, S. M., Vieira, M. L. C. (2010) Extension of the core map of common bean with EST-SSR, RGA, AFLP, and putative functional markers. Molecular Breeding, 25 (1), 25-45. DOI: 10.1007/s11032-009-9306-7

Kalia, R. K., Rai, M. K., Kalia, S., Singh R., Dhawan A. K. (2011). Microsatellite markers: an overview of the recent progress in plants. Euphytica, 177 (3), 309-334. DOI: 10.1007/s10681-010-0286-9

Khaidizar, M. I., Haliloglu, K., Elkoca, E., Aydin, M., Kantar, F. (2012) Genetic diversity of common bean (*Phaseolus vulgaris* L.) landraces grown in northeast Anatolia of Turkey assessed with simple sequence repeat markers. Turkish Journal of Field Crops, 17 (2), 145-150.

Koenig, R., Gepts, P. (1989) Allozyme diversity in wild *Phaseolus vulgaris*: further evidence for two major centers of genetic diversity. Theoretical and Applied Genetics, 78 (6), 809-817. DOI: 10.1007/BF00266663

Koenig, R. L., Singh, S. P., Gepts, P. (1990) Novel phaseolin types in wild and cultivated common bean (*Phaseolus vulgaris*, Fabaceae). Economic Botany, 44 (1), 50-60. DOI: 10.1007/BF02861066

Kole, C. (2011) Wild crop relatives: genomic and breeding resources, legume crops and forages. New York: Springer. DOI: 10.1007/978-3-642-14387-8

Kwak, M., Gepts, P. (2009) Structure of genetic diversity in the two major gene pools of common bean (*Phaseolus vulgaris* L., Fabaceae). Theoretical and Applied Genetics, 118 (5), 979-992. DOI: 10.1007/s00122-008-0955-4

Leitão, T. S., Dinis, M., Veloso, M. M., Šatović, Z., Vaz Patto, M. C. (2017) Establishing the bases for introducing the unexplored Portuguese common bean germplasm into the breeding world. Frontiers in Plant Science, 8, 1296. DOI: 10.3389/fpls.2017.01296

Lobo Burle, M., Fonseca, J. R., Kami, J. A., Gepts, P. (2010) Microsatellite diversity and genetic structure among common bean (*Phaseolus vulgaris* L.) landraces in Brazil, a secondary center of diversity. Theoretical and Applied Genetics, 121 (5), 801-813. DOI: 10.1007/s00122-010-1350-5

Maras, M., Šuštar-Vozlič, J., Kainz, W., Meglič, V. (2013) Genetic diversity and dissemination pathways of common bean in Central Europe. Journal of the American Society for Horticultural Science, 138 (4), 297-305.

McClean, P., Kami, J., Gepts, P. (2004) Genomics and genetic diversity in common bean. In: R. F. Wilson, H. T. Stalker, E. C. Brummer, eds. (2004) Legume crop genomics. Champaign, Illinois, USA: AOCS Press, 61-82. DOI: 10.1201/9781439822265.ch4

McClean, P. E., Terpstra, J., McConnell, M., White, C., Lee, R., Mamidi, S. (2012) Population structure and genetic differentiation among the USDA common bean (*Phaseolus vulgaris* L.) core collection. Genetic Resources and Crop Evolution, 59 (4), 499-515. DOI: 10.1007/s10722-011-9699-0

Melotto, M., Coelho, M. F., Pedrosa-Harand, A., Kelly, J. D., Camargo, L. E. A. (2004) The anthracnose resistance locus *Co-4* of common bean is located on chromosome 3 and contains putative disease resistance-related genes. Theoretical and Applied Genetics, 109 (4), 690-699. DOI: 10.1007/s00122-004-1697-6

Melotto, M., Monteiro-Vitorello, C. B., Bruschi, A. G., Camargo, L. E. A. (2005) Comparative bioinformatic analysis of genes expressed in common bean (*Phaseolus vulgaris* L.) seedlings. Genome, 48 (3), 562-570. DOI: 10.1139/g05-010

Métais, I., Hamon, B., Jalouzot, R., Peltier, D. (2002) Structure and level of genetic diversity in various bean types evidenced with microsatellite markers isolated from a genomic enriched library. Theoretical and Applied Genetics, 104 (8), 1346-1352. DOI: 10.1007/s00122-002-0901-9

Miklas, P. N., Singh, S. P. (2007) Common bean. In: C. Kole, ed. (2007) Genome mapping and molecular breeding in plants. 3. Pulses, sugar and tuber crops. Heidelberg, Germany: Springer-Verlag Berlin Heidelberg, 1-31. DOI: 10.1007/978-3-540-34516-9

Müller, B. S. F., Sakamoto, T., Menezes, I. P. P., Prado, G. S., Martins, W. S., Brondani C., Barros, E. G., Vianello, R. P. (2014) Analysis of BAC-end sequences in common bean (*Phaseolus vulgaris* L.) towards the development and characterization of long motifs SSRs. Plant Molecular Biology, 86 (4-5), 455-470. DOI: 10.1007/s11103-014-0240-7

Nodari, R. O., Tsai, S. M., Gilbertson, R. L, Gepts, P. (1993a) Towards an integrated linkage map of common bean. II. Development of an RFLP-based linkage map. Theoretical and Applied Genetics, 85 (5), 513-520. DOI: 10.1007/BF00220907

Nodari, R. O., Tsai, S. M., Guzmán, P., Gilbertson, R. L., Gepts, P. (1993b) Towards an integrated linkage map of common bean. III. Mapping genetic factors controlling host-bacteria interactions. Genetics, 134 (1), 341-350.

Ochoa, I. E., Blair, M. W., Lynch, J. P. (2006) QTL analysis of adventitious root formation in common bean under contrasting phosphorus availability. Crop Science, 46 (4),1609-1621. DOI: 10.2135/cropsci2005.12-0446

Okii, D., Tukamuhabwa, P., Kami, J., Namayanja, A., Paparu, P., Ugen, M., Gepts, P. (2014) The genetic diversity and population structure of common bean (*Phaseolus vulgaris* L) germplasm in Uganda. African Journal of Biotechnology, 13 (29), 2935-2949. DOI: 10.5897/AJB2014.13916

Papa, R., Gepts, P. (2003) Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica. Theoretical and Applied Genetics, 106 (2), 239-250. DOI: 10.1007/s00122-002-1085-z

Pathania, A., Kumar Sharma, S., Nath Sharma, P. (2014) Common bean. In: M. Singh, I. Singh Bisht, M. Dutta, eds. (2014) Broadening the genetic base of grain legumes. New Delhi, India: Springer, 11-50. DOI: 10.1007/978-81-322-2023-7

Pérez-Vega, E., Trabanco, N., Campa, A., Ferreira, J. J. (2013) Genetic mapping of two genes conferring resistance to powdery mildew in common bean (*Phaseolus vulgaris* L.). Theoretical and Applied Genetics, 126 (6), 1503-1512. DOI: 10.1007/s00122-013-2068-y

Ramírez, M., Graham, M. A., Blanco-López, L., Silvente, S., Medrano-Soto, A., Blair, M. W., Hernández, G., Vance, C. P., Lara, M. (2005) Sequencing and analysis of common bean ESTs. Building a foundation for functional genomics. Plant Physiology, 137 (4), 1211-1227. DOI: 10.1104/pp.104.054999

Rodríguez-Suárez, C., Méndez-Vigo, B., Pañeda, A., Ferreira, J. J., Giraldez, R. (2007) A genetic linkage map of *Phaseolus vulgaris* L. and localization of genes for specific resistance to six races of anthracnose (*Colletotrichum lindemuthianum*). Theoretical and Applied Genetics, 114 (4), 713-722. DOI: 10.1007/s00122-006-0471-3

Rodríguez-Suárez, C., Ferreira, J. J., Campa, A., Pañeda, A., Giraldez, R. (2008) Molecular mapping and intra-cluster recombination between anthracnose race-specific resistance genes in the common bean differential cultivars Mexico 222 and Widusa. Theoretical and Applied Genetics, 116 (6), 807-814. DOI: 10.1007/s00122-008-0714-6

Selkoe K. A., Toonen R. J. (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology Letters, 9 (5), 615-629. DOI: 10.1111/j.1461-0248.2006.00889.x

Senan, S., Kizhakayil, D., Sasikumar, B., Sheeja, T. E. (2014) Methods for development of microsatellite markers: an overview. Notulae Scientia Biologicae, 6 (1), 1-13. DOI: 10.15835/nsb.6.1.9199

Singh, S. P. (1989) Patterns of variation in cultivated common bean (*Phaseolus vulgaris*, Fabaceae). Economic Botany, 43 (1), 39-57. DOI: 10.1007/BF02859324

Singh, S. P., Gutiérrez, J. A., Molina, A., Urrea, C., Gepts, P. (1991) Genetic diversity in cultivated common bean: II. marker-based analysis of morphological and agronomic traits. Crop Science, 31 (1), 23-29. DOI: 10.2135/cropsci1991.0011183X003100010005x

Tóth, G., Gáspári, Z., Jurka, J. (2000) Microsatellites in different eukaryotic genomes: survey and analysis. Genome Research, 10, 967-981. DOI: 10.1101/gr.10.7.967

Vallejos, C. E., Sakiyama, N. S., Chase, C. D. (1992) A molecular-marker based linkage map of *Phaseolus vulgaris* L. Genetics, 131 (3), 733-740.

Yaish, M. W. F., Pérez de la Vega, M. (2003) Isolation of $(GA)_n$ microsatellite sequences and description of a predicted MADS-box sequence isolated from common bean (*Phaseolus vulgaris* L.). Genetics and Molecular Biology, 26 (3), 337-342. DOI: 10.1590/S1415-47572003000300019

Yu, K., Park, S. J., Poysa, V. (1999) Abundance and variation of microsatellite DNA sequences in beans (*Phaseolus* and *Vigna*). Genome, 42 (1), 27-34. DOI: 10.1139/gen-42-1-27

Yu, K., Park, S. J., Poysa, V., Gepts, P. (2000) Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaseolus vulgaris* L.). Journal of Heredity, 91 (6), 429-434. DOI: 10.1093/jhered/91.6.429

Zane, L., Bargelloni, L., Patarnello, T. (2002) Strategies for microsatellite isolation: a review. Molecular Ecology, 11 (1), 1-16.