THIAGO LÍVIO PESSOA OLIVEIRA DE SOUZA

OBTENÇÃO DE SNPs, REAÇÃO DO FEIJOEIRO COMUM À FERRUGEM DA SOJA E PIRAMIDAÇÃO DE GENES DE RESISTÊNCIA A *Uromyces appendiculatus*

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento, para obtenção do título de *Doctor Scientiae*.

VIÇOSA
MINAS GERAIS - BRASIL
2009

Ficha catalográfica preparada pela Seção de Catalogação e Classificação da Biblioteca Central da UFV

T
S729o
2009

Souza, Thiago Lívio Pessoa Oliveira de, 1981-
Obtenção de SNPs, reação do feijoeiro comum à ferrugem da soja e piramidação de genes de resistência a *Uromyces appendiculatus* / Thiago Lívio Pessoa Oliveira de Souza. – Viçosa, MG, 2009.
xv, 114f. : il ; 29cm.

Orientador: Everaldo Gonçalves de Barros.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Feijão - Ferrugem. 2. Polimorfismo, único nucleotídeo. 3. Feijão - Melhoramento genético. 4. *Phaseolus vulgaris*. 5. *Uromyces appendiculatus*. 6. *Phakopsora pachyrhizi*. 7. Marcadores genéticos. 8. Herança de caracteres adquiridos. 9. Banco de genes de plantas. I. Universidade Federal de Viçosa. II. Título.

CDD 22.ed. 635.652

THIAGO LÍVIO PESSOA OLIVEIRA DE SOUZA

OBTENÇÃO DE SNPs, REAÇÃO DO FEIJOEIRO COMUM À FERRUGEM DA SOJA E PIRAMIDAÇÃO DE GENES DE RESISTÊNCIA A *Uromyces appendiculatus*

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento, para obtenção do título de *Doctor Scientiae*.

APROVADA: 24 de agosto de 2009.

Dr. Trazilbo José de Paula Júnior

Dra. Eveline Teixeira Caixeta

Prof. Maurilio Alves Moreira
(Co-Orientador)

Prof. José Eustáquio de Souza Carneiro
(Co-Orientador)

Prof. Everaldo Gonçalves de Barros
(Orientador)

À minha esposa Ingrid.

Aos meus pais Ademir e Maria da Glória.

Aos meus irmãos, cunhados, sogros e sobrinhos.

AGRADECIMENTOS

A Deus, o grande Deus, por ter me concedido a benção desta encarnação, a perseverança e o equilíbrio emocional para alcançar mais esta conquista profissional e intelectual.

À Universidade Federal de Viçosa (UFV) e, particularmente, ao Instituto de Biotecnologia Aplicada à Agropecuária (BIOAGRO) e ao Programa de Pós-Graduação em Genética e Melhoramento, pela oportunidade de realização deste trabalho.

Ao *Soybean Genomics and Improvement Laboratory*, *United States Department of Agriculture*/*Agricultural Research Service* (USDA/ARS), BARC-West (Beltsville, Maryland, EUA), pela oportunidade, infra-estrutura e apoio financeiro para o desenvolvimento do meu projeto de doutorado sanduíche.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e à Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), pelo auxílio financeiro, indispensável para a realização deste trabalho. À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e ao CNPq, pela concessão das bolsas de pesquisa: iniciação científica, mestrado, doutorado e doutorado sanduíche no exterior.

À minha querida esposa Ingrid, pelo apoio constante, companheirismo, cumplicidade, carinho, afeto e compreensão.

Aos meus pais, Ademir e Maria da Glória, pelo incentivo, pela educação precisa e atenta e, sobretudo, pela dedicação e amor incondicional aos filhos.

Aos meus irmãos, cunhados, sogros e sobrinhos, pelo carinho, apoio, amizade e solidariedade.

Ao meu orientador, professor Everaldo Gonçalves de Barros, pela orientação constante e competente, pela confiança e amizade, pelo apoio e incentivo desde a Iniciação Científica. Além disso, agradeço o exemplo de

ética e comprometimento profissional, e sua colaboração em minha formação humana e profissional.

Aos meus co-orientadores no Brasil, os professores Maurilio Alves Moreira, José Eustáquio de Sousa Carneiro e Cosme Damião Cruz, pelas sugestões, pela amizade e atenção sempre a mim dispensadas.

Aos meus co-orientadores nos EUA, Dr. Perry B. Cregan e Dr. Marcial A. Pastor-Corrales, pela oportunidade, pelos ensinamentos, confiança e amizade.

Aos colegas do Programa de Melhoramento do Feijoeiro da UFV, em especial, aos amigos Suelen e Demerson, pelo companheirismo, amizade e auxílio no dia-a-dia da pesquisa.

A todos os amigos e funcionários do Laboratório de Genética Molecular de Plantas do BIOAGRO/UFV, pelo convívio amistoso e sempre muito agradável.

Aos amigos e funcionários do *Soybean Genomics and Improvement Laboratory*, pelo auxílio na condução da pesquisa e na interpretação dos resultados. Além disso, por me ajudarem a me familiarizar com a língua inglesa e a me adaptar nos EUA.

Ao Sr. José Pinto, pelo auxílio na rotina de atividades na casa de vegetação, e ao Sr. Gilberto, pela amizade e ajuda nos trabalhos de campo.

Aos professores do Programa de Pós-Graduação em Genética e Melhoramento da UFV, e aos colegas de curso, pelos importantes ensinamentos.

À Coordenação do curso de Genética e Melhoramento, pelo apoio.

Aos amigos da Ordem Espiritualista Cristã Vale do Amanhecer, pelo convívio edificante, pelo apoio fraterno e por me proporcionarem momentos tão especiais em todos estes anos que compartilhamos.

Aos meus familiares, amigos, colegas e, enfim, a todos que contribuíram de alguma forma com a realização deste trabalho.

BIOGRAFIA

THIAGO LÍVIO PESSOA OLIVEIRA DE SOUZA, filho de Ademir Oliveira de Sousa e Maria da Glória Pessoa Oliveira de Souza, nasceu em Goiânia, Goiás, em 13 de abril de 1981. Concluiu o ensino fundamental em 1995, na Escola Municipal Ana das Neves de Freitas, em Goiânia, Goiás. Cursou o ensino médio no Colégio Visão, município de Formosa, Goiás, o qual foi concluído em 1998.

Em 1999, iniciou o curso de Agronomia na Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, colando grau em janeiro de 2004 como Engenheiro Agrônomo. No período de sua graduação, desenvolveu atividades de pesquisa como bolsista de Iniciação Científica do Departamento de Biologia Geral por três anos consecutivos (2000 a 2003), atuando nas áreas de "Genética Molecular e Melhoramento Vegetal" e "Genética da Interação Planta-Patógeno". Além disso, foi estagiário nos Laboratórios de Genética Molecular de Plantas do Instituto de Biotecnologia Aplicada à Agropecuária da UFV (BIOAGRO/UFV) e da Embrapa Cerrados, Planaltina, Distrito Federal. Também foi monitor da disciplina Genética Básica na UFV (2003). Ainda durante sua graduação, foi coordenador geral do Centro Acadêmico de Agronomia, na gestão 2002/2003.

Em março de 2004, iniciou o curso de Mestrado no Programa de Pós-Graduação em Genética e Melhoramento da UFV, na área de "Genética Molecular de Plantas e de Microorganismos", defendendo sua dissertação em agosto de 2005. Neste mesmo período, iniciou o curso de Pós-Graduação em nível de Doutorado, também no Programa de Genética e Melhoramento da UFV, na área de "Genética Molecular Aplicada ao Melhoramento de Plantas".

Durante seu doutorado, por três semestres, foi representante discente e membro da Comissão Coordenadora do Programa de Pós-Graduação em Genética e Melhoramento da UFV, além de coordenador geral do GenMelhor (Grupo de Estudos em Genética e Melhoramento da UFV).

Parte de seu projeto de doutorado foi desenvolvida no *Soybean Genomics and Improvement Laboratory*, USDA/ARS, BARC-West (Beltsville, Maryland, EUA), como bolsista de doutorado sanduíche do CNPq, sob a orientação do Dr. Perry B. Cregan e do Dr. Marcial A. Pastor-Corrales, no período de fevereiro de 2008 a fevereiro de 2009.

Defendeu sua tese de doutorado em agosto de 2009. Neste mesmo período, foi contratado como Cientista pelo Grupo Du Pont do Brasil S.A. - Divisão Pioneer Sementes, para atuar na Equipe "Pesquisa Soja", sendo lotado na Unidade de Brasília, Distrito Federal. Assumiu o programa de melhoramento molecular de soja no Brasil, visando à identificação de novos genes e a implementação e ampliação do uso das técnicas moleculares nos programas de melhoramento de Brasília, Sorriso e Sul. Além disso, passou a liderar o departamento de patologia de soja da Pioneer Sementes no Brasil.

ÍNDICE

RESUMO

SOUZA, Thiago Lívio Pessoa Oliveira de, D.Sc., Universidade Federal de Viçosa, agosto de 2009. **Obtenção de SNPs, reação do feijoeiro comum à ferrugem da soja e piramidação de genes de resistência a *Uromyces appendiculatus***. Orientador: Everaldo Gonçalves de Barros. Co-Orientadores: Maurilio Alves Moreira, José Eustáquio de Sousa Carneiro e Cosme Damião Cruz.

Este trabalho apresenta resultados de pesquisas desenvolvidas no âmbito do Programa de Melhoramento do Feijoeiro conduzido na Universidade Federal de Viçosa (UFV), sendo este composto por seções ou subprojetos distintos. Desta forma, optou-se por redigi-lo na forma de artigos científicos, sendo um de revisão e quatro de resultados originais, os quais já foram ou serão submetidos para publicação em periódicos internacionais. Por esse motivo, decidiu-se por escrevê-los no idioma inglês, em congruência com as Normas de Redação de Teses e Dissertações da UFV. Os resumos de tais artigos contendo suas justificativas, seus objetivos e principais resultados são apresentados a seguir: 1) Identificação de SNPs (*Single Nucleotide Polymorphisms*) no feijoeiro – SNPs, diferenças de um único nucleotídeo ou pequenas inserções/deleções (*indels*) entre fragmentos homólogos de DNA, foram identificados no feijoeiro (*Phaseolus vulgaris* L.) por meio do re-sequenciamento de STSs (*Sequence-Tagged Sites*) gerados por *primers* desenhados para amplificar sequências *shotgun* e BAC-*end* de soja e regiões gênicas e microssatélites de feijão. Os fragmentos de DNA contendo SNPs foram identificados em produtos de PCR (bandas únicas de DNA) obtidos a partir de seis linhagens de feijão, três de origem Andina (‘Jalo EEP558’, ‘G19833’ e ‘AND 277’) e três de origem Mesoamericana (‘BAT93’, ‘DOR 364’ e ‘Rudá’). O tamanho final do alinhamento das sequências de DNA obtidas foi de 134.653 pb, onde foram identificados 677 SNPs, incluindo 555 mudanças de base (295 transições e 260 transversões) e 122 *indels*. Verificou-

se uma frequência de 5,16 SNPs/kb. A diversidade nucleotídica média expressa pelo coeficiente teta de Halushka foi de 0,00226. Este trabalho representa um esforço pioneiro para a identificação de SNPs no feijoeiro. 2) Resistência à ferrugem asiática da soja no feijoeiro – A incidência da ferrugem asiática da soja (FAS), incitada pelo fungo *Phakopsora pachyrhizi*, tem sido constatada em cultivares de feijão. Neste trabalho, 44 acessos de feijoeiro foram testados para resistência ao *P. pachyrhizi*. Entre estes estavam incluídas fontes de resistência a diferentes doenças do feijoeiro, cultivares comerciais dos grupos carioca, preto e vermelho, e linhagens avançadas desenvolvidas pelo programa de melhoramento do BIOAGRO/UFV. Foram identificadas 14 fontes de resistência. 'PI181996', 'Pérola' e 'Redlands Pioneer' apresentaram os menores graus médios de reação ao patógeno. A herança da resistência à FAS no acesso 'PI181996' foi estudada e os resultados indicam que esta característica é monogênica e dominante. 3) Melhoramento do feijoeiro para resistência à ferrugem no BIOAGRO/UFV, Brasil – O feijoeiro é uma cultura de reconhecida importância social, nutricional e econômica. No entanto, quando comparada a outras espécies leguminosas de interesse agronômico, sua produtividade média ainda é considerada baixa. Um dos fatores que explicam esta situação é o grande número de doenças que acometem o feijoeiro e causam danos severos. Entre elas destaca-se a ferrugem, incitada pelo fungo *Uromyces appendiculatus*, o qual apresenta alta variabilidade. O controle da ferrugem por meio do uso de cultivares resistentes é uma estratégia viável, econômica e de fácil adoção, a ser usada de forma integrada às outras práticas. Neste trabalho são apresentadas importantes informações sobre a doença, além de relevantes iniciativas de melhoramento visando o desenvolvimento de cultivares resistentes à ferrugem no BIOAGRO/UFV. 4) Caracterização do gene de resistência à ferrugem presente na cultivar de feijoeiro 'Ouro Negro', a principal fonte de resistência usada no Brasil – A identificação e caracterização de novas fontes de resistência (R) constituem etapas fundamentais para os programas de melhoramento que visam ao desenvolvimento de cultivares com resistência efetiva a patógenos. A cultivar

‘Ouro Negro’, de origem Mesoamericana, é a principal fonte de resistência à ferrugem utilizada no Brasil. No entanto, seu gene R (*Ur-ON*) ainda não foi completamente caracterizado em relação a outros já identificados. Neste trabalho, inicialmente, ‘Ouro Negro’ e linhagens portadoras de genes R já caracterizados foram inoculadas com nove raças de *U. appendiculatus*. Além disso, o DNA de todas as linhagens de feijão foi analisado com marcadores moleculares ligados a *Ur-ON*, visando identificar evidências adicionais para a presença de alelos para esse lócus nas fontes R. Finalmente, foi testada a relação alélica entre *Ur-ON* e os genes de resistência à ferrugem presentes nas linhagens que foram resistentes a pelo menos uma das raças do patógeno. Testes de alelismo entre ‘Ouro Negro’ e importantes fontes de resistência à ferrugem no Brasil, as quais possuem genes R não caracterizados, foram também realizados. Os resultados indicam que o gene de efeito principal que condiciona resistência ao *U. appendiculatus* na cultivar ‘Ouro Negro’ é distinto daqueles com os quais ele foi comparado. 5) Melhoramento do feijoeiro assistido por marcadores moleculares visando à piramidação de genes de resistência à ferrugem – A piramidação de genes R assistida por marcadores moleculares foi usada pelo programa de melhoramento do feijoeiro conduzido no BIOAGRO/UFV como uma estratégia para acelerar o desenvolvimento de linhagens com resistência durável e de amplo espectro à ferrugem. Os genes *Ur-5* (‘Mexico 309’), *Ur-11* (‘BelMiDak RR-3’) e *Ur-ON* (‘Vi-4899’, linhagem do tipo carioca derivada do cruzamento ‘Rudá’ × ‘Ouro Negro’) foram combinados na cultivar ‘Rudá’, a qual possui grãos do tipo carioca. Foram obtidas linhagens apresentando todas as marcas moleculares associadas aos genes R, as quais confirmaram-se resistentes quando inoculadas com raças específicas de *U. appendiculatus*. Estas linhagens também foram resistentes em avaliações realizadas em condições de campo. Ensaios de rendimento demonstraram que as linhagens obtidas são tão produtivas quanto seu genitor recorrente (‘Rudá’) e outras cultivares com grãos do tipo carioca atualmente plantadas no Brasil.

ABSTRACT

SOUZA, Thiago Lívio Pessoa Oliveira de, D.Sc., Universidade Federal de Viçosa, August, 2009. **SNP discovery, reaction of the common bean to soybean rust, and pyramiding of resistance genes to *Uromyces appendiculatus***. Advisor: Everaldo Gonçalves de Barros. Co-Advisors: Maurilio Alves Moreira, José Eustáquio de Souza Carneiro, and Cosme Damião Cruz.

The present work reports results from researches developed at the Common Bean Breeding Program of the Federal University of Viçosa (UFV). It is composed by distinct sectors or sub-projects. We have decided to write it in the format of scientific papers; one of them is a paper review and the other four articles are original reports. They either have been submitted or will be submitted for publication in international refereed journals. For this reason, we have decided to write them in English, according to the Thesis and Dissertations Writing Standards from UFV. Abstracts of these articles containing their justifications, objectives, and main results are presented below: 1) Single nucleotide polymorphisms (SNPs) discovery in the common bean – SNPs were discovered in common bean (*Phaseolus vulgaris* L.) via resequencing of sequence-tagged sites (STSs) developed by PCR primers designed to soybean shotgun and BAC-end sequences, and to common bean genes and microsatellite flanking regions. DNA fragments harboring SNPs were identified in single amplicons from six contrasting *P. vulgaris* genotypes of the Andean ('Jalo EEP558', 'G19833', and 'AND277') and Mesoamerican ('BAT 93', 'DOR 364', and 'Rudá') gene pools. In the 131,120 bp of aligned sequence, a total of 677 SNPs were identified, including 555 single-base changes (295 transitions and 260 transversions) and 122 small nucleotide insertions/deletions (indels). The frequency of SNPs was 5.16 SNPs/Kb and the mean nucleotide diversity expressed as Halushka's theta was 0.00226. This work represents one of the pioneer efforts aiming to detect SNPs in *P.*

vulgaris. 2) Resistance to soybean rust in the common bean – Soybean rust (SBR) incited by *Phakopsora pachyrhizi* has been reported in common bean cultivars. In this work a set of 44 *P. vulgaris* genotypes were tested for resistance to *P. pachyrhizi* including resistance sources to several fungal common bean diseases, carioca-type, black-type and red-type Brazilian commercial cultivars, and advanced lines developed by the BIOAGRO/UFV breeding program. Fourteen resistance sources to *P. pachyrhizi* were identified. 'PI 181996', 'Pérola', and 'Redlands Pioneer' presented the lowest mean scores of disease reaction. The inheritance of SBR resistance in 'PI181996' was studied and the results support the hypothesis that this resistance is monogenic and dominant. 3) Breeding for common bean rust resistance in the BIOAGRO/UFV, Brazil – Common bean is an economically, nutritionally, and socially important crop. Unfortunately, compared to other important grain legumes common bean yields are quite low. One of the several factors contributing to this situation is the high number of destructive diseases that attack *P. vulgaris* and cause serious damage to the crop. Among them is bean rust, incited by the highly variable fungus *Uromyces appendiculatus*. Bean rust control by plant resistance is an easy and economical strategy to be used in association to other rust management practices. This review presents an overview about the bean rust and reports some breeding initiatives aiming at the development of rust resistant cultivars in the BIOAGRO/UFV. 4) Characterization of the rust resistance gene present in the common bean cultivar 'Ouro Negro', the main rust resistance source used in Brazil – Identification and characterization of new resistance sources are basic steps for plant breeding programs aiming to develop modern cultivars with effective resistance to pathogens. The Mesoamerican black seeded common bean cultivar 'Ouro Negro' is the main rust resistance (RR) source used in Brazil, but its resistance gene (*Ur-ON*) has not been fully characterized yet. Here we have compared the RR spectrum presented by 'Ouro Negro' with those of other bean lines harboring known RR genes when inoculated with nine races of *U. appendiculatus*. In addition, all bean lines

were screened with molecular markers linked to *Ur-ON* aiming to identify additional evidence for the presence of alleles for this locus in the screened RR sources. Finally, we tested the allelic relationships of *Ur-ON* with already characterized RR genes from lines resistant to at least one race of the pathogen. We also accomplished allelism tests between 'Ouro Negro' and important RR sources in Brazil harboring unnamed RR genes. The results showed that the major dominant gene conditioning RR in 'Ouro Negro' is positioned at a locus distinct from those with which it was compared. 5) DNA marker-assisted breeding aiming rust resistance gene pyramiding in the common bean – In our common bean breeding program we used a gene pyramiding approach assisted by DNA markers aiming to develop bean lines with durable and ample resistance spectrum to rust. The RR genes *Ur-5* (from 'Mexico 309'), *Ur-11* (from 'BelMiDak RR-3'), and *Ur-ON* (from 'Vi-4899', a carioca seeded line derived from the cross 'Rudá' × 'Ouro Negro') were combined in the carioca-type bean cultivar 'Rudá'. Carioca seeded beans are the most consumed in the Brazilian market. We were able to select lines presenting all the DNA markers associated to the genes of interest and resistant to all of the specific isolates of *U. appendiculatus* which were tested. They were also resistant under field conditions. Yield evaluations show that these selected lines are as productive as the recurrent parent 'Rudá' and other high performance cultivars currently grown in Brazil.

Single Nucleotide Polymorphisms (SNPs) Discovery in the Common Bean

Abstract: Single nucleotide polymorphisms (SNPs) were discovered in common bean (*Phaseolus vulgaris* L.) via resequencing of sequence-tagged sites (STSs) developed by PCR primers designed to soybean shotgun and BAC-end sequences, and to common bean genes and microsatellite flanking regions. DNA fragments harboring SNPs were identified in single amplicons from six contrasting *P. vulgaris* genotypes of the Andean ('Jalo EEP558', 'G19833', and 'AND277') and Mesoamerican ('BAT 93', 'DOR 364', and 'Rudá') gene pools. These genotypes are the parents of three common bean RIL mapping populations. From an initial set of 1,880 PCR primer pairs tested, 265 robust STSs were obtained, amplified and sequenced in each one of the six common bean genotypes. In the resulting 131,120 bp of aligned sequence, a total of 677 SNPs were identified, including 555 single-base changes (295 transitions and 260 transversions) and 122 small nucleotide insertions/deletions (indels). The frequency of SNPs was 5.16 SNPs/Kb and the mean nucleotide diversity expressed as Halushka's theta was 0.00226. This work represents one of the pioneer efforts aiming to detect SNPs in *P. vulgaris*. The SNPs identified are an important resource to common bean geneticists for quantitative trait loci (QTLs) discovery, marker-assisted selection and for map-based cloning. They will be also useful for diversity analysis and microsynteny studies among legume species.

Key words: DNA polymorphisms – genome variations – molecular markers – PCR primers – *Phaseolus vulgaris* – sequence tagged sites

1. Introduction

Common bean (*Phaseolus vulgaris* L.), the most important legume directly used as a human food, is grown and consumed worldwide but mainly in developing countries in Latin America, Africa, and Asia. It is largely a subsistence crop used as a major source of dietary protein in these countries, as complement to carbohydrate-rich sources such as rice, maize, and cassava. The common bean is also an important source of minerals, i.e., iron and zinc, and of certain vitamins (Wortmann et al. 1998; Broughton et al. 2003).

Efficient molecular tools for genetic studies in the common bean are still greatly needed. Single DNA base differences between homologous DNA fragments and small nucleotide insertions and deletions (indels) – collectively referred as single nucleotide polymorphisms (SNPs) – are highly desirable as molecular markers because they can be used for in-depth genetic analysis. SNPs provide a rich source of DNA polymorphisms in eukaryotic species because they are an abundant form of genome variation. SNPs are distinguished from rare genome variations by a requirement that the least abundant allele has to present a frequency of 1% or more (Brookes 1999; Avise 2004).

SNPs can be used as biallelic and codominant DNA markers for a variety of tasks in crop improvement including genes and quantitative trait loci (QTL) discovery, assessment of genetic diversity, association analysis, and marker-assisted selection. SNP markers have two main advantages over other molecular markers: (i) they are the most abundant form of genetic variation within genomes (Zhu et al. 2003), and (ii) a wide array of technologies have now been developed for high throughput SNP analysis (Fan et al. 2006). In addition, SNPs are less unstable than simple sequence repeats (SSR) or microsatellite markers because they present lower mutation rate. For this reason, SNPs are useful for population genetics and phylogeny studies (Jin et al. 2003). Another relevant characteristic of the SNP markers is that they can

be transferred between closely related species and utilized for microsynteny analysis.

Large numbers of SNP markers are currently available for many plant species including *Arabidopsis thaliana* (Jander et al. 2002; Schmid et al. 2003, 2005; Nordborg et al. 2005), maize (*Zea mays* L.) (Tenaillon et al. 2001; Ching et al. 2002; Wright et al. 2005), rice (*Oryza sativa* L.) (Feltus et al. 2004), barley (*Hordeum vulgare* L.) (Kanazin et. al. 2002; Rostoks et al. 2005), sorghum (*Sorghum bicolor* L.) (Hamblin et al. 2004), soybean (*Glycine max* L.) (Zhu et al. 2003; Choi et al. 2007), sugarbeet (*Beta vulgaris* L.) (Schneider et al. 2001), poplar (*Populus trichocarpa* Torr. & Gray) (Tuskan et al. 2006), apple (*Malus domestica* Borkh.) (Chagné et al. 2008), and *Vitis* spp. (Fernández et al. 2008). SNPs have also been detected in the common bean. In a recent work, Gaitán-Solís et al. (2008) conducted a sequence analysis of 10 genotypes of cultivated and wild Mesoamerican and Andean beans. A total of 20,964 bp were analyzed and compared, resulting in the discovery of 372 SNPs in 41 distinct STSs from coding and noncoding regions of common bean DNA sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov) and generated by degenerate oligonucleotide primers (DOP).

More than 20,000 SNPs have been discovered in soybean by sequence analysis and comparison of sequence tagged sites (STSs) from a small set of diverse genotypes. An initial phase of a large-scale soybean SNP discovery effort resulted in the identification of 5,551 SNPs (Zhu et al. 2003; Choi et al. 2007). Since then, approximately 12,000 additional soybean SNPs have been identified using the same approach (unpublished data). Because of the relatively close relationship between soybean and common bean (Zhu et al. 2005), it has been reported that many of the soybean-derived STS primers would be useful to amplify single amplicons in the common bean genome.

In this work we used available PCR primers designed to amplify soybean shotgun and BAC-end sequences for SNP discovery in common bean. This approach aimed to save time and money spent in the initial steps of primer development. In addition, we also used available PCR primers

designed to common bean microsatellite flanking regions. As reported by Zhu et al. (2003), a higher amount of polymorphism is present in non-coding DNA. For this reason, microsatellite regions could be a good source of SNPs. Finally, we also used PCR primers designed to specific *P. vulgaris* genes.

2. Material and Methods

2.1. Plant material and DNA extraction

Six contrasting Andean ('Jalo EEP558', 'G19833', and 'AND277') and Mesoamerican ('BAT 93', 'DOR 364', and 'Rudá') common bean lines were used for SNP discovery. These lines are the parents of three common bean RIL mapping populations: 'BAT 93' × 'Jalo EEP558', 'DOR 364' × 'G19833', and 'Rudá' × 'AND277'. Seeds from 'Rudá' and 'AND277' were provided by the *P. vulgaris* Active Germplasm Bank of the BIOAGRO/UFV (Viçosa, MG, Brazil). Seeds from the other common bean lines were provided by the Active Germplasm Bank of the Beltsville Agricultural Research Center, USDA/ARS (Beltsville, MD, USA).

Genomic DNA was extracted from bulked leaf tissue of five plants of each common bean line using the 'DNeasy Plant Mini' DNA extraction kit (Qiagen, Valencia, CA, USA), according to the manufacturer's protocol.

2.2. Sources of PCR primers for STS amplification

Sequences of the soybean PCR primers were obtained from the Beltsville Agricultural Research Center Soybean Map and SNP Database, at http://bfgl.anri.barc.usda.gov/soybean/ (Choi et al. 2007). They were randomly selected among STS primers designed to soybean shotgun and BAC-end sequences.

Common bean PCR primer sets designed to microsatellite flanking regions were obtained from published data available as of August 2008 on the BIC (Bean Improvement Cooperative) website (http://www.css.msu.edu/bic/Genetics.cfm). Aiming to generate significant

information for mapping purpose, SSR primers were selected that have not been mapped on the common bean core mapping population ('BAT 93' × 'Jalo EEP558') as they did not show size polymorphisms between the parent cultivars.

PCR primers were also designed to amplify common bean candidate gene sequences available at pertinent literature to represent a range of important agronomic functions. Full-length gene sequences randomly selected from GenBank (http://www.ncbi.nlm.nih.gov) were also used as template to design STS primers. Primers were designed using the software Primer3 (Whitehead Inst., Cambridge, MA, USA) and Array Designer 2 (Premier Biosoft International, Palo Alto, CA, USA) with predicted PCR products length adjusted to 400 to 800 bp.

2.3. Preliminary test of PCR primers and DNA sequence analysis

All primers pairs designed were initially tested via PCR and agarose gel analysis aiming to identify those producing single amplicons. Amplification reactions were performed with 30 ng of genomic DNA from cultivar 'Jalo EEP558', 1.0 μM of forward and reverse primers, 1.0 μL of 2× 'BioLine B' PCR buffer (BioLine USA Inc., Taunton, MA, USA), 1.5 mM $MgCl_2$, and 2 U of homemade *Taq* DNA polymerase in a 10 μL reaction volume. DNA amplification assays consisted of: one initial denaturation step at 94ºC for 5 min; 30 cycles at 94ºC for 45 s, 58ºC (soybean primers) or 54ºC (common bean primers) for 1 min, and 72ºC for 1 min; and a final step at 72ºC for 7 min. The amplified products were visualized under UV light after electrophoresis on 1.5% agarose gels containing ethidium bromide (0.2 μg/mL), immersed in a 1× sodium boric acid (SB) buffer (10 mM sodium hydroxide, pH adjusted to 8.5 with boric acid). Reactions with soybean primers that gave no products were reamplified at annealing temperature of 48ºC. The primer sets that amplified a single discrete product were selected for DNA sequence analysis.

The single amplicons from selected PCR primer pairs were prepared for sequence analysis by treatment with 4 U of shrimp alkaline phosphatase (SAP) and 4 U of exonuclease I (ExoI) incubated at 37ºC for 1 h followed by 72ºC for 15 min to inactivate the enzymes. Labeling reactions were performed with 1 μL of PCR product, 1.0 μL (4 U) of BigDye Terminator version 3.1 (Applied Biosystems, Foster City, CA, USA), 1.5 μM of one of the original PCR primers (forward and reverse primers in separate reactions), 0.8 μL of 10× 'Promega' *Taq* DNA polymerase buffer (Promega, Madison, WI, USA), and 1.75 mM of $MgCl_2$ in a 10 μL reaction volume. Labeling reactions cycling conditions were as follows: one initial denaturation step at 90ºC for 30 s; 40 cycles at 90ºC for 10 s, 50ºC for 5 s, and 60ºC for 1 min. The PCR products were labeled from both ends and the resulting termination products were sequenced on an ABI 3730 DNA Analyzer. The two resulting sequence traces derived from opposite ends of each amplicon were analyzed and aligned with standard DNA analysis software Phred (Ewing and Green 1998) and Phrap (http://www.phrap.org/). Resulting alignments and trace data were visually inspected in the Consed viewer (Gordon et al. 1998) to distinguish those amplicons that were locus specific and those that apparently resulted from amplification of two or more loci. The primer pairs that produced single amplicons with good quality sequence data were used for PCR amplification and sequencing of genomic DNA of the other five common bean genotypes: 'BAT 93', 'DOR 364', 'G19833', 'Rudá', and 'AND277'. Resulting amplicons were treated with SAP/ExoI, sequenced and analyzed as described for 'Jalo EEP558'. Forward and reverse sequence traces from the six genotypes were aligned.

2.4. STS verification and SNP discovery

SNP discovery was carried out by sequence alignment using the SNP-PHAGE software (Matukumalli et al. 2006). Sequence traces for each putative SNP identified were visually inspected to confirm the sequence polymorphisms.

Single-DNA base changes and indels present in each alignment were recorded as described by Matukumalli et al. (2006).

A diagram summarizing the SNP discovery strategy used in this work is shown in Figure 1.

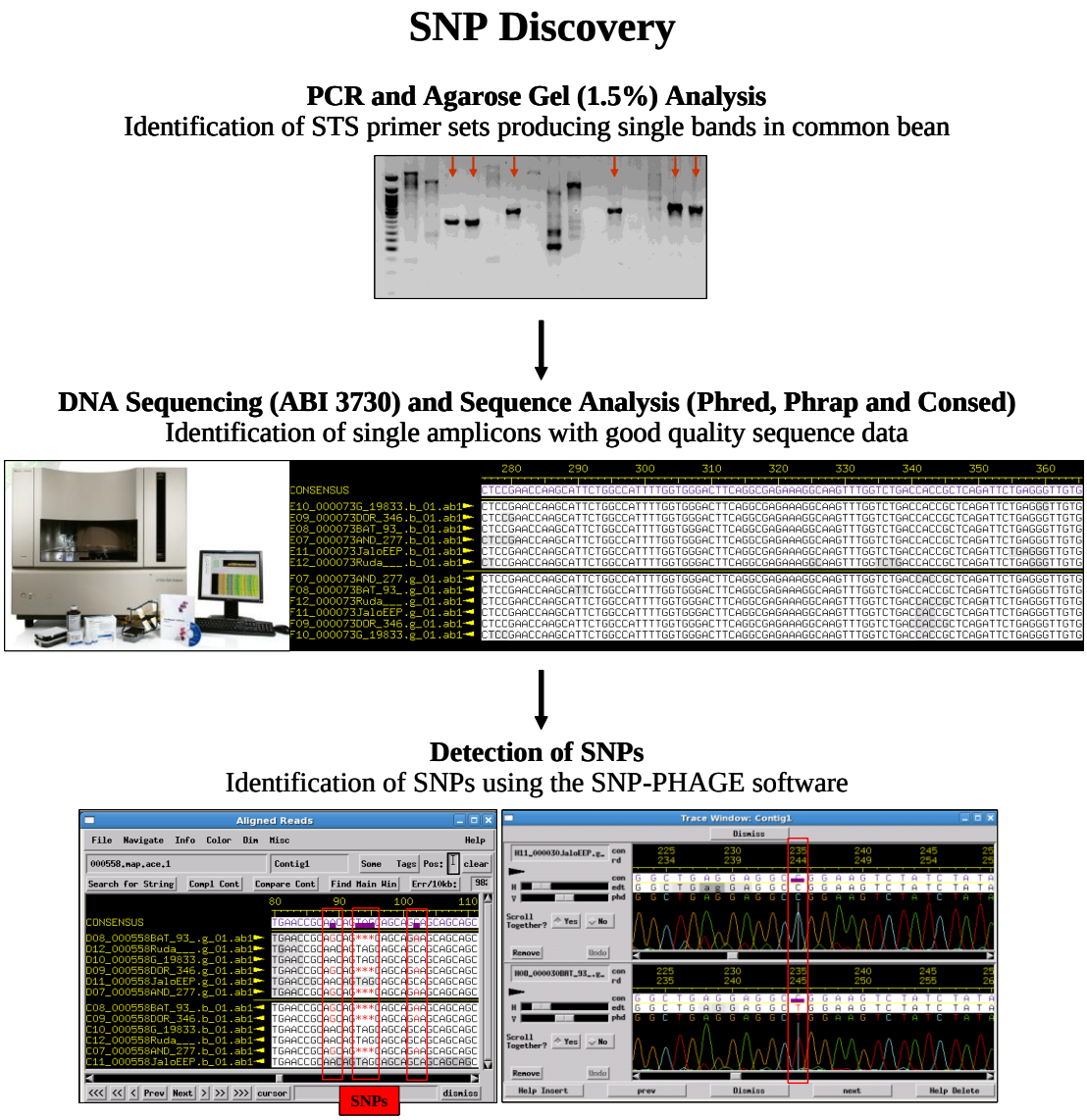

Figure 1. Diagram representing the discovery strategy used to identify SNPs in sequence alignments of amplicons from genomic DNA of six contrasting *P. vulgaris* genotypes ('BAT 93', 'Jalo EEP558', 'DOR 364', 'G19833', 'Rudá' and 'AND277'). The amplicons were generated by PCR primers designed to soybean shotgun and BAC-end sequences, to common bean genes and SSR flanking regions.

2.5. Nucleotide diversity

Nucleotide diversity (θ) was estimated according to Halushka et al. (1999): $\theta = K/aL$; where 'K' is the number of SNPs identified in an alignment of 'n' genotypes, 'L' is the total length of aligned sequences in bp, and $a = \Sigma\ 1/(i - 1)$, with i = 2-to-n.

3. Results

A total of 1,499 soybean STS primer pairs were tested. Out of them, 513 (34.22%) amplified each a single PCR product in the common bean genome. Out of those, 128 (8.54%) produced high quality sequence data. The total length of aligned sequences was 66,085 bp and the mean length of each amplicon was 516 bp. Two hundred and seventy-seven SNPs were identified in 81 (5.40%) distinct DNA fragments. The frequency of SNPs was 4.19 SNPs/Kb and the nucleotide diversity expressed as Halushka's theta was 0.00184 (Table 1).

Out of the 168 PCR primer pairs designed for common bean gene sequences, 109 (64.88%) amplified each a single discrete amplicon and 66 (39.29%) of them were selected after the DNA sequence analysis. The total length of aligned sequences was 38,167 bp and the mean length of STSs was 578 bp. A total of 237 SNPs were identified in 48 (28.57%) distinct DNA fragments. The frequency of SNPs was 6.21 SNPs/Kb and the nucleotide diversity was $\theta = 0.00272$ (Table 2).

Seven hundred and fifty-eight *P. vulgaris* SSR primer pairs were tested and 477 (62.93%) of them amplified a single DNA band. Because the DNA sequencing platform used (ABI 3730) was not efficient to sequence small amplicons (< 200 bp), only bands > 200 bp (213) were selected for DNA sequence analysis. Out of them, 71 (33.33%) produced high quality sequencing data. The total length of aligned sequences was 26,868 bp and the mean length of single amplicons was 378 bp. One hundred and sixty-three SNPs were identified in 44 (20.66%) distinct fragments. The frequency of

SNPs was 6.07 SNPs/Kb and the nucleotide diversity was $\theta = 0.00266$ (Table 3).

The results obtained using all 1880 distinct PCR primer pairs are summarized in Table 4. The aligned sequence of the 265 (14.10%) STSs resulted in the discovery of 677 SNPs in 173 (9.20%) distinct DNA fragments. The final length of aligned sequences was 131,120 bp and the mean length of the single amplicons was 495 bp. The final value of the frequency of SNPs was 5.16 SNPs/Kb and the mean nucleotide diversity was $\theta = 0.00226$.

The SNP classes are shown in Table 5. Out of the 277 SNPs identified in DNA fragments generated with soybean STS primers, 123 (44.40%) are transitions (A↔G and C↔T), 118 (42.60%) are transversions (A↔C, A↔T, C↔G, and G↔T), and 36 (13.00%) are indels. Among the 237 SNPs found in amplicons produced by primers designed to common bean genes, 109 (46.00%) transitions, 88 (37.13%) transversions, and 40 (16.87%) indels were detected. Of the 163 SNPs identified in PCR products generated by common bean SSR primers, 63 (38.65%) are transitions, 54 (33.13%) are transversions, and 46 (28.22%) are indels. Of a total of 677 SNPs identified in this work, 295 (43.57%) are transitions, 260 (38.41%) transversions, and 122 (18.02%) indels.

Table 1. Detection of SNPs in common bean DNA fragments generated by soybean STS primers. Number of PCR primer primers tested and results of PCR and sequence analysis in six genotypes of *P. vulgaris*.

	Primers designed to shotgun sequences		Primers designed to BAC-end sequences		Total	
	No.	% of total	No.	% of total	No.	% of total
Primer pairs tested[a]	731		768		1499	
PCR and agarose gel analysis						
Primer pairs producing no product	49	6.70	246	32.03	295	19.68
Primer pairs producing multiple bands	463	63.34	228	29.69	691	46.10
Primer pairs producing a single band[b]	219	29.96	294	38.28	513	34.22
DNA sequence analysis						
Multiple amplicons	10	1.37	07	0.91	17	1.13
Single amplicon (STS)	73	9.97	55	7.16	128	8.54
Poor or no sequence data	136	18.62	232	30.21	368	24.55
Fragments with at least one SNP	46	6.29	35	4.56	81	5.40
Length of aligned sequence						
Total (bp)	40,121		25,964		66,085	
Mean STS length	550		472		516	
SNPs						
Total	119		158		277	
Frequency (SNPs/Kb)	2.97		6.08		4.19	
Nucleotide diversity (θ^c x 1000)	1.30		2.67		1.84	

[a] The primer pairs were initially used to amplify the DNA of the common bean cultivar 'Jalo EEP 558' followed by DNA sequence analysis of the resulting amplicon. When high quality sequence data were obtained, the STS primers were then used to amplify and sequence genomic DNA of the other five genotypes that are parents of three mapping populations: 'AND277', 'BAT 93', 'DOR 364', 'G19833', and 'Rudá'.

[b] Primer pairs that amplified a single band at 58°C or 48°C (annealing temperature).

[c] $\theta = K / aL$; where 'K' is the number of SNPs identified in an alignment of 'n' genotypes, 'L' is the total length of aligned sequences in bp, and $a = \Sigma\ 1/(i - 1)$, with i = 2-to-n.

Table 2. Detection of SNPs in common bean gene sequences. Number of PCR primer pairs tested and results of PCR and sequence analysis in six genotypes of *P. vulgaris*.

	Primers designed to selected candidate gene sequences		Primers designed to random gene sequences (GenBank)		Total	
	No.	% of total	No.	% of total	No.	% of total
Primer pairs tested[a]	72		96		168	
PCR and agarose gel analysis						
Primer pairs producing no product	14	19.45	36	37.50	50	29.76
Primer pairs producing multiple bands	06	08.33	03	03.12	09	5.36
Primer pairs producing a single band[b]	52	72.22	57	59.38	109	64.88
DNA sequence analysis						
Multiple amplicons	03	04.17	02	02.08	05	2.97
Single amplicon (STS)	25	34.72	41	42.71	66	39.29
Poor or no sequence data	24	33.33	14	14.59	38	22.62
Fragments with at least 1 SNP	16	22.22	32	33.33	48	28.57
Length of aligned sequence						
Total (bp)	13,549		24,618		38,167	
Mean STS length	542		600		578	
SNPs						
Total	98		139		237	
Frequency (SNPs/Kb)	7.23		5.65		6.21	
Nucleotide diversity (θ^c x 1000)	3.17		2.47		2.72	

[a] The primer pairs were initially used to amplify the DNA of the common bean cultivar 'Jalo EEP 558' followed by DNA sequence analysis of the resulting amplicon. When high quality sequence data were obtained, the STS primers were then used to amplify and sequence genomic DNA of the other five genotypes that are parents of three mapping populations: 'AND277', 'BAT 93', 'DOR 364', 'G19833', and 'Rudá'.

[b] Primer pairs that amplified a single band at 54°C (annealing temperature).

[c] $\theta = K / aL$; where 'K' is the number of SNPs identified in an alignment of 'n' genotypes, 'L' is the total length of aligned sequences in bp, and $a = \Sigma\ 1/(i - 1)$, with i = 2-to-n.

Table 3. Detection of SNPs in common bean DNA fragments generated by PCR primers designed to microsatellite flanking regions. Number of PCR primer pairs tested and results of PCR and sequence analysis in six genotypes of *P. vulgaris*.

	Primers designed to microsatellite flanking regions	
	No.	% of total
Primer pairs tested[a]	758	
PCR and agarose gel analysis		
Primer pairs producing no product	110	14.51
Primer pairs producing multiple bands	171	22.56
Primer pairs producing a single band[b]	477	62.93
Primer pairs producing a band > 200 bp	213	28.10
Primer pairs tested (band > 200 bp)	213	
DNA sequence analysis		
Multiple amplicons	08	3.76
Poor or no sequence data	134	62.91
Single amplicon (STS)	71	33.33
Fragments with at least 1 SNP	44	20.66
Length of aligned sequence		
Total (bp)	26,868	
Mean STS length	378	
SNPs		
Total	163	
Frequency (SNPs/Kb)	6.07	
Nucleotide diversity (θ^c x 1000)	2.66	

[a] The primer pairs were initially used to amplify the DNA of the common bean cultivar 'Jalo EEP 558' followed by DNA sequence analysis of the resulting amplicon. When high quality sequence data were obtained, the STS primers were then used to amplify and sequence genomic DNA of the other five genotypes that are parents of three mapping populations: 'AND277', 'BAT 93', 'DOR 364', 'G19833', and 'Rudá'.

[b] Primer pairs that amplified a single band at 54°C (annealing temperature).

[c] $\theta = K / aL$; where 'K' is the number of SNPs identified in an alignment of 'n' genotypes, 'L' is the total length of aligned sequences in bp, and $a = \Sigma\ 1/(i - 1)$, with i = 2-to-n.

Table 4. Results summary of SNP discovery in *P. vulgaris* DNA fragments generated by common bean and soybean-derived PCR primers.

	Source of primers[a]			Total
	Soybean STSs	Common bean genes	Common bean SSRs	
No. of tested primers	1499	168	213[b]	1880
No. of single amplicons – STS (%)	128 (8.54)	66 (39.29)	71 (33.33)	265 (14.10)
Fragments with at least 1 SNP (%)	81 (5.40)	48 (28.57)	44 (20.66)	173 (9.20)
Sequence length (bp)	66,085	38,167	26,868	131,120
Mean STS length	516	578	378	495
No. of SNPs	277	237	163	677
SNP Frequency (SNPs/Kb)	4.19	6.21	6.07	5.16
Nucleotide diversity (θ x 1000)	1.84	2.72	2.66	2.26

[a] See Tables 1, 2, and 3 for more details.
[b] Primer pairs producing a single band > 200 bp selected in a total of 758 tested common bean SSR primers to attend the requirement of the DNA sequencing platform utilized (ABI 3730).

Table 5. Characteristics of SNPs identified in *P. vulgaris* DNA fragments generated by common bean and soybean-derived PCR primers.

Source of primers[a]	SNPs	Single-base changes				Indels[d]	
		Transitions[b]		Transversions[c]			
		No.	% of total	No.	% of total	No.	% of total
Soybean STSs	277	123	44.40	118	42.60	36	13.00
Common bean genes	237	109	46.00	88	37.13	40	16.87
Common bean SSRs	163	63	38.65	54	33.13	46	28.22
Total	677	295	43.57	260	38.41	122	18.02

[a] See Tables 1, 2, and 3 for more details.
[b] A↔G and C↔T.
[c] A↔C, A↔T, C↔G, and G↔T.
[d] Small nucleotide insertions and deletions.

4. Discussion

As part of an international effort led by the Soybean Genomics and Improvement Laboratory (SGIL), USDA/ARS, aiming to develop SNP markers for *P. vulgaris*, primers designed to soybean shotgun and BAC-end sequences, to common bean genes and flanking SSR regions were examined for locus-specific PCR amplification followed by resequencing of resulting amplicons. The use of available PCR primers (soybean STSs and common bean SSR primers) aimed to save time and money spent in the initial steps of primer development. However, considering the soybean-derived primers, only 5.40% of them produced DNA fragments with at least one SNP (Table 1). Despite the advantages mentioned above and the relatively close relationship of the soybean and common bean genomes (Zhu et al. 2005), the approach using soybean-derived primers was not as productive as originally anticipated. The low rate of SNP discovery reported here was, mainly, the result of the inability to develop robust STSs using these primers (Table 1).

The recent development of a large number of common bean SSR markers provided a valuable resource of STSs for SNP discovery. Out of the SSR primer sets available as of August 2008 and with sequences deposited in the BIC website (http://www.css.msu.edu/bic/Genetics.cfm), we selected those that had not been mapped in the bean core mapping population as they did not reveal size polymorphisms. Our goal with this selection was to generate information useful to increase the saturation of *P. vulgaris* core genetic map. Of a total of 785 SSR primer pairs tested in this work, 62.93% were determined to produce a single discrete PCR product. This high percentage was already expected since the primers had been previously identified and/or validated in common bean. However, because the utilized DNA sequencing platform (ABI 3730) was not efficient to sequence small amplicons, we were able to use only 28.10% (213) of the SSR primer sets (PCR products > 200 bp). These 213 SSR primer pairs were used for sequence analysis of the six contrasting *P. vulgaris* genotypes, and 20.66% produced single amplicons

with good quality DNA sequence data and containing at least one SNP (Table 3).

Of a total of 168 primer sets designed to common bean gene sequences and examined for locus-specific PCR amplification followed by resequencing for SNP discovery, 28.57% were determined to contain a sequence variant (Table 2). The efficiency of the common bean-derived PCR primers in result an SNP-containing amplicon was higher than that observed in soybean (21.50%) using the same strategy (Choi et al. 2007). The high level of duplication in the soybean genome complicates the development of robust STSs and can be the main explanation for this discrepancy.

The aligned sequence from all 265 STSs (131,120 bp) obtained from the six common bean genotypes resulted in the discovery of 677 SNPs in 173 DNA fragments (Table 4). Approximately 81.98% of the SNPs were single-base changes of which 43.57% were transitions and 38.41% transversions. The remaining 18.02% SNPs were indels (Table 5). The proportion of SNP classes detected here is similar to that reported in soybean (47.28% transitions, 37.61% transversions, and 15.11% indels) by Choi et al. (2007), but it differs significantly from that previously reported in common bean (31.45% transitions, 35.75% transversions, and 32.80% indels) by Gaitán-Solís et al. (2008).

The nucleotide diversity verified in this work (θ = 0.00226) in the analyzed 131,160 bp of common bean DNA sequence is approximately twice as higher than that detected in soybean by Zhu et al. (2003) (θ = 0.00086), Hyten et al. (2006) (θ = 0.00115), and Choi et al. (2007) (θ = 0.00010). However, a similar level of nucleotide diversity was verified in rice (θ = 0.00181) (Feltus et al. 2004), barley (θ = 0.00250) (Kanazin et al. 2002), and sorghum (θ = 0.00230) (Hamblin et al. 2004). Higher nucleotide diversity levels have also been reported in maize (θ = 0.00627) (Wright et al. 2005) and sugarbeet (θ = 0.00770) (Schneider et al. 2001). A high nucleotide diversity value (θ = 0.00627) was reported for common bean (Gaitán-Solís et al. 2008), similar to that detected in maize. This higher diversity level detected by these

authors can be explained by the use of 10 highly diverse cultivated and wild bean genotypes from Mesoamerican and Andean origins.

It is important to highlight that the SNPs identified in the present work are not putative genome variations. They are real DNA polymorphisms that will be useful for genetic mapping, diversity analysis, and microsynteny studies among legume species. Additional information about these SNPs, i.e., their forward and reserve primers and respective SNP-containing amplicon sequences are available upon request. Currently, SNP discovery approaches using reduced representation libraries and high throughput sequence analysis on the Illumina Genome Analyzer are being undertaken at SGIL. In the near future analysis of the common bean SNPs developed in this work and others will be tested in the common bean germplasm using the SNP GoldenGate assay on the Ilumina BeadStation. In this assay it is possible to multiplex from 96 to 1,536 SNPs in a single reaction over a 3-day period (Fan et al. 2003). For this reason, it can be used for high throughput SNP genotyping. The Illumina GoldenGate assay has been demonstrated to function well with the complex paleopolyploid genome of soybean (Hyten et al. 2008), thus it should also work for a less complex genome as is the case of the common bean. These data will certainly boost the common bean genetic mapping efforts aiming at QTL discovery and map-based cloning, as well as the use of marker-assisted selection for cultivar development.

Acknowledgments

This work was supported by grants from United States Department of Agriculture/Agriculture Research Service - USDA/ARS (American Government). The first author was supported by a PhD fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (Brazilian Government). The support from CNPq was greatly appreciated. We also thank Alicia Bertles from Bovine Functional Genomics Laboratory, BARC- East, USDA/ARS (Beltsville, MD, USA) for assistance with the DNA sequencing.

References

Avise JC (2004) Molecular markers, natural history and evolution. Sunderland, Sinauer Associates, 2ª ed., 684 pp

Brookes AJ (1999) The essence of SNPs. Gene 234:177–186

Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J (2003) Beans (*Phaseolus* spp.) - model food legumes. Plant and Soil 252:55–128

Chagné D, Gasic K, Crowhurst RN, Han Y, Bassett HC, Bowatte DR, Lawrence TJ, Rikkerink EHA, Gardiner SE, Korban SS (2008) Development of a set of SNP markers present in expressed genes of the apple. Genomics 92:353–358

Ching A, Caldwell KS, Jung M, Dolan M, Smith OS, Tingey S, Morgante M, Rafalski AJ (2002) SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. BMC Genetics 3:19

Choi IY, Hyten DL, Matukumalli LK, Song Q, Chaky JM, Quigley CV, Chase K, Lark KG, Reiter RS, Yoon MS, Hwang EY, Yi SI, Young ND, Shoemaker RC, VanTassell CP, Specht JE, Cregan PB (2007) A soybean transcript map: gene distribution, haplotype and SNP analysis. Genetics 176:685–696

Ewing B, Green P (1998) Base-calling of automated sequencer traces using Phred. II. Error probabilities. Genome Research 8:186–194

Fan JB, Oliphant A, Shen R, Kermani BG, Garcia F, Gunderson KL, Hansen M, Steemers F, Butler SL, Deloukas P, Galver L, Hunt S, McBride C, Bibikova M, Rubano T, Chen J, Wickham E, Doucet D, Chang W, Campbell D, Zhang B, Kruglyak S, Bentley D, Haas J, Rigault P, Zhou L, Stuelpnagel J, Chee MS (2003) Highly parallel SNP genotyping. Cold Spring Harbor Symposium on Quantitative Biology 68:69–78

Fan JB, Chee MS, Gunderson KL (2006) Highly parallel genomic assays. Nature Reviews Genetics 7:632–644

Feltus FA, Wan J, Schulze SR, Estill JC, Jiang N, Paterson AH (2004) An SNP resource for rice genetics and breeding based on subspecies indica and japonica genome alignments. Genome Research 14:1812–1819

Fernández MP, Núñez Y, Ponz F, Hernáiz S, Gallego FJ, Ibáñez J (2008) Characterization of sequence polymorphisms from microsatellite flanking regions in *Vitis* spp. Molecular Breeding 22:455–465

Gaitán-Solís E, Choi IY, Quigley C, Cregan P, Tohme J (2008) Single nucleotide polymorphisms in common bean: their discovery and genotyping using a multiplex detection system. The Plant Genome 1(2):125–134

Gordon D, Abajian C, Green P (1998) Consed: a graphical tool for sequence finishing. Genome Research 8:195–202

Halushka MK, Fan JB, Bentley K, Hsie L, Shen N, Weder A, Cooper R, Lipshutz R, Chakravarti A (1999) Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homeostasis. Nature Genetics 22:239–247

Hamblin MT, Mitchell SE, White GM, Gallego J, Kukatla R, Wing RA, Paterson AH, Kresovich S (2004) Comparative population genetics of the panicoid grasses: sequence polymorphism, linkage disequilibrium and selection in a diverse sample of sorghum bicolor. Genetics 167:471–483

Hyten DL, Song Q, Zhu Y, Choi IY, Nelson RL, Costa JM, Specht JE, Shoemaker RC, Cregan PB (2006) Impacts of genetic bottlenecks on soybean genome diversity. Proceedings of the National Academy of Science USA 103:16666–16671

Hyten DL, Song Q, Choi I, Yoon M, Specht JE, Matukumalli LK, Shoemaker RC, Young ND, Cregan PB (2008) High-throughput genotyping with the GoldenGate assay in the highly complex genome of soybean. Theoretical and Applied Genetics 116:945–952

Jander G, Norris SR, Rounsley SD, Bush DF, Levin IM, Last RL (2002) *Arabidopsis* map-based cloning in the post-genome era. Plant Physiology 129:440–450

Jin Q, Waters D, Cordeiro GM, Henry RJ, Reinke RF (2003) A single nucleotide polymorphism (SNP) marker linked to the fragrance gene in rice (*Oryza sativa* L.). Plant Science 165:359–364

Kanazin V, Talbert H, See D, DeCamp P, Nevo E, Blake T (2002) Discovery and assay of single-nucleotide polymorphisms in barley (*Hordeum vulgare*). Plant Molecular Biology 48:529–537

Matukumalli LK, Grefenstette JJ, Hyten DL, Choi IY, Cregan PB, VanTassell CP (2006) SNP-PHAGE: high throughput SNP discovery pipeline. BMC Bioinformatics 7:468–474

Nordborg M., Hu TT, Ishino Y, Jhaveri J, Toomajian C, Zheng H, Bakker E, Calabrese P, Gladstone J, Goyal R, Jakobsson M, Kim S, Morozov Y, Padhukasahasram B, Plagnol V, Rosenberg NA, Shah C, Wall JD, Wang J, Zhao K, Kalbfleisch T, Schulz V, Kreitman M, Bergelson J (2005) The pattern of polymorphism in *Arabidopsis thaliana*. PLoS Biology 3:e196

Rostoks N, Mudie S, Cardle L, Russell J, Ramsay L, Booth A, Svensson JT, Wanamaker SI, Walia H, Rodriguez EM, Hedley PE, Liu H, Morris J, Close TJ, Marshall DF, Waugh R (2005) Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress. Molecular Genetics and Genomics 274:515–527

Schmid KJ, Ramos-Onsins S, Ringys-Beckstein H, Weisshaar B, Mitchell-Olds T (2005) A multilocus sequence survey in *Arabidopsis thaliana* reveals a genomewide departure from a neutral model of DNA sequence polymorphism. Genetics 169:1601–1615

Schmid KJ, Sorensen TR, Stracke R, Torjek O, Altmann T, Mitchell-Olds T, Weisshaar B (2003) Large-scale identification and analysis of genomewide single-nucleotide polymorphisms for mapping in Arabidopsis thaliana. Genome Research 13:1250–1257

Schneider K, Weisshaar B, Borchardt DC, Salamini F (2001) SNP frequency and allelic haplotype structure of *Beta vulgaris* expressed genes. Molecular Breeding 8:63–74

Tenaillon MI, Sawkins MC, Long AD, Gaut RL, Doebley JF (2001) Patterns of DNA sequence polymorphism along chromosome 1 of maize (*Zea mays* ssp. *mays* L.). Proceedings of the National Academy of Science USA 98:9161–9166.

Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A, Schein J, Sterck L, Aerts A, Bhalerao RR, Bhalerao RP, Blaudez D, Boerjan W, Brun A, Brunner A, Busov V, Campbell M, Carlson J, Chalot M, Chapman J, Chen GL, Cooper D, Coutinho PM, Couturier J, Covert S, Cronk Q, Cunningham R, Davis J, Degroeve S, Déjardin A, Pamphilis C, Detter J, Dirks B, Dubchak I, Duplessis S, Ehlting J, Ellis B, Gendler K, Goodstein D, Gribskov M, Grimwood J, Groover A, Gunter L, Hamberger B, Heinze B, Helariutta Y, Henrissat B, Holligan D, Holt R, Huang W, Islam-Faridi N, Jones S, Jones-Rhoades M, Jorgensen R, Joshi C, Kangasjarvi J, Karlsson J, Kelleher C, Kirkpatrick R, Kirst M, Kohler A, Kalluri U, Larimer F, Leebens-Mack J, Leplé JC, Locascio P, Lou Y, Lucas S, Martin F, Montanini B, Napoli C, Nelson DR, Nelson C, Nieminen K, Nilsson O, Pereda V, Peter G, Philippe R, Pilate G, Poliakov A, Razumovskaya J, Richardson P, Rinaldi C, Ritland K, Rouzé P, Ryaboy D, Schmutz J, Schrader J, Segerman B, Shin H, Siddiqui A, Sterky F, Terry A, Tsai CJ, Uberbacher E, Unneberg P, Vahala J, Wall K, Wessler S, Yang G, Yin T, Douglas C, Marra M, Sandberg G, Van de Peer Y, Rokhsar D (2006) The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science 313:1596–1604

Wortmann CS, Kirkby RA, Eledu CKA, Allen DJ (1998) Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa. Cali, CIAT, 17 pp

Wright SI, Bi IV, Schroeder SG, Yamasaki M, Doebley JF, McMullen MD, Gaut BS (2005) The effects of artificial selection on the maize genome. Science 308:1310–1314

Zhu H, Choi HK, Cook DR, Shoemaker RC (2005) Bridging model and crop legumes through comparative genomics. Plant Physiology 137:1189–1196

Zhu YL, Song QJ, Hyten DL, VanTassell CP, Matukumalli LK, Grimm DR, Hyatt SM, Fickus EW, Young ND, Cregan PB (2003) Single-nucleotide polymorphisms in soybean. Genetics 163:1123–1134

Resistance to Soybean Rust in the Common Bean

Abstract: Soybean rust (SBR) incited by *Phakopsora pachyrhizi* has been reported in common bean (*Phaseolus vulgaris*) cultivars inoculated under controlled and natural-field conditions in South Africa, the United States, Argentina and Brazil. Although the SBR is currently not a serious problem to the common bean crop, many bean breeders are concerned about this possibility because of the high severity and virulence diversity of *P. pachyrhizi*, and its broad host range. In this work a set of 44 *P. vulgaris* genotypes were tested for resistance to *P. pachyrhizi* including resistance sources to several fungal common bean diseases, carioca-type, black-type and red-type Brazilian commercial cultivars, and advanced lines developed by the BIOAGRO/UFV breeding program. Fourteen resistance sources to *P. pachyrhizi* were identified. 'PI 181996', 'Pérola', and 'Redlands Pioneer' presented the lowest mean scores of disease reaction. 'PI181996' was crossed with susceptible cultivars aiming to study the inheritance of resistance in this common bean accession. The results support the hypothesis that resistance to *P. pachyrhizi* in 'PI181996' is monogenic and dominant.

Key words: *Phakopsora pachyrhizi – Phaseolus vulgaris* – disease resistance – resistance source – inheritance study – prebreeding

1. Introduction

Soybean rust (SBR), incited by the highly variable fungus *Phakopsora pachyrhizi* H. Sydow & P. Sydow, is reported as the major disease limiting soybean (*Glycine max* L.) production in Asia (Hartman et al. 1992) and, most recently, in the Americas. Since the first detection of *P. pachyrhizi* incidence associated with rust epidemics in the American continent, the pathogen has moved swiftly from one country to the other. SBR was first reported in Paraguay and Brazil in 2001, after in the Argentina (2002), Bolivia (2003), Uruguay and the US (2004), and in Mexico (2005) (Ivancovich 2005; Pivonia and Yang 2005; Schneider et al. 2005; Yorinori et al. 2005). In Brazil, SBR was first found and disseminated in the south-central areas. In May 2001, it was detected in the west region of the state of Paraná. In the 2001/2002 crop season, the disease was already found on all soybean fields of Paraná and in some areas of the states of Rio Grande do Sul, Goiás, Mato Grosso, and Mato Grosso do Sul. Yield losses due to soybean rust ranged from 30 to 75% (Yorinori et al. 2005). Currently, SBR is endemic in almost all Brazilian soybean growing areas and all soybean commercial cultivars are susceptible to *P. pachyrhizi*.

Control measures for SBR include cultural practices (crop rotation, soil incorporation of soybean infected debris, planting within recommended dates, etc.), growing tolerant cultivars, and timely spraying of fungicides (Hartman et al. 1992). Compared to chemical control, the most utilized method to date, the use of resistant cultivars not only is harmless to the environment but also an economically sound strategy. But the high severity and variability of the pathogen prevent the efficient control of *P. pachyrhizi* by plant resistance and the identification of effective-resistance sources in soybean.

The host range of *P. pachyrhizi* is broad, with over 90 species, including some economically important crops (Ono et al. 1992; Rytter et al. 1984). Among them is the common bean (*Phaseolus vulgaris* L.), the food legume most used for direct human consumption worldwide. The

pathogenicity of *P. pachyrhizi* has been reported on *P. vulgaris* cultivars gown under field and controlled conditions, and tested by natural and artificial inoculations with the pathogen. The first report on the virulence of *P. pachyrhizi* on common bean lines inoculated under controlled conditions was done by Stavely et al. (1985). Most recently, SBR was reported on common bean accessions grown under natural-field conditions in South Africa, Brazil, and the USA (2005), and Argentina (2006) (DuPreez et al. 2005; Nunes Junior et al. 2005; Lynch et al. 2006; Pastor-Corrales et al. 2006, 2007; Ivancovich et al. 2007).

In the research developed by Miles et al. (2007), common bean cultivars resistant to bean rust, incited by *Uromyces appendiculatus* F. Strauss (syn. *U. phaseoli* G. Winter), were tested against six isolates of *P. pachyrhizi* from Asia, Africa, and South America. Resistance to all six isolates was identified. As previously reported by Stavely et al. (1985), when lesion sizes and spore production were compared between soybean and common bean susceptible cultivars inoculated with virulent *P. pachyrhizi* isolates, the bean cultivars presented smaller lesions and less spores. According to Stavely et al. (1985), when these same rust symptoms were compared on susceptible common bean cultivars inoculated with *P. pachyrhizi* and *U. appendiculatus*, the SBR pathogen incited smaller lesions with less spore production than those incited by the bean rust pathogen. It was concluded that *P. pachyrhizi* is not a threat to common bean production unlike *U. appendiculatus*. The smaller severity of SBR on *P. vulgaris* compared to *G. max* accessions using natural inoculation assays under field conditions was also reported by DuPreez et al. (2005), Nunes Junior et al. (2005), Lynch et al. (2006), Pastor-Corrales et al. (2006, 2007), and Ivancovich et al. (2007). Nevertheless, because the high virulence diversity of *P. pachyrhizi*, in addition to its broad host range and great dispersal capacity, many bean breeders are concerned about the possibility that SBR also becomes a serious problem to the common bean crop in endemic areas.

A similar phenomenon was already reported in the common bean growing system. The angular leaf spot, incited by the fungus *Pseudocercospora griseola* (Sacc.) Crous & U. Braun, was already considered as one of the least important diseases for the bean crop until approximately 20 years ago. However, to date angular leaf spot is one of the most destructive fungal diseases affecting the common bean production in Brazil and other tropical and sub-tropical growing areas of the world (Sartorato 2002). In Brazil, yield losses caused by *P. griseola* may reach between 70% and 100% (Jesus Junior et al. 2001).

Based on the reported information, the identification and use of SBR resistance sources in *P. vulgaris* is being considered as strategic for the common bean breeding programs focused on disease resistance. In addition, considering the current possibilities presented by the modern biotechnology tools, this effort may also lead to the development of transgenic soybean cultivars with effective resistance to *P. pachyrhizi*.

The main goal of the present work was to identify SBR resistance sources among common bean accessions maintained in the *P. vulgaris* Active Germplasm Bank of the BIOAGRO/UFV (Instituto de Biotecnologia Aplicada à Agropecuária of the Universidade Federal de Viçosa). To better understand the genetic control of plant resistance in the pathosystem *P. pachyrhizi–P. vulgaris*, we also determined the inheritance mode of SBR resistance in the common bean accession 'PI181996', one of the resistant sources identified in this work.

2. Material and Methods

2.1. Plant genetic material

Characteristics of the common bean accessions screened for SBR resistance in the present work are summarized in Table 1. Among the accessions there are: resistance sources to common bean diseases, such as rust, anthracnose, and angular leaf spot; Brazilian commercial cultivars with carioca-type, black-type, and red-type genetic backgrounds; and advanced lines developed by the BIOAGRO/UFV breeding program.

Seeds from the differential cultivars for bean rust (Table 1) were provided by the Centro Internacional de Agricultura Tropical - CIAT (Cali, Colombia). Seeds from cultivars 'IAPAR 14', 'IAPAR 16', and 'IAPAR 57' were provided by Dr. Vania Moda-Cirino of the Instituto Agronômico do Paraná - IAPAR (Londrina, Paraná, Brazil). Seeds from the other common bean lines were supplied by BIOAGRO/UFV (Viçosa, Minas Gerais, Brazil).

Soybean cultivars 'CAC-1' and 'Cristalina' were used as susceptible control in the SBR resistance screening as they are highly susceptible to *P. pachyrhizi* under field condition in Brazil. Soybean lines harboring the four identified single resistance genes to the pathogen were also included in the inoculation assays: 'PI200492' (gene *Rpp1*); 'PI547878' (gene *Rpp2*), line derived from 'Willians' (susceptible) and 'PI230970' (gene *Rpp2*); 'PI462312' (gene *Rpp3*); and 'PI459025' (gene *Rpp4*). Seeds from all soybean accessions were also provided by BIOAGRO/UFV.

In order to standardize their vigor and germination power, seeds from all common bean and soybean accessions were multiplied under greenhouse conditions before the disease screening tests. Ten plants from each accession were inoculated with the pathogen.

Table 1. Description of the common bean accessions screened for resistance to *Phakopsora pachyrhizi*.

Accession	Gene pool	Note[a]
AB 136	Mesoamerican	Differential cultivar for bean anthracnose. Possesses the resistance genes *Co-6* and *Co-8* (Pastor-Corrales 1991).
AND 277	Andean	Resistance source to bean angular leaf spot. Possesses the gene *Phg-1* (Caixeta et al. 2005; Ragagnin et al. 2009).
Aurora	Mesoamerican	Differential cultivar for bean rust. Possesses the resistance gene *Ur-3* (Steadman et al. 2002). Resistance source to soybean rust (Miles et al. 2007; Pastor-Corrales et al. 2007).
BAT 332	Mesoamerican	Differential cultivar for bean angular leaf spot. Possesses the gene *Phg-6*^{2} (Pastor-Corrales and Jara 1995).
Brow Beauty	Andean	Resistance source to bean rust. Possesses the gene *Ur-4* (Souza et al. 2008).
Compuesto Negro Chimaltenango (CNC)	Mesoamerican	Resistance source to bean rust. Possesses one single gene conferring resistance mainly to Andean *U. appendiculatus* races (Souza et al. 2008). Resistance source to soybean rust (Miles et al. 2007; Pastor-Corrales et al. 2007). According to Pastor-Corrales and Frederick (2008), soybean rust resistance in 'CNC' is controlled by the interaction of two genes with complete dominance at both gene pairs.
Cornell 49-242	Mesoamerican	Differential cultivar for anthracnose and angular leaf spot. Possesses the resistance genes *Co-2* (anthracnose) and *Phg-3* (angular leaf spot) (Pastor-Corrales 1991; Pastor-Corrales and Jara 1995).
California Small White 643	Mesoamerican	Resistance source to bean rust. Possesses unknown resistance genes. It is speculated that 'CSW 643' may have the gene *Ur-2* and heterozygosis for the locus *Ur-Red=Ur-13* (Liebenberg et al. 2006).
Diamante Negro	Mesoamerican	Brazilian black-type cultivar.
Dorado	Mesoamerican	Resistance source to bean rust. Possesses two uncharacterized resistance genes temporary named as *Ur-Dorado53* and *Ur-Dorado108* (Souza et al. 2008).
G 2333	Mesoamerican	Differential cultivar for bean anthracnose. Possesses the resistance genes *Co-4*^{2}, *Co-5*, and *Co-7* (Pastor-Corrales 1991; Caixeta et al. 2005).
Golden Gate Wax	Andean	Differential cultivar for bean rust. Possesses the resistance gene *Ur-6* (Steadman et al. 2002).
IAPAR 14 IAPAR 16 IAPAR 57	Mesoamerican	Brazilian cultivars developed by IAPAR. 'IAPAR 14' is tolerant to rust, anthracnose, and common bacterial blight under field conditions, 'IAPAR 16' is resistance to common bacterial blight, and 'IAPAR 57' is tolerant to bean common mosaic virus under field conditions (Dr. Vania Moda-Cirino, personal communication). These cultivars showed to be resistance to soybean rust in the field-evaluating experiment conduced in Brazil by Nunes Junior et al. (2005).
Jalo EEP 558	Andean	Parental line of the common bean core mapping population; 'BAT 93' x 'Jalo EEP 558' RIL population (Souza et al. 2008).

Table 1. Continuation...

Accession	Gene pool	Note[a]
MAR-2	Mesoamerican	Resistance source to bean angular leaf spot. Possesses the genes *Phg-4* and *Phg-5*^{2} (Caixeta et al. 2005).
Mexico 235	Mesoamerican	Differential cultivar for bean rust. Possesses the resistance gene *Ur-3*$^{+}$ (Steadman et al. 2002).
Mexico 309	Mesoamerican	Differential cultivar for bean rust. Possesses the resistance gene *Ur-5* (Steadman et al. 2002).
Mexico 54	Mesoamerican	Differential cultivar for bean angular leaf spot. Possesses the resistance genes *Phg-2*, *Phg-5*, and *Phg-6* (Pastor-Corrales and Jara 1995; Caixeta et al. 2005).
Montcalm	Andean	Differential cultivar for angular leaf spot and rust. Possesses resistance genes currently unknown (Pastor-Corrales and Jara 1995; Steadman et al. 2002).
Ouro Negro	Mesoamerican	Brazilian black-type cultivar. Resistance source to rust, anthracnose and angular leaf spot. Possesses de gene *Co-10* (anthracnose) and the uncharacterized resistance genes temporary named as *Ur-ON* (rust) and *Phg-ON* (angular leaf spot) (Faleiro et al. 2004; Ragagnin et al. 2009).
Ouro Vermelho	Mesoamerican	Brazilian red-type cultivar.
P-33-5-1 P-49-8-2	Mesoamerican	Advanced lines developed by BIOAGRO/UFV. They have the genetic background of the Brazilian carioca-type cultivar 'Pérola' but with simultaneous resistance to rust (gene *Ur-ON*), anthracnose (genes *Co-4*, *Co-6*, and *Co-10*), and angular leaf spot (gene *Phg-1*) (Ragagnin et al. 2009).
Pérola	Mesoamerican	Brazilian carioca-type cultivar.
PI181996	Mesoamerican	Differential cultivar for bean rust. Possesses the resistance gene *Ur-11* (Steadman et al. 2002). Resistance source to soybean rust (Miles et al. 2007; Pastor-Corrales et al. 2007).
PI260418	Andean	Differential cultivar for bean rust. Possesses a single resistance gene tentatively named as *Ur-14* (Steadman et al. 2002; Pastor-Corrales 2005).
Pinto Olathe	Mesoamerican	Resistance source to bean rust. Possesses the gene *Ur-6*$^{+}$ (Souza et al. 2008).
R-127-4-13 R-127-10-14 R-97-13-5 R-97-13-6	Mesoamerican	Advanced lines developed by BIOAGRO/UFV. They have the genetic background of the Brazilian carioca-type cultivar 'Rudá' but with simultaneous resistance to rust (gene *Ur-ON*), anthracnose (genes *Co-4*, *Co-6*, and *Co-10*), and angular leaf spot (gene *Phg-1*) (Ragagnin et al. 2009).
Redlands Pioneer	Andean	Differential cultivar for bean rust. Possesses the resistance gene *Ur-13*. Although 'Redlands Pioneer' has been considered as an Andean cultivar, *Ur-13* appears to be of Mesoamerican origin (Steadman et al. 2002; Liebenberg et al. 2006).
Rudá	Mesoamerican	Brazilian carioca-type cultivar.
SEL 1308	Mesoamerican	Resistance source to bean anthracnose. Possesses the gene *Co-4*^{2}.

Table 1. Continuation...

Accession	Gene pool	Note[a]
Talismã	Mesoamerican	Brazilian carioca-type cultivar.
TO	Mesoamerican	Differential cultivar for bean anthracnose. Possesses the resistance gene *Co-4* (Pastor-Corrales 1991).
TU	Mesoamerican	Differential cultivar for bean anthracnose. Possesses the resistance gene *Co-5* (Pastor-Corrales 1991).
US Pinto 111	Mesoamerican	Cultivar used as a universal susceptible control in the bean rust screening assays conduced by the BIOAGRO/UFV breeding program.
Valente	Mesoamerican	Brazilian black-type cultivar.
Vermelhinho	Mesoamerican	Brazilian red-type cultivar.
Vermelho 2157	Mesoamerican	Obsolete Brazilian red-type cultivar.
Vi-4899 (Pioneiro)	Mesoamerican	Carioca-type cultivar resistant to anthracnose (gene *Co-10*) and rust (*Ur-ON*) developed by BIOAGRO/UFV (Faleiro et al. 2004).

[a] Additional online references: Bean Improvement Cooperative - BIC, List of *Phaseolus vulgaris* L. Genes, Version 2009: http://www.css.msu.edu/bic/PDF/Bean_Genes_List_2009.pdf; and USDA/ARS National Genetic Resources Program, Germplasm Resources Information Network - GRIN: http://www.ars-grin.gov/npgs/index.html.

2.2. *P. pachyrhizi* inoculum

The initial inoculum of *P. pachyrhizi* was obtained from soybean cultivar 'CAC-1' plants infected under field conditions in UFV experimental stations located in Viçosa, Coimbra and Tocantins, state of Minas Gerais, Brazil. The spores were periodically multiplied on cultivar 'CAC-1' in order to increase the inoculum and maintain its virulence. Since the inoculum is not a single-pustule isolate, i.e., it did not originate from a single lesion, it is possible that it represents a mixture of more than one pathotype. Therefore, all multiplications of the inoculum as well as disease screening tests were monitored for the appearance of mixed lesions or highly contrasting reaction degrees on a same soybean or common bean genotype.

Although *P. pachyrhizi* does not grow in artificial culture as it is an obligate parasite, viable spores can be preserved under artificial conditions. In the BIOAGRO/UFV, dry spores in plastic or glass tubes are being successfully maintained under dark conditions at -80°C or in liquid nitrogen.

Genomic DNA of spores obtained from the inoculum used in this work was utilized for PCR analysis with specific primers designed to amplify ITS (Internal Transcribed Spacer) regions of *P. pachyrhizi* (Asian SBR) and *P. meibomiae* (Arthur) Arthur (American SBR) (Frederick et al. 2002). Differentiation between Asian SBR and American SBR symptoms by visual evaluation only is not efficient (Ono et al. 1992). Fungal DNA extraction and PCR conditions were as described by Frederick et al. (2002). The primers used in the test were: Ppm1 (5'-GCAGAATTCAGTGAATCATCAAG-3') and Ppa2 (5'-GCAACACTCAAAATCCAACAAT-3'), for the *P. pachyrhizi*-specific assay, and Ppm1 and Pme2 (5'-CTCAAACAGGTGTACCTTTTGG-3'), for the *P. meibomiae*-specific assay. Each DNA amplification assay consisted of: one initial denaturation step at 94ºC for 3 min; 30 cycles at 94ºC for 1 min, 57ºC for 1 min, 72ºC for 1 min and 30 s; and a final step at 72ºC for 7 min. The electrophoresis analyses were performed in 3.0% agarose gel containing ethidium bromide (0.2 μg/mL), immersed in a 1X sodium boric acid (SB) buffer (10 mM sodium hydroxide, pH adjusted to 8.5 with boric

acid). DNA template control of *P. pachyrhizi* and *P. meibomiae* were provided by Dr. Ricardo Abdelnoor of the Embrapa Soja (Londrina, Paraná, Brazil).

2.3. Inoculation and disease screening

The primary leaves and the first trifolium of all plants were inoculated about 15 days after sowing under greenhouse conditions (20 ± 5°C). Prior to inoculation, spores were removed from storage conditions (-80°C), heat shocked at 40ºC for 10 min, and hydrated by incubating in a small plastic weigh boat over water for 24 h. The inoculum concentration was 3.0×10^5 *P. pachyrhizi* spores/mL distilled water containing Tween-20 (0.05%, v:v). The inoculum was sprayed on both leaf surfaces using a manual atomizer (De Vilbiss n^o 15) powered by an electric compressor. After inoculation the plants were transferred to a mist chamber (20 ± 1°C and relative humidity > 95%) where they were kept for 48 h under a 12-hour light regime. After this period the plants were transferred to a greenhouse (20 ± 5°C), where they were kept until symptom evaluation.

Disease symptoms were evaluated at 15, 20 and 25 days after the inoculations based on a 1-to-5 scale modified from Stavely (1985). In this scale, 1 = no sporulation, 2 = sporulation present but less than 10% of fully sporulating lesions, 3 = sporulation present and 11 to 25% of fully sporulating lesions, 4 = sporulation present and 26 to 40% of fully sporulating lesions, and 5 = sporulation present and 65 to 100% of fully sporulating lesions.

Rust lesions in both surfaces of the inoculated leaves were determined visually by at least two independent evaluators. Individual plants with degrees 1-to-3 were considered resistance, whereas those with degrees 4 or 5 were considered susceptible. When different plants of the same accession were evaluated, accessions presenting mean scores of disease reaction ≤ 3.00 were considered resistance and those with mean scores > 3.00, susceptible.

2.4. Crosses and genetic analysis of F_1, F_2 and F_3 generations

Inheritance of SBR resistance in the common bean accession 'PI181996' was studied by crossing this resistance source with the susceptible cultivars 'US Pinto 111' and 'Mexico 309'. The plants were grown and artificially crossed under greenhouse condition.

All F_1 plants from the cross 'PI181996' × 'US Pinto 111' were analyzed morphologically (flower color). Because 'PI181996' and 'Mexico 309' are black seeded beans with similar flower color and other morphological traits, the F_1 plants derived from the crosses between them were analyzed with RAPD markers as proposed by Alzate-Marin et al. (1996). As 'Mexico 309' was used as female parent, the presence in the F_1 plant of a PCR product which was present only in 'PI181996' confirmed that the plant was indeed a hybrid. Plant DNA samples were extracted according to Doyle and Doyle (1990). DNA amplification by the RAPD technique was accomplished according to Alzate-Marin et al. (1996) and the electrophoresis analyses were done on 1.2% agarose gel containing ethidium bromide (0.2 μg/mL), immersed in a 1X SB buffer. All selected F_1 plants were used to obtain the F_2 and then the F_3 populations.

Plants from F_2 and F_3 populations, soybean control cultivars 'CAC-1' and 'Cristalina', and the parental common bean lines were inoculated with *P. pachyrhizi* and screened for SBR reaction. The ratio of resistant and susceptible reactions observed in the segregating population was tested for goodness-of-fit to theoretical ratios with Chi-square (χ^2) test.

3. Results

3.1. *Phakopsora* spp. molecular detection test

PCR analysis using genomic DNA of spores from the inoculum used in this work and ITS region-specific primers for *P. pachyrhizi* and *P. meibomiae* detection, previously reported by Frederick et al. (2002), was efficiently accomplished. The results are shown in Figure 1. The amplification of PCR products only with *P. pachyrhizi*-specific primers confirmed that the inoculum used for SBR resistance screening is indeed from *P. pachyrhizi*.

3.2. Reaction of common bean accessions to *P. pachyrhizi*

Out of the 44 common bean accessions tested against the SBR pathogen, 14 were considered resistant (mean scores of disease reaction ≤ 3.00) and the other 30 accessions were considered to be susceptible (mean scores of disease reaction > 3.00) (Table 2).

None of the common bean accessions presented immune reaction to *P. pachyrhizi* (Table 2). However, three resistant lines, 'PI181996', 'Pérola' and 'Redlands Pioneer', were considered to be promising sources for resistance to SBR. They presented mean scores of disease reaction of 2.25 ± 0.26, 2.20 ± 0.41, and 2.20 ± 0.35, respectively (Table 2). On the other hand, 15 *P. vulgaris* susceptible accessions were identified: 'AND 277', 'Cornell 49-242', 'California Small White 643', 'Diamante Negro', 'Dorado', 'Mexico 309', 'Mexico 54', 'Ouro Vermelho', 'PI260418', 'Pinto Olathe', 'Rudá', 'SEL 1308', 'TU', 'US Pinto 111', and 'Vermelhinho'. They presented mean scores of disease reaction equal or greater than 4.50 (Table 2).

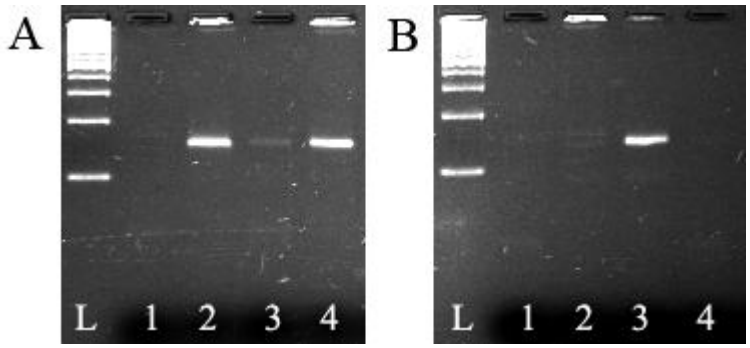

Figure 1. *Phakopsora* spp. molecular detection test. Electrophoretic analysis on 3.0 % agarose gel of amplification products obtained with *P. pachyrhizi*-specific (A) and *P. meibomiae*-specific (B) primers (Frederick et al. 2002). Lanes are as follows: L, size marker (100-bp DNA ladder); 1, no DNA template control; 2, *P. pachyrhizi* DNA control; 3, *P. meibomiae* DNA control; and 4, genomic DNA of spores obtained from the *P. pachyrhizi* inoculum used in this work. PCR products between 100 and 200-bp are present in lanes 2A and 4A, and 3B.

All soybean cultivars presented susceptible reaction to the SBR pathogen, showing high mean scores of disease reaction, including the lines harboring the resistance genes *Rpp1*, *Rpp2*, *Rpp3*, and *Rpp4* (Table 2). As previously reported by Stavely et al. (1985) and Miles et al. (2007), the soybean cultivars presented lesion sizes and spore production greater than those presented by the common bean susceptible accessions (data not shown).

Although the inoculum used for disease screening tests did not originate from a single pustule, no mixed lesion types or highly contrasting reaction degrees were observed on plants from a same soybean or common bean accession. This could be verified by analyzing the standard error values associated to the mean scores of disease reaction (Table 2).

Table 2. Reaction of common bean accessions and soybean control cultivars to *Phakopsora pachyrhizi* expressed in mean scores of disease reaction (1-to-5 degrees) with their respective standard error (SE) values.

Common bean accession	Disease reaction[a]		Common bean line	Disease reaction[a]	
	Mean	SE		Mean	SE
AB 136	3.75	0.35	Ouro Vermelho	4.55	0.37
AND 277	4.55	0.37	P-33-5-1	3.00	0.00
Aurora	4.15	0.34	P-49-8-2	3.65	0.24
BAT 332	3.20	0.35	Pérola	2.20	0.41
Brow Beauty	3.00	0.00	PI181996	2.25	0.26
CNC	3.75	0.26	PI260418	4.50	0.33
Cornell 49-242	4.65	0.34	Pinto Olathe	4.70	0.42
California Small White 643	4.65	0.34	R-127-4-13	2.85	0.24
Diamante Negro	4.55	0.37	R-127-10-14	2.95	0.37
Dorado	4.65	0.41	R-97-13-5	3.80	0.26
G 2333	4.15	0.24	R-97-13-6	2.95	0.16
Golden Gate Wax	4.20	0.35	Redlands Pioneer	2.20	0.35
IAPAR 14	2.95	0.44	Rudá	4.65	0.34
IAPAR 16	2.95	0.16	SEL 1308	4.60	0.32
IAPAR 57	3.00	0.33	Talismã	4.00	0.24
Jalo EEP 558	2.75	0.42	TO	3.80	0.26
MAR-2	4.15	0.24	TU	4.55	0.44
Mexico 235	4.15	0.34	US Pinto 111	4.75	0.26
Mexico 309	4.55	0.16	Valente	2.75	0.35
Mexico 54	4.65	0.41	Vermelhinho	4.60	0.46
Montcalm	4.40	0.39	Vermelho 2157	4.20	0.35
Ouro Negro	3.35	0.24	Vi-4899 (Pioneiro)	3.00	0.33
Soybean control cultivar	Disease reaction[a]		Soybean control cultivar	Disease reaction[a]	
	Mean	SE		Mean	SE
CAC-1	5.00	0.00	PI547878 (gene *Rpp2*)	5.00	0.00
Cristalina	4.95	0.16	PI462312 (gene *Rpp3*)	4.25	0.16
PI200492 (gene *Rpp1*)	4.75	0.20	PI459025 (gene *Rpp4*)	4.58	0.13

[a] Resistant, mean score of disease reaction ≤ 3.00; susceptible, mean score of disease reaction > 3.00. Mean scores of disease reaction were obtained by evaluating 10 plants from each common bean accession or soybean control cultivar.

3.3. Inheritance study

Two hundred and forty-six F_2 plants derived from the cross 'US Pinto 111' × 'PI181996' and 46 F_2 plants from the cross 'Mexico 309' × 'PI181996' were tested against the SBR pathogen. The segregation for *P. pachyrhizi* resistance on both F_2 populations fit a 3 resistant: 1 susceptible ratio (3R_:1rr) with χ^2 values of 0.0487 and 0.0289, and probability (*P*) values of 82.52% and 86.48%, respectively (Table 3).

One hundred and seven F_3 plants from the crosses between 'US Pinto 111' and 'PI181996' and 162 F_3 plants resulting from the cross 'Mexico 309' × 'PI181996' were also inoculated. A 5R_:3rr segregation ratio was observed on the F_3 populations with χ^2 values of 0.0006 and 0.5942, and *P* values of 98.01% and 44.08%, respectively (Table 3).

These results support the hypothesis that resistance to *P. pachyrhizi* in the common bean accession 'PI181996' is controlled by a single gene with intra-allelic relationship of complete dominance.

Table 3. Inheritance of soybean rust resistance in the common bean accession 'PI181996'.

Cross	Population	No. of tested plants	Expected ratio[a]	Observed ratio	χ^2	*P*(%)[d]
US Pinto 111 ×	F_2	246	3(R):1(S)[b]	183(R):63(S)	0.0487	82.52
PI181996	F_3	107	5(R):3(S)[c]	67(R):40(S)	0.0006	98.01
Mexico 309 ×	F_2	46	3(R):1(S)	34(R):12(S)	0.0289	86.48
PI181996	F_3	162	5(R):3(S)	106(R):56(S)	0.5942	44.08

[a] R, resistant; S, susceptible.
[b] Dominant monogenic resistance/susceptibility segregation in a F_2 generation.
[c] Dominant monogenic resistance/susceptibility segregation in a F_3 generation.
[d] Percent probability of the Chi-square (χ^2) test.

4. Discussion

In a pilot experiment aiming to identify SBR resistance sources in *P. vulgaris* to be better characterized and potentially explored by common bean and soybean breeding programs, here we screened 44 accessions from the *P. vulgaris* Active Germplasm Bank of the BIOAGRO/UFV with *P. pachyrhizi*. Three accessions were selected as promising resistance sources because their low disease reactions expressed in mean scores of infection degree (1-to-5 degrees) and their respective standard error values: 'PI181996' (2.25 ± 0.26), 'Pérola' (2.20 ± 0.41), and 'Redlands Pioneer' (2.20 ± 0.35) (Table 2). 'PI181996' has already been reported as resistance to six isolates of *P. pachyrhizi* from Taiwan, Thailand, Zimbabwe, Paraguay, and Brazil when inoculated under controlled conditions in the USA (Miles et al. 2007). This resistance source also presented no visible SBR symptoms in experiments using natural-field inoculation carried out in Brazil and South Africa (Nunes Junior et al. 2005; Pastor-Corrales et al. 2006, 2007).

Because the lowest mean score of disease reaction of 'PI181996' observed in this work, considering its associated standard error value, it was used in inheritance studies to elucidate the genetic control of its resistance to *P. pachyrhizi*. Crosses between 'PI181996' and the susceptible cultivars 'US Pinto 111' (4.75 ± 0.26) and 'Mexico 309' (4.55 ± 0.16) (Table 2) were done. The segregating populations F_2 and F_3 were obtained from these two crosses and screened with the pathogen. The results showed that SBR resistance in 'PI181996' is monogenic and dominant.

It was also verified in this work that, in general, resistance to *P. pachyrhizi* is not correlated to resistance to other fungal common bean diseases, such as rust, anthracnose and angular leaf spot (Tables 1 and 2). Although some carioca-type advanced lines developed by BIOAGRO/UFV presented resistance, it is possible that most bean lines now being developed in Brazil will be susceptible to the pathogen, as the carioca-type, black-type and

red-type cultivars (parental lines) currently grown in the country were susceptible to *P. pachyrhizi* (Tables 1 and 2).

SBR is not a serious problem to bean crop to date, but the results reported here indicate that works aiming to identify and explore resistance sources in *P. vulgaris* may be considered as strategic for the common bean breeding programs. Additionally, this effort may also help the development of transgenic soybean cultivars with effective resistance to *P. pachyrhizi* since the single dominant resistance genes identified in soybean (*Rpp1*, *Rpp2*, *Rpp3*, and *Rpp4*) condition resistance to a limited set of rust pathotypes and the resistance has not shown to be durable (Hartman et al. 2005). This would obviously demand the cloning of the gene(s) conferring resistance to SBR in the common bean and its (their) transfer to the soybean genome. The differential response to *P. pachyrhizi* presented by the *P. vulgaris* accessions also suggests that some of them could be used as differentials cultivars for classification of the fungus physiological races.

The *Phakopsora* spp. molecular detection test confirms that the spores used in our inoculation assays are indeed from *P. pachyrhizi* (Figure 1). Since we did not use a single-pustule isolate for the SBR resistance screening, it was possible that the inoculum represented a mixture of many pathotypes difficulting the disease evaluating process. But no mixed lesions or highly contrasting reaction degrees on a same soybean or common bean genotype were observed in the greenhouse inoculations, as shown by the standard error values associated to the mean scores of disease reaction presented in Table 2. The results from our inheritance studies also showed that the inoculum was efficient to detect the monogenic dominant nature of the resistance to *P. pachyrhizi* in 'PI181996' (Table 3). However, we cannot discard that a gene cluster or complex loci (closely linked genes) governs SBR resistance in this common bean line.

Based on the results obtained from Stavely et al. (1985), Miles et al. (2007), and Pastor-Corrales et al. (2007) that indicated the common bean line 'CNC' as a promising resistance source to *P. pachyrhizi*, Pastor-Corrales and

Frederick (2008) analyzed the segregating resistance pattern to *P. pachyrhizi* among 241 F_2 plants derived from crosses between susceptible cultivar 'Mexico 309' and 'CNC'. The results showed that the SBR resistance in 'CNC' is controlled by the interaction of two genes with complete dominance at both gene pairs: one dominant allele of each two genes is necessary to produce the resistant phenotype but either recessive homozygote is epistatic to the other gene. Additional assays analyzing other segregating generation are being carried out to confirm these results (Dr. Marcial A. Pastor-Corrales, personal communication).

Currently, fungicide spraying is the only effective method to control SBR worldwide. This strategy increases production costs and exposes the environment to high levels of chemicals. As a first step towards the development of common bean cultivars resistant to SBR, we identified resistance sources and studied the genetic basis of this resistance in *P. vulgaris*. To date we are working on the identification of molecular markers linked to the resistance gene present in 'PI181996' aiming their utilization for marker-assisted selection. We are also conducing additional inheritance studies using a *P. pachyrhizi* single-pustule isolate recently obtained by our group. Additionally, other *P. vulgaris* accessions are being screened aiming at the identification of effective resistance to the disease.

Acknowledgments

This work was supported by grants from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais); Brazilian Government. CNPq provided a PhD fellowship to the first author. The authors thank Dr. Vania Moda-Cirino (IAPAR, Londrina, Brazil) and CIAT (Cali, Colombia) for provided seeds from some of the common bean accessions used in present work. We are also thankful to Dr. Ricardo Abdelnoor (Embrapa Soja, Londrina, Brazil) for providing DNA template control for *P. pachyrhizi* and *P. meibomiae*.

References

Alzate-Marin, A. L., G. S. Baía, S. Martins Filho, T. J. Paula Junior, C. S. Sediyama, E. G. Barros, and M. A. Moreira, 1996: Use of RAPD-cPCR to identify true hybrid plants from crosses between closely related progenitors. Rev. Bras. Genet. 19, 621–623.

Caixeta, E. T., A. Borém, A. L. Alzate-Marin, A. S. Fagundes, M. G. M. Silva, E. G. Barros, and M. A. Moreira, 2005: Allelic relationships for genes that confer resistance to angular leaf spot in common bean. Euphytica 143, 237–245.

Doyle, J. J., and J. L. Doyle, 1990: Isolation of plant DNA from fresh tissue. Focus 12, 13–15.

DuPreez, E. D., N. C. van Rij, K. F. Lawrence, M. R. Miles, and R. D. Frederick, 2005: First report of soybean rust (*Phakopsora pachyrhizi*) on dry beans in South Africa. Plant Dis. 89, 206 (abstract).

Faleiro, F. G., V. A. Ragagnin, M. A. Moreira, and E. G. Barros, 2004: Use of molecular markers to accelerate the breeding of common bean lines resistant to rust and anthracnose. Euphytica 138, 213–218.

Frederick, R. D., C. Snyder, G. L. Peterson, and M. R. Bonde, 2002: Polymerase chain reaction assays for the detection and discrimination of the soybean rust pathogens *Phakopsora pachyrhizi* and *P. meibomiae*. Phytopathology 92, 217–227.

Hartman, G. L., E. M. Saadaoui, A. T. Tschanz, and Scientific Editors, 1992: Annotated bibliography of soybean rust (*Phakopsora pachyrhizi* Sydow). AVRDC Library Bibliography Series 4–1, Asian Vegetable Research and Development Center, Tropical Vegetable Information Service. Taipei, Taiwan.

Hartman, G. L., M. R. Miles, and R. D. Frederick, 2005: Breeding for resistance to soybean rust. Plant Dis. 89, 664–666.

Ivancovich, A. J., 2005: Soybean rust in Argentina. Plant Dis. 89, 667–668.

Ivancovich, A. J., G. Botta, M. Rivadaneira, E. Saieg, L. Erazzú, and E. Guillin, 2007: First report of soybean rust caused by *Phakopsora pachyrhizi* on *Phaseolus* spp. in Argentina. Plant Dis. 91, 111 (abstract).

Jesus Junior, W. C., F. X. R. do Vale, R. R. Coelho, B. Hau, L. Zambolim, L. C. Costa, and A. Bergamin Filho, 2001: Effects of angular leaf spot and rust on yield losses of *Phaseolus vulgaris*. Phytopathology 91, 1045–1053.

Liebenberg, M. M., C. M. S. Mienie, A. Z. Pretorius, 2006: The occurrence of rust resistance gene *Ur-13* in common bean cultivars and lines. Euphytica 150, 365–386.

Lynch, T. N., J. J. Marois, D. L. Wright, P. F. Harmon, C. L. Harmon, M. R. Miles, and G. L. Hartman, 2006: First report of soybean rust caused by *Phakopsora pachyrhizi* on *Phaseolus* species in the United States. Plant Dis. 90, 970 (abstract).

Miles, M. R., M. A. Pastor-Corrales, G. L. Hartman, and R. D. Frederick, 2007: Differential response of common bean cultivars to *Phakopsora pachyrhizi*. Plant Dis. 91, 698–704.

Nunes Junior, J., H. D. Campos, A. Sartorato, M. J. del Peloso, M. A. Pastor-Corrales, and P. A. Arraes Pereira, 2005: Ferrugem asiática da soja em cultivares de feijoeiro comum. In: VIII Congresso Nacional de Pesquisa de Feijão, 466–469. Embrapa Arroz e Feijão, Goiânia, GO, Brazil.

Ono, Y., P. Buritica, and J. F. Hennen, 1992: Delimitation of *Phakopsora*, *Physopella* and *Cerotelium* and their species on Leguminosae. Mycol. Res. 96, 825–850.

Pastor-Corrales, M. A., 1991: Estandarización de variedades diferenciales y de designación de razas de *Colletotrichum lindemuthianum*. Phytopathology 81, 694 (abstract).

Pastor-Corrales, M. A., C. E. Jara, 1995: La evolucion de *Phaeoisariopsis griseola* con el frijol comum en América Latina. Fitopatol. Col. 19, 15–22.

Pastor-Corrales, M. A., 2005: Inheritance of resistance in PI260418, an Andean bean resistant to most races of the bean rust pathogen. Annu. Rep. Bean Improv. Coop. 48, 134–135.

Pastor-Corrales, M. A., M. M. Liebenberg, A. Sartorato, P. A. Arraes-Pereira, 2006: Reaction of common bean cultivars to the Asian soybean rust pathogen, *Phakopsora pachyrhizi*, under field conditions in South Africa and Brazil. Annu. Rep. Bean Improv. Coop. 49, 31–32.

Pastor-Corrales, M. A., A. Sartorato, M. M. Liebenberg, M. J. del Peloso, P. A. Arraes Pereira, J. Nunes Junior, and H. Dinis Campos, 2007: Evaluation of common bean cultivars from the United States for their reaction to soybean rust under field conditions in Brazil and South Africa. Annu. Rep. Bean Improv. Coop. 50, 123–124.

Pastor-Corrales, M. A., and R. D. Frederick, 2008: Resistance to the soybean rust pathogen (*Phakopsora pachyrhizi*) in common bean cultivar CNC. Annu. Rep. Bean Improv. Coop. 51, 20–21.

Pivonia, S., and X. B. Yang, 2005: Assessment of epidemic potential of soybean rust in the United States. Plant Dis. 89, 678–682.

Ragagnin, V. A., T. L. P. O. Souza, D. A. Sanglard, K. M. A. Arruda, M. R. Costa, A. L. Alzate-Marin, J. E. S. Carneiro, M. A. Moreira, and E. G. Barros, 2009: Development and agronomic performance of common bean lines simultaneously resistant to anthracnose, angular leaf spot and rust. Plant Breeding 128, 156–163.

Rytter, J. L., W. M. Dowler, and K. R. Bromfield, 1984: Additional alternative hosts of *Phakopsora pachyrhizi*, causal agent of soybean rust. Plant Dis. 68, 818–819.

Sartorato, A., 2002: Identification of *Phaeoisariopsis griseola* pathotypes from five states in Brazil. Fitopatol. Bras. 27, 78-81.

Schneider, R. W., C. A. Hollier, H. K. Whitam, M. E. Palm, J. M. McKemy, J. R. Hernández, L. Levy, and R. DeVries-Paterson, 2005: First report of soybean rust caused by *Phakopsora pachyrhizi* in the continental United States. Plant Dis. 89, 774 (abstract).

Souza, T. L. P. O., A. L. Alzate-Marin, F. G. Faleiro, and E. G. Barros, 2008: Pathosystem common bean–*Uromyces appendiculatus*: host resistance, pathogen specialization, and breeding for rust resistance. Pest Technology 2, 56–69.

Stavely, J. R., 1985: The modified Cobb Scale for estimating bean rust intensity. Annu. Rep. Bean Improv. Coop. 28, 31–32.

Stavely, J. R., J. L. Rytter, and M. H. Royer, 1985: Virulence of the soybean rust pathogen, *Phakopsora pachyrhizi*, on *Phaseolus vulgaris* cultivars. Annu. Rep. Bean Improv. Coop. 28, 35–36.

Steadman, J. R., M. A. Pastor-Corrales, and J. S. Beaver, 2002: An overview of the 3rd Bean Rust and 2nd Bean Common Bacterial Blight International Workshops, March 4-8, 2002, Pietermaritzburg, South Africa. Annu. Rep. Bean Improv. Coop. 45, 120–125.

Yorinori, J. T., W. M. Paiva, R. D. Frederick, L. M. Costamilan, P. F. Bertagnolli, G. E. Hartman, C. V. Godoy, J. Nunes Junior, 2005: Epidemics of soybean rust (*Phakopsora pachyrhizi*) in Brazil and Paraguay from 2001 to 2003. Plant Dis. 89, 675–677.

Breeding for Common Bean Rust Resistance in the BIOAGRO/UFV, Brazil

Abstract: Common bean (*Phaseolus vulgaris* L.) is an economically, nutritionally, and socially important crop. It is grown in distinct regions and different seasons around the world by subsistence level farmers with low-technology input as well as by farmers that use high input technologies. Unfortunately, compared to other important grain legumes common bean yields are quite low. One of the several factors contributing to this situation is the high number of destructive diseases that attack *P. vulgaris* and cause serious damage to the crop. Among them is bean rust, incited by the highly variable fungus *Uromyces appendiculatus*. This disease is distributed throughout the world, but it effectively causes major production problems in humid tropical and subtropical regions. In Brazil, rust causes major losses in south, southeast, and central regions of the country. Bean rust control by plant resistance is an easy and economical strategy to be used in association to other rust management practices. The pyramiding of different race-specific resistance genes in association with other genes conferring adult plant resistance, slow rusting, and reduced pustule size can prolong the lifespan of a common bean cultivar by creating a more durable resistance complex against the rust pathogen. This review presents an overview about the bean rust and reports some breeding initiatives aiming at the development of rust resistant cultivars in the BIOAGRO/UFV, Minas Gerais, Brazil.

Keywords: gene pyramiding – host-pathogen interaction – marker-assisted selection – *Phaseolus vulgaris* – plant resistance – *Uromyces appendiculatus*

1. Introduction

The common bean (*Phaseolus vulgaris* L.) is the most important legume directly used for human consumption worldwide. Among the five domesticated and grown species of the genus *Phaseolus*, a genus comprised of some 70 species (Freytag and Debouck 2002), *P. vulgaris* accounts for more than 90% of the cultivated crop in the world (Singh *et al.* 1991a, 2001). Dry bean cultivars of the species *P. vulgaris* were grown and consumed on approximately 27 million hectares in more than 120 countries in 2007 (http://faostat.fao.org). The social value of the common bean is extremely high to millions of people in many countries and most especially in developing countries of Latin America, and Eastern and Southern Africa (Pachico 1989; Wortmann *et al*. 1998; Broughton *et al.* 2003). In Brazil, the main producer and consumer country (http://www.fao.org), dry beans are the main source of vegetable protein, minerals, and vitamins for a major proportion of the population. The set of essential amino acids present in dry bean seeds is complementary to that in grains of cereals such as rice (*Oryza sativa* L.).

Some bean seed properties have been shown to contribute positively to major health issues, such as the control of type II diabetes. Regular bean consumption also lowers glycemic and cholesterolemic indices and decreases the incidence of certain types of cancer (Andersen *et al.* 1984; Hangen and Bennink 2003).

The attack of pests and pathogens is one of the main causes of yield and quality losses in the common bean crop worldwide (Stavely and Pastor-Corrales 1989). This is especially true for small farmers with low-technology inputs, which play an important role, as they account for the greatest fraction of the product for the world market supply (http://faostat.fao.org). Among the most serious diseases that attack common bean we find bean rust, incited by the highly variable fungus *Uromyces appendiculatus* F. Strauss (syn. *U. phaseoli* G. Winter). This disease is distributed throughout the world, but it effectively causes major production problems in humid tropical and

subtropical areas and periodic severe epidemics in humid temperate regions (Souza *et al.* 2008). Severe bean rust epidemics have been reported in Brazil and other Latin American countries as well as in Australia, China, the United States, and some areas of Europe (Stavely and Pastor-Corrales 1989).

According to Lindgren *et al.* (1995) a 1% increase in bean rust severity leads to a yield loss of approximately 19 kg/ha. The major losses in Brazil occur in south, southeast and central areas, including the states of Paraná, Rio Grande do Sul, Santa Catarina, Minas Gerais, São Paulo, and Goiás (Souza *et al.* 2005a). Yield losses higher than 68% have been detected in the state of Minas Gerais located in the southeast region of the country (Vieira *et al.* 2005).

No single cost-effective or efficient control measure can be recommended to prevent rust infection in all cases or different regions. Disease management practices for bean rust control include crop rotation, soil incorporation of bean debris, planting within recommended dates, growing resistant cultivars, and timely spraying of fungicides (Mmbaga *et al.* 1996).

Compared to chemical control the use of plant resistance not only is harmless to the environment but also an economically sound strategy. However, the wide variability of *U. appendiculatus* represents an obstacle to breeders aiming at the development of common bean cultivars with durable resistance to rust. The combination of different rust resistance (RR) genes in the same cultivar has been proposed as an important strategy for obtaining effective and durable genetic resistance to rust (Johnson 1984; Stavely and Pastor-Corrales 1989; Kelly *et al.* 1995; Souza *et al.* 2005b, 2007a). This can be accomplished by gene pyramiding, when distinct genes are combined in the same cultivar, or by using multilines, when different genes are transferred to different lines of the same cultivar. For gene pyramiding, knowledge about the inheritance and organization of the genes and also the use of proper selection tools are of paramount importance (Souza *et al.* 2008).

Molecular markers have been used to assist different steps of common bean breeding programs aimed at developing cultivars resistant to rust.

Isozymes and DNA-based markers have been used to study the genetic diversity of the rust fungus (Lu and Groth 1988; Linde *et al.* 1990a, 1990b; McCain *et al.* 1992; Groth *et al.* 1995; Maclean *et al.* 1995; Faleiro *et al.* 1998) and also for mapping and characterizing resistance genes to *U. appendiculatus* and to other important bean pathogens (Freyre *et al*. 1998; Miklas *et al*. 2002; Kelly *et al*. 2003; Miklas *et al*. 2006; Souza *et al.* 2008).

In this review we describe and discuss important aspects about the bean rust. In addition, we present common bean breeding initiatives aiming at the development of rust resistant cultivars in Brazil. We have focused mainly the routine of the BIOAGRO/UFV Common Bean Breeding Program, which is assisted by molecular marker. This program is the main bean breeding program aiming rust resistance conducted in Brazil. Its objectives include the genetic study of host-pathogen interaction, identification and validation of molecular markers linked to RR genes, and the effective use of these markers for the development of cultivars with durable resistance to rust.

2. Characterization and Maintenance of the Pathogen

2.1. Differential cultivars for *U. appendiculatus*

During the "Bean Rust Workshop" (BRW), held in 1983, 35 researchers from different countries proposed a series of 20 cultivars as the international differential standard for *U. appendiculatus* (Stavely *et al.* 1983) (Table 1). In 1984, cv. 'Mountainer White Half Runner' was eliminated from this series due to its similarity with 'Kentucky Wonder 780' (Stavely 1984a). Characterization of Brazilian isolates based on those 19 differential cultivars was accomplished by Mora-Nuñes *et al.* (1992), Santos and Rios (2000) and Souza *et al.* (2005a). In their work, Mora-Nuñes *et al.* (1992) concluded that eight out of the 19 cultivars – 'Kentucky Wonder 814', 'Early Gallatin', '51051', 'NEP 2', 'Ecuador 299', 'Olathe', 'Mexico 309' and 'Redlands Pioneer' – were sufficient to discriminate and classify isolates collected in

Brazil. Using these eight cultivars, Faleiro *et al.* (1999a) characterized 13 races of this fungus in the Brazilian state of Minas Gerais.

Table 1. Series of common bean cultivars adopted as differentials for *Uromyces appendiculatus* at the 1983 Bean Rust International Workshop (Stavely *et al.* 1983).

Common bean cultivar	
1. U.S. 3	11. Ecuador 299
2. California Small White 643	12. Mexico 235
3. Pinto 650	13. Mexico 309
4. Kentucky Wonder 765	14. Brown Beauty
5. Kentucky Wonder 780	15. Olathe
6. Kentucky Wonder 814	16. AxS 37
7. Golden Gate Wax	17. NEP 2
8. Early Gallatin	18. Aurora
9. Mountaineer White Half Runner[a]	19. 51051
10. Redlands Pioneer	20. CNC

[a]Deleted of the list because of its similarity to 'Kentucky Wonder 780' (Stavely 1984a).

In the 3rd BRW held in 2002, a new differential series was proposed for characterization of *U. appendiculatus* isolates (Steadman *et al.* 2002). This series contains six Andean and six Mesoamerican bean cultivars (Table 2). Cultivars 'Early Gallatin', 'Redlands Pioneer', 'Golden Gate Wax', 'Aurora', 'Mexico 309', 'Mexico 235' and 'CNC', which were proposed in the 1983 BRW, were maintained in the present differential series. Cultivars 'Montcalm', 'PC-50', 'PI 260418', 'Great Northern 1140' and 'PI 181996' were added to the series. The wide adoption of this system can contribute to the elaboration of an internationally standardized classification methodology, and facilitate the exchange of information, and the cooperative use of the results obtained by different research groups throughout the world.

2.2. Obtaining of single-pustule isolates

Obtaining single-pustule isolates from *U. appendiculatus* primary inoculum is an important initial step before the characterization and use of the pathogen in rust resistance tests. Single-pustule isolates are pure lines of the pathogen;

specific genotypes of the fungus. They are essential in the study of host-pathogen interaction, host resistance and pathogen specialization.

Table 2. Differential series and the binary system of nomenclature adopted at the 3rd Bean Rust International Workshop as international standard for classification of *Uromyces appendiculatus* physiological races (Steadman *et al.* 2002).

Binary system value	Differential cultivar	Resistance gene	Gene pool
1	Early Gallatin	*Ur-4*	Andean
2	Redlands Pioneer	*Ur-13*	
4	Montcalm	*Ur-?*[a]	
8	PC-50	*Ur-9*, *Ur-12*	
16	Golden Gate Wax	*Ur-6*	
32	PI 260418	*Ur-?*	
1	Great Northern 1140	*Ur-7*	Mesoamerican
2	Aurora	*Ur-3*	
4	Mexico 309	*Ur-5*	
8	Mexico 235	*Ur-3*$^{+}$	
16	CNC	*Ur-?*	
32	PI 181996	*Ur-11*	

[a]*Ur-?* = unnamed gene.

U. appendiculatus single-pustule isolates can be obtained from inoculum previously collected and maintained in fungal collections. But most often they are isolated from fungal spores collected from plant tissues infected under natural conditions in bean growing areas or experimental stations. For single-pustule isolation, a lower than usual inoculum concentration is used for inoculation in order to increase the chance of getting individual pustules. The spores collected in these single-pustules are multiplied in susceptible varieties for three or more consecutive cycles, using the regular inoculum concentration. After this procedure, the uredospores are collected and stored under controlled conditions (Souza *et al.* 2007a).

2.3. Physiological races

Classification of *U. appendiculatus* into physiological races and the consequent knowledge of its virulence diversity is a basic step towards

understanding the dynamics of the pathogen distribution and the development of resistant cultivars. In this step it is also possible to identify which pathogen isolates can be used to monitor the introgression of resistance genes in breeding programs (Pastor-Corrales 2001; Pastor-Corrales and Stavely 2002; Souza *et al.* 2007a).

Steadman *et al.* (2002) proposed a binary nomenclature system for designation of the races in which the evaluation scale was divided in only two reaction degrees: resistant and susceptible. Each race is designated by two numbers separated by a hyphen. The first number is obtained by the sum of the binary values attributed to the susceptible Andean cultivars of the differential series. The second number is obtained by the sum of the binary values of the susceptible Mesoamerican cultivars (see Table 2).

Characterization of *U. appendiculatus* isolates collected in the USA, South Africa, Honduras, Argentina and Mozambique has been accomplished based on the procedure proposed in the 3rd BRW (Steadman *et al.* 2002; Acevedo *et al.* 2004; Jochua *et al.* 2004). Souza *et al.* (2007a) reported the first work using the standard system for classification of *U. appendiculatus* physiological races in Brazil using *U. appendiculatus* single-pustule isolates obtained from the fungal collection of BIOAGRO/UFV.

2.4. Fungal collection

Although *U. appendiculatus* does not grow in artificial culture as it is an obligate parasite, viable spores can be preserved under laboratory conditions. Dry uredospores in plastic or glass tubes have been successfully maintained under dark conditions at 5 ± 1°C and relative humidity < 50% for about one year at BIOAGRO/UFV. Alternatively, uredospores of the fungus frozen at -80°C or submersed in liquid nitrogen can be stored for a longer period of time (Souza *et al.* 2008). Periodical inoculation of the preserved spores on susceptible common bean varieties is highly recommended, in order to increase the inoculum virulence.

3. Disease Screening

3.1. *U. appendiculatus* inoculation

U. appendiculatus inoculation is often done when the primary leaves of the bean plants reach approximately 2/3 of their full development, about 10 days after sowing under greenhouse conditions (20 ± 5°C). The standard concentration of inoculum is 2.0×10^4 uredospores/mL of distilled water containing 0.05% Tween-20. The inoculum solution can be applied on both leaf surfaces with a brush or sprayed using a manual atomizer (e.g., atomizer De Vilbiss n^o 15) adapted to an electric compressor. After inoculation the plants are transferred to a mist chamber (20 ± 1°C and relative humidity > 95%) where they are kept for approximately 48 h under a 12-hour light regime. In order to avoid contamination, plants inoculated with different isolates are kept in separate compartments of the chamber. After this period the plants are transferred to a greenhouse (20 ± 5°C), where they are kept until symptom evaluation, about 14 days after inoculation (Carrijo *et al.* 1980; Souza *et al.* 2005a, 2007a).

The inoculation can also be conduced using common bean excised leaves, as reported by Souza *et al.* (2005c). In this alternative method, after inoculation, each leaf is placed in a Petri dish (90 × 15 mm) on a sterile filter paper previously moistened with 3.0 mL distilled water. The dishes are incubated in a BOD at 20°C, under a 12-hour light regime. Each filter paper is moistened again with 1.5 mL distilled water every three-days until disease symptom evaluation, about 10 days after inoculation.

Both inoculation methods are efficient for evaluating the reaction of the common bean to *U. appendiculatus*. The only differences observed on the results by the two methods is that the number of pustules is higher in the conventional method and disease symptoms appear earlier in the alternative method (Souza *et al.* 2005c). It is suggested that the conventional inoculation method is more appropriate for spore multiplication and the excised leaf method can be used in the cases where the same plant needs to be assayed

several times. Another advantage of the alternative procedure refers to costs and safety; the whole method can be conducted in the laboratory without the need of exposing other plants to the pathogen. In addition, the tested plants are still able to grow and produce seeds.

3.2. Evaluation of rust symptoms

The fungus *U. appendiculatus* may infect leaves, pods, and, rarely, stems and branches. Symptoms usually appear first on the lower leaf surface as minute, whitish slightly raised spots about five or six days after inoculation. These spots enlarge to form mature reddish brown uredia which rupture the epidermis about two days later. Sporulation begins and the uredia may attain a diameter of 1-2 mm about 8-12 days after inoculation. In some cases, secondary and tertiary uredia develop around the perimeter of these primary uredia. The entire infection cycle occurs within approximately 10-15 days (Stavely and Pastor-Corrales 1989; Souza *et al.* 2008).

The reaction degrees to the disease are usually determined based on a scale of six infection degrees: 1- no pustules (immunity); 2 - necrotic spots without sporulation; 3 - pustules undergoing sporulation with a diameter of < 300 μm; 4 - pustules undergoing sporulation with a diameter ranging from 300 μm to 499 μm; 5 - pustules undergoing sporulation with a diameter ranging from 500 μm to 800 μm; and 6 - pustules undergoing sporulation with a diameter of > 800 μm (Stavely *et al.* 1983).

The infection degrees are determined approximately about 10-15 days after inoculation when up to 50 % of the pustules are sporulating. The lesions in both surfaces of the primary leaves should be determined visually by at least two evaluators. The cultivars that predominantly presented degrees 3 or lower were classified as resistant, whereas those with predominant degrees 4 or higher are considered to be susceptible.

4. Pathosystem *U. appendiculatus–P. vulgaris*

4.1. Host-pathogen coevolution

Alloenzymes studies, variation patterns of the common bean seed protein phaseolin, and morphological evidences revealed the existence of a Mesoamerican and an Andean gene pool in common bean (Gepts *et al*. 1986; Singh *et al.* 1991a, 1991b). The Andean cultivars originated in the Andean region of South America, while the Mesoamerican beans were domesticated from wild populations in Mexico and Central America.

Using phenotypic (virulence diversity) and genotypic (RAPD markers) analyses of 90 *U. appendiculatus* isolates from thirteen Latin American countries, Araya *et al*. (2004) were able to divide them into two major groups, namely the Andean and the Mesoamerican, and one intermediate group. In general, Andean and Mesoamerican isolates showed virulence specificity to landraces belonging to their respective gene pools, however, the Andean isolates tended to display greater geographic specificity than the Mesoamerican isolates. This phenomenon, previously observed by Sandlin *et al*. (1999), suggests a parallel evolution in the bean rust pathosystem. The intermediate virulence group of *U. appendiculatus* races, observed by Braithwaite *et al.* (1994), Maclean *et al*. (1995), Sandlin *et al*. (1999), and Araya *et al*. (2004), provide evidence of a transition area between these two gene pools in both the common bean host and the rust pathogen. It is therefore possible that ongoing adaptation between pathogen and host will eventually include isolates from the intermediate group into the other two major groups (Araya *et al*. 2004).

The virulence pattern analysis of 41 *U. appendiculatus* isolates from Brazil, demonstrated they were compatible with both Mesoamerican and Andean common bean cultivars (Souza *et al.* 2005a) suggesting that these isolates belong to the intermediate virulence group.

4.2. Genetics of the rust resistance in common bean

Resistance to bean rust can be controlled by major single dominant genes (Augustin *et al.* 1972; Ballantyne 1978; Christ and Groth 1982a; Sayler *et al*. 1995; Corrêa *et al*. 2000; Faleiro *et al*. 2000a, 2000b; Alzate-Marin *et al*. 2004; Souza *et al*. 2007b, 2007c), single recessive gene (Zaiter *et al.* 1989), two genes (Finke *et al*. 1986), two complementary dominant genes (Grafton *et al*. 1985), or by genes with minor effect (Edington *et al*. 1994). The gene-to-gene relationship proposed by Flor (1971) has been shown to occur in the *U. appendiculatus–P. vulgaris* interaction (Christ and Groth 1982a, 1982b). Resistance genes effective against multiple races of the pathogen are organized in clusters or complex loci (Stavely 1984b; Stavely and Grafton 1985). To date at least 13 dominant RR genes have been identified (genes *Ur-1* to *Ur-13*), which are described in Table 3. These genes are named according to a nomenclature proposed by Kelly *et al*. (1996). In addition to these 13 genes, other important unnamed RR genes, in 'BAC6' (Jung *et al.* 1996), 'Ouro Negro' (Corrêa *et al*. 2000; Faleiro *et al.* 2000a), 'Dorado' (Miklas *et al.* 2000, 2002), and 'PI 260418' (Pastor-Corrales 2005; Pastor-Corrales *et al.* 2008) have been identified. The proper characterization of the RR genes from Mesoamerican and Andean gene pools is essential for the future of the common bean breeding aiming rust resistance. It will broaden the spectra of the RR genes presently used (Liebenberg *et al*. 2006; Pastor-Corrales *et al.* 2008).

5. Gene Tagging

Several RAPD markers associated with genes conferring resistance to rust in common bean have been identified, as described in Table 3. Many SCAR markers have been developed to increase the reproducibility of the RAPD markers (Table 3). These molecular markers have been used for mapping *Ur* genes in the integrated common bean map (Freyre *et al*. 1998; Miklas *et al*. 2002; Kelly *et al*. 2003; Miklas *et al*. 2006).

Table 3. Common bean rust resistance genes and linked molecular markers.

Resistance gene	Gene pool[b]	Cultivar source	LG[d]	Molecular marker	Note
Ur-1	MA	B 1627 (Gallaroy Genotype I)	?	-	Identified by Ballantyne (1978). 'Gallaroy' derives from '643' x 'Sanilac'. *Ur-1=Ur-A.*
Ur-2	MA	B2090 (Gallaroy Genotype II)	?	-	Identified by Ballantyne (1978). 'Gallaroy' derives from '643' x 'Sanilac'. *Ur-2=Ur-B.*
Ur-2^{2}	MA	B2055 and AxS 37	?	-	'B2055' possesses only the *Ur-E* gene derived from 'AxS 37' ('Actopan' x 'Sanilac Selection 37', with genes *Ur-E* and *Ur-F*). *Ur-B* and *Ur-E* are allelic (Ballantyne 1978).
Ur-3	MA	Aurora[c]	B11	-	Identified by Ballantyne (1978). 'Aurora' possesses two linked genes (*Ur-M* and *Ur-N*); *Ur-M =Ur-3*.
Ur-3	MA	NEP 2	B11	RAPD-K14$_{620}$ (Haley *et al.* 1994) and SCAR-K14$_{620}$ (Nemchinova and Stavely 1998) – 2.2 cM/Coupling	'NEP 2' possesses the genes *Ur-F*, *Ur-I*, *Ur-J* and *Ur-K*. *Ur-J* is allelic or closely linked in repulsion phase to gene *Ur-H* of 'Cornell 49242'. Gene *Ur-I* is allelic to *Ur-3* (Ballantyne 1978).
Ur-3^{+}	MA	Mexico 235[c]	B11	-	It is distinct from the other *Ur-3* sources ('Aurora', 'Nep-2', and '51051') because it presents slightly different reaction profile across a differential set of *U. appendiculatus* races (Miklas *et al.* 2002).
Ur-4	A	Early Gallatin[c]	B6	RAPD-A14$_{1,100}$ – 0.0 cM/Coupling (Miklas *et al.* 1993), and SCAR-A14$_{1079/800}$ – 0.0 cM/Codominant (Mienie *et al.* 2004)	Identified by Ballantyne (1978). *Ur-4=Ur-C.*
Ur-5	MA	Mexico 309[c] and B-190	B4	RAPD-F10$_{970}$ – 2.1 cM/Coupling (Haley *et al.* 1993); RAPD-I19$_{460}$ (Haley *et al.* 1993) and SCAR-I19$_{460}$ (Melloto and Kelly 1998) – 0.0 cM/Coupling; SCAR-I19$_{460}$ – 3.3 cM/Coupling (Souza *et al.* 2007b)	Block of eight tightly linked rust resistance genes identified by Stavely (1984a).
Ur-6	A	Golden Gate Wax[c] and Olathe	B11	RAPD-BC06$_{308}$ and SCAR-BC06$_{308}$ – 1.3 cM/Coupling (Park *et al.* 2003a, 2004); RAPD-AG15$_{300}$ – 2.0 cM/ Coupling, and RAPD-AY15$_{200}$ – 7.7 cM/ Repulsion (Park *et al.* 2003a, 2004)	Identified by Ballantyne (1978) and Grafton *et al.* (1985). *Ur*-6=*Ur-G*.

Table 3. Continuation...

Resistance gene	Gene pool[b]	Cultivar source	LG[d]	Molecular marker	Note
Ur-7	MA	Great Northern 1140[c]	B11	RAPD-AD12$_{537}$ and SCAR-AD12$_{537}$ – 0.0 cM/Coupling; RAPD-AA11$_{500}$ – 0.0 cM/Coupling (Park *et al.* 1999a, 2003b); RAPD-AF17$_{900}$ – 0.0 cM/Coupling, RAPDAB16$_{850}$ – 2.2 cM/Coupling, RAPD-AD9$_{550}$ – 2.2 cM/Coupling, and RAPD-AB18$_{650}$ – 2.4 cM/Repulsion (Park *et al.* 1999a)	Identified by Augustin *et al.* (1972). Also found in cultivar 'Pinto US-5'.
Ur-8	A	U.S. 3	?	-	Identified by Christ and Groth (1982a, b).
Ur-9	A	PC-50[c]	B1	RAPD-J13$_{1,100}$ – 5.0 cM/Coupling (Jung *et al.* 1998); RAPD-A04$_{1,050}$ – 8.6 cM/Coupling (Park *et al.* 1999b)	Identified by Finke *et al*. (1986).
Ur-10	A/MA	Cape and Resisto	?	-	Identified by Webster and Ainsworth (1988).
Ur-11	MA	PI 181996[c]	B11	RAPD-AC20$_{490}$ – 0.0 cM/Coupling, and RAPD-AE19$_{890}$ – 6.2 cM/Repulsion (Johnson *et al.* 1995); RAPD-AE19$_{890}$ (Souza *et al.* 2002) and SCAR-AE19$_{890}$ (Queiroz *et al.* 2004; Liebenberg *et al.* 2008) – 1.0 cM/Repulsion; RAPD-GT02$_{450}$ and SCAR-GT02$_{450}$ – 0.0 and 5.4 cM/Coupling (Boone *et al.* 1999)	Identified by Stavely (1998) as *Ur-3*^{2}. Tightly linked to *Ur-3*.
Ur-12	A	PC-50[c]	B7	-	Identified by Jung *et al.* (1998). Conditions adult plant resistance (APR).
Ur-13	A	Kranskop	B8	SCAR-KB126$_{430/405}$ – 1.6 cM/ Codominant, SCAR-KB85$_{310/288}$ – 9.2 cM/Codominant, and SCAR-KB4$_{250/186}$ – 13.8 cM/Codominant (Mienie *et al.* 2005)	Identified by Liebenberg and Pretorius (2004). 'Kranskop' shares an ancestor with 'Redlands Pioneer' (Liebenberg *et al.* 2006).
Ur-13	A/MA(?)	Redlands Pioneer[c]	B8	-	Described by Liebenberg and Pretorius (2004). Although 'Redlands Pioneer' has been considered as an Andean cultivar (Steadman *et al*. 2002), *Ur-13* appears to be of Mesoamerican origin (Liebenberg *et al.* 2006).

Table 3. Continuation...

Resistance gene	Gene pool[b]	Cultivar source	LG[d]	Molecular marker	Note
Ur-?[a]	A	PI 260418[c]	?	-	Important Andean source from Bolivia (Pastor-Corrales 2005). Tentatively named as *Ur-14*.
Ur-?	A	Montcalm[c]	?	-	Pedigree: 'Great Northern #1' x 'Dark Bed Kidney' (McClean and Myers 1990).
Ur-?	MA	CNC[c]	?	-	Composite of Guatemalan black beans (McClean and Myers 1990). One single gene conferring resistance to race 49 was detected by Rasmussen *et al*. (2002). Confers resistance to Andean *U. appendiculatus* races (Sandlin *et al*. 1999).
Ur-?	MA	Ouro Negro	B4	RAPD-BA08$_{530}$ and SCAR-BA08$_{530}$ – 6.0 cM/Coupling, RAPD-F10$_{1,072}$ and SCAR-F10$_{1,072}$ – 7.0 cM/ Coupling (Corrêa *et al.* 2000; Faleiro *et al*. 2000a); RAPD-X11$_{550}$ – 5.8 cM / Coupling (Faleiro *et al.* 2000a)	Identified by Faleiro *et al*. (2000a, b). It has shown a wide spectrum resistance in Brazil (Faleiro *et al.* 1999a) and USA (Alzate-Marin *et al.* 2004). Temporarily named as *Ur-OuroNegro* or *Ur-ON*. It is the main rust resistance source used in Brazil. *Ur-ON* does not have allelic relationship with *Ur-3*$^{+}$, *Ur-5* or *Ur-11* (Alzate-Marin *et al.* 2004; Souza *et al.* 2007c).
Ur-?	MA	Dorado (DOR 346)	B4	-	Reported by Miklas *et al.* (2000). Temporarily named as *Ur-Dorado108* (Miklas *et al.* 2002).
Ur-?	MA	Dorado (DOR 346)	B11	-	Reported by Miklas *et al.* (2000). Temporarily named as *Ur-Dorado53* (Miklas *et al.* 2002).
Ur-?	MA	BAC6	B11	RAPD-AJ16$_{250}$ – 12.5 cM/ Coupling (Jung *et al*. 1996)	Described by Jung *et al.* (1996). Temporarily named as *Ur-BAC6* (Miklas *et al.* 2002).

[a]*Ur-?* = unnamed gene.
[b]Andean (A) and Mesoamerican (MA) *P. vulgaris* gene pools.
[c]Differential cultivar for *U. appendiculatus* (Steadman *et al.* 2002).
[d]The linkage groups (LG) designated as B1-to-B11 in the BJ common bean core map (Freyre *et al.* 1998; Miklas *et al.* 2002; Kelly *et al.* 2003; Miklas *et al.* 2006) correspond to the *P. vulgaris* chromosomes 1-to-11, respectively (Pedrosa *et al.* 2003, 2006, 2008).

The groups of Mesoamerican genes *Ur-5/Ur-Dorado53/Ur-ON* and *Ur-3/Ur-7/Ur-11/Ur-Dorado108/Ur-BAC6* have been mapped in linkage groups (LG) B4 and B11, respectively. *Ur-3* and *Ur-11*, and also *Ur-Dorado108* map to the end of LG B11, next to the *Co-2* locus, which is related to resistance to anthracnose. *Ur-BAC6* is located near to the *Ur-7* locus, and they do not appear to be close to *Ur-Dorado108*, *Ur-3*, and *Ur-11*. The Andean genes *Ur-4*, *Ur-6*, *Ur-9*, *Ur-12*, and *Ur-13* were mapped to LG B6, B11, B1, B7, and B8, respectively (Miklas *et al.* 2002; Kelly *et al.* 2003; Miklas *et al.* 2006; Wright *et al*. 2008). Park *et al*. (2008) observed a possible allelic relationship between *Ur-7* present in Mesoamerican cultivar 'Great Northern 1140' and *Ur-6* present in Andean cultivar 'Olathe', based on the fact that the band generated by SCAR AD12 linked to *Ur-7* was also present in cultivar 'Olathe'.

Clustering is also observed between RR genes and those conferring resistance to anthracnose (*Co*) and BCMV (Miklas *et al*. 2006). For instance, the Andean RR gene *Ur-9* and the anthracnose resistance gene *Co-1* co-localize on LG B1 (Kelly and Vallejo 2004; Miklas *et al.* 2006). The Mesoamerican genes *Ur-5* and *Co-3*/*Co-9*, and gene *Ur-ON* from cultivar 'Ouro Negro' and *Co-10* co-localize on LG B4 (Faleiro *et al.* 2000b; Alzate-Marin *et al*. 2003), and *Ur-3* co-localize with *Co-2* on LG B11, suggesting that these genes derived from common ancestral gene sequences (Geffroy *et al*. 1999; Faleiro *et al.* 2000b, 2003; Miklas *et al.* 2006). Recent works show that SCAR SQ4 linked to the *Co-2* anthracnose resistance gene is closely linked to *Ur-11* (Awale *et al.* 2008).

The linkage groups designated as B1-to-B11 in the BJ common bean core map correspond to the chromosomes 1-to-11, respectively (Pedrosa *et al.* 2003, 2006, 2008).

6. Gene Pyramiding Aiming at Development of Common Bean Lines Resistant to Rust

The use of resistant cultivars is certainly the main component of the integrated bean rust management. Pyramiding of resistance genes from both Andean and Mesoamerican gene pools is an important strategy for developing complementary and durable resistance to a large number of *U. appendiculatus* races (Stavely and Pastor-Corrales 1989; Pastor-Corrales and Stavely 2002; Araya *et al.* 2004). The large number of virulence patterns of *U. appendiculatus*, some of which are unique to certain countries, requires the use of specific resistance genes in different regions (Ballantyne 1978; Araya *et al.* 2004; Souza *et al.* 2005a; Liebenberg *et al.* 2006; Acevedo *et al.* 2008; Alleyne *et al.* 2008).

Gene pyramiding using only conventional breeding methods has not been effective mainly due to the difficulties in selecting genotypes harboring different resistance genes which demand multiple or serial inoculations of the same plant or population (Michelmore 1995). This limitation affects the breeding process as a whole and also decreases the accuracy and efficiency of the selection process (Bigirimana and Höfte 2001; Souza *et al.* 2005d). Epistatic interactions between different resistance genes can also affect the selection process (Singh *et al.* 2001).

These limitations can be overcome by the use of molecular markers linked to the resistance genes. With the use of molecular markers not only the multiple and sequential inoculations can be avoided but also the confounding effect of potential epistatic interactions among the different resistance genes present in the same genetic background (Michelmore 1995; Bigirimana and Höfte 2001; Singh *et al.* 2001; Toenniessen *et al.* 2003). However, for each resistance allele a specific marker or markers need to be identified. The use of flanking markers tightly linked to the locus of interest makes selection even more robust (Faleiro *et al.* 2003).

Molecular markers can also be used to accelerate the recovery of the recurrent parent's genome in backcross breeding programs. Simulation studies and real data indicate that only three or four backcrosses are necessary to recover the recurrent parent's genome when molecular markers are used (Openshaw *et al.* 1994; Faleiro *et al.* 2004; Ragagnin *et al.* 2009). Separate backcross programs assisted by molecular marker fingerprinting can be used for the individual introgression of resistance genes in commercial cultivars. This strategy can be the initial step for pyramiding of RR alleles.

Experimental evidence demonstrates that gene pyramiding confers more effective resistance to the host plant than that conferred by the sum of the resistance present in the progenitor plants (Yoshimura *et al.* 1995; Huang *et al.* 1997; Singh *et al.* 2001). According to Schafer and Roelfs (1985), the probability that a pathogen will overcome a gene pyramid of four to six genes is extremely low. In order for this to happen, independent mutations in the pathogen genome must occur and they should be combined in the same genetic background, or they could occur simultaneously or sequentially in the genome of a specific pathogen isolate. Nelson (1979) argues that resistance resulting from the partial action of several resistance genes exerts a low selection pressure on the pathogen and for this reason it tends to last for a long period of time. Although this concept is not fully accepted by the scientific community there are experimental data supporting the existence of partial effects of different resistance genes in some pathosystems (Brondy *et al.* 1986; Pedersen and Leath 1988). According to the theory presented the duration of resistance will depend on the number of genes to be overcome by the pathogen.

Epidemiology data also support the use of gene pyramiding as an effective strategy for disease control. According to Thrall and Burdon (2003), there is an inverse correlation between pathogen fitness, as measured by the number of spores produced, and the number of avirulence genes present in its genome. The authors observed that the pathogen populations which were able to infect a greater number of host populations were less aggressive than

pathogen populations which were able to infect a lower number of host populations. This indicates that the inactivation of several avirulence genes in the pathogen compromises its adaptability. This is a positive aspect from the epidemiological perspective because it indicates that gene pyramiding can potentially keep the disease below an economical damage level and also prevent its fast dissemination.

The main steps of a gene pyramiding breeding program assisted by molecular markers aiming at disease resistance are: (i) identification of the most prevalent and virulent races of the pathogen in the region of interest and characterization of the most promising resistance sources for that region; (ii) determination of the disease resistance inheritance mode by crossing the resistance sources and the susceptible cultivar; (iii) identification of molecular markers tightly linked to the various disease resistance alleles; (iv) development of lines harboring the R genes and the molecular markers of interest; this process is often done by backcrossing; (v) identification of markers that can specifically identify the resistance alleles to avoid false positives; and (vi) pyramiding of resistance alleles by intercrossing the lines obtained. During this process, the following activities must also be considered: (i) continuous characterization of the variability of the pathogen and the host; (ii) characterization and introduction of new resistance sources in the breeding program; and (iii) identification of molecular markers linked to the resistance genes present in the new resistance sources (Alzate-Marin *et al.* 2005; Souza *et al.* 2008).

In the BIOAGRO-UFV Common Bean Breeding Program, molecular fingerprinting based on the RAPD-PCR technique has been used to accelerate the development of common bean lines resistant to rust, anthracnose and angular leaf spot. Molecular marker-assisted selection has been also used to combine the different resistance genes in a single genetic background (Faleiro *et al.* 2004; Ragagnin *et al.* 2009).

In the specific case of rust, the breeding program conduced at BIOAGRO/UFV is also using the MAS (molecular-assisted selection) for

developing lines with specific RR genes (*Ur-ON*, *Ur-5*, and *Ur-11*) aiming at their introgression and pyramiding in Brazilian commercial cultivars (Alzate-Marin *et al*. 2004; Faleiro *et al*. 2004; Souza *et al*. 2005d, 2007b; Ragagnin *et al*. 2009). Initially, the gene *Ur-ON* has been used as the only source for resistance to *U. appendiculatus* in that breeding program. The RAPD marker OPX11 (Faleiro *et al.* 2000a) and the SCAR markers SF10 and SBA08 have been used for the indirect selection of *Ur-ON* and its introgression in the genetic background 'Rudá' (Corrêa *et al.* 2000). Later on, another RR gene was characterized, the gene *Ur-11*, which was then also introgressed into 'Rudá' (Souza *et al.* 2002). To assist the selection of *Ur-11*, the RAPD marker OPAE19 was validated in a F_2 population derived from the cross 'Rudá' × 'Belmidak RR-3' (Alzate-Marin *et al.* 2004). Then, this marker was converted into a SCAR marker (SCAR AE19) by Queiroz *et al.* (2004). In the study of Souza *et al.* (2007b) the SCAR marker SI19 was validated as linked to gene *Ur-5* from cultivar 'Mexico 309'. It was also verified that this marker can be used for the indirect selection of gene *Ur-5* in the presence of genes *Ur-ON* and *Ur-11*.

Using separate backcross programs and gene pyramiding approaches assisted by molecular markers, the BIOAGRO/UFV Common Bean Breeding Program was able to create advanced carioca-type common bean lines with wide and potentially durable resistance to *U. appendiculatus*. These lines show the same resistance spectra present in the donor parents and no yield penalty in relation to the commercial cultivars used as recurrent parents (Souza *et al.* 2005b, 2009).

The yield components of the common bean lines developed by the BIOAGRO/UFV are evaluated in at least two distinct growing seasons. The evaluation assays include resistant control lines and high yielding reference cultivars widely planted in Brazil. Randomized complete block or lattice designs with three repetitions (plots) are used. Each plot consists of two rows each 2.0 m long, spaced by 0.5 m, with about 15 seeds per meter. Fertilizer application is done according to the recommendations for dry bean cultivation

in the State of Minas Gerais. Harvest of the whole plot is done manually. The yield components usually evaluated are: grain yield, determined for each plot and expressed as g/plot or Kg/ha; plant height, the mean height (in centimeters) of the plants at stage R8 in each plot; mean number of seeds per pod in each plot; mean number of pods per plant in each plot (Ragagnin et al. 2009).

The lines selected based on molecular markers and yield components are also tested against the pathogen under greenhouse (specific races) and field (natural incidence) conditions. The lines with resistance spectra similar to those of the donor parents and as productive as the recurrent parents and control cultivars are evaluated in different regions by the Brazilian Bean Assay Network. If the superior agronomic performance of these lines is confirmed they are recommended as new cultivars. In addition, the resistance gene pyramid can be transferred to other Brazilian commercial cultivars.

7. Perspectives

Yield and disease resistance are the main foci of common bean breeders throughout the world. The large number of pathogens affecting this crop is one of the main causes of the low yield observed in many bean growing regions.

The concept of gene pyramiding assisted by molecular markers has been successfully used by the BIOAGRO/UFV Common Bean Breeding Program to create lines with wide and potentially durable resistance. Separate backcross programs assisted by molecular markers have allowed the individual introgression of resistance genes in recurrent cultivars after only three or four backcross cycles. The lines obtained have shown the same resistance spectra present in the donor parents. Evaluation of yield components of the advanced lines have demonstrated that no yield penalty in relation to recurrent cultivars was observed after the pyramiding process (Faleiro *et al.* 2004; Ragagnin *et al.* 2009; Souza *et al.* 2005b, 2009).

To be effective, the gene pyramiding strategy must be a continuous effort. Permanent monitoring for the presence of new virulent races in the field and search for new resistance sources are inherent steps in this breeding strategy.

A significant variability of *U. appendiculatus* isolates has been observed in the Brazilian state of Minas Gerais (Faleiro *et al.* 1998, 1999a; Souza *et al.* 2005a, 2007a). Isolates collected by these authors have been used to support the bean breeding programs of different research institutions in Brazil. As a result of this work, it was possible to select resistant cultivars, like 'Ouro Negro' (Faleiro *et al.* 2000b; Vieira *et al.* 2005), and to develop other cultivars and advanced lines harboring rust resistance genes (Faleiro *et al.* 2004; Ragagnin *et al.* 2009). Periodical collection, maintenance and classification of *U. appendiculatus* isolates are essential steps of the bean breeding program as these steps inform about the variability of the pathogen in a growing area over time. Thus new sources of resistance can be identified and validated with adequate monitoring and identification of the most frequent races of the pathogen (Souza *et al.* 2007a).

Frequent monitoring of prevalent races of pathogens presenting high variability is very helpful in studies involving genetic dynamics, as the geographical origin of the isolates may provide indirect evidences of their diversity, because of their adaptation to different environment conditions.

In this way, a consistent effort of monitoring and classification of *U. appendiculatus* isolates has been established in Minas Gerais, Brazil, to attend the breeding programs of the "Minas Gerais Common Bean Covenant". This agreement includes four instituitions: Universidade Federal de Viçosa (UFV), Universidade Federal de Lavras (UFLA), Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), and Embrapa Arroz e Feijão. This effort also includes analyses of the geographical variability of *U. appendiculatus* in association with molecular markers.

Acknowledgements

The BIOAGRO/UFV Common Bean Breeding Program is supported by grants from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais) – Brazilian Government. CNPq provided a fellowship to the first author.

References

Acevedo M, Alleyne AT, Fenton J, Steadman JR (2004) Phenotypic and genotypic variation in *Uromyces appendiculatus* from regions of commercial production and centers of common bean domestication. *Annual Report of the Bean Improvement Cooperative* 47, 115-116

Acevedo M, Steadman JR, Rosas JC, Venegas J (2008) Co-evolution of the bean rust pathogen *Uromyces appendiculatus* with its wild, weedy and domesticated hosts (*Phaseolus* spp.) at a center of diversity. *Annual Report of the Bean Improvement Cooperative* 51, 22-23

Alleyne AT, Steadman JR, Eskridge KM (2008) Monitoring changing virulence patterns of *Uromyces appendiculatus* in the resistant pinto bean cultivar Olathe by rep-PCR. *European Journal of Plant Pathololology* 122, 315-319

Alzate-Marin AL, Costa MR, Arruda KM, Barros EG, Moreira MA (2003) Characterization of the anthracnose resistance gene present in Ouro Negro (Honduras 35) common bean cultivar. *Euphytica* 133, 165-169

Alzate-Marin AL, Souza TLPO, Ragagnin VA, Moreira MA, Barros EG (2004) Allelism tests between the rust resistance gene present in common bean cultivar Ouro Negro and genes *Ur-5* and *Ur-11*. *Journal of Phytopathology* 152, 60-64

Alzate-Marin AL, Cervigni GDL, Moreira MA, Barros EG (2005) Seleção assistida por marcadores moleculares visando ao desenvolvimento de plantas resistentes a doenças, com ênfase em feijoeiro e soja. *Fitopatologia Brasileira* 30, 333-342

Andersen JW, Story L, Sieling B, Chen WJL, Petro MS, Story J (1984) Hypocholesterolemic effects of oat-bran or bean intake for hypercholesterolemic men. *American Journal of Clinical Nutrition* 40, 1146-1155

Araya CM, Alleyne AT, Steadman JR, Eskridge KM, Coyne DP (2004) Phenotypic and genotypic characterization of *Uromyces appendiculatus* from *Phaseolus vulgaris* in the Americas. *Plant Disease* 88, 830-836

Augustin E, Coyne DP, Schuster ML (1972) Inheritance of resistance in *Phaseolus vulgaris* to *Uromyces phaseoli typica* Brazilian rust race B11 and of plant habit. *Journal of the American Society for Horticultural Science* 97, 526-529

Awale HE, Ismail SM, Vallejo VA, Kelly JD (2008) SQ4 SCAR marker linked to the *Co-2* gene on B11 appears to be linked to the *Ur-11* gene. *Annual Report of the Bean Improvement Cooperative* 51, 174-175

Ballantyne BJ (1978) The genetic bases of resistance to rust, caused by *Uromyces appendiculatus* in beans (*Phaseolus vulgaris*). PhD thesis, University of Sydney, Sydney, 262 pp. Available online: http://www.css.msu.edu/bic/PDF/Ballantyne%20Thesis%20Bean%20Rust.pdf

Bigirimana J, Höfte M (2001) Bean anthracnose: inoculation methods and influence of plant stage on resistance of *Phaseolus vulgaris* cultivars. *Journal of Phytopathology* 149, 403-408

Boone WE, Stavely JR, Weeden NF (1999) Development of a sequence-tagged site (STS) marker for *Ur-11*, a gene conferring resistance to the bean rust fungus, *Uromyces appendiculatus*. *Annual Report of the Bean Improvement Cooperative* 42, 33-34

Braithwaite KS, Manners JM, Erwin JAG, Maclean DJ (1994) DNA markers reveal hybrids between two diverse background genotypes in Australian collections of the bean rust fungus *Uromyces appendiculatus. Australian Journal Botany* 42, 255-267

Brondy U, Nelson RR, Gregory LV (1986) The residual and interactive expression of "defeated" wheat stem rust resistance genes. *Phytopatology* 76, 546-549

Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J (2003) Beans (*Phaseolus* spp.) - model food legumes. *Plant and Soil* 252, 55-128

Carrijo IV, Chaves GM, Pereira AA (1980) Reação de vinte e cinco variedades de *Phaseolus vulgaris* a trinta e nove raças fisiológicas de *Uromyces phaseoli* var. *typica* Arth. em condições de casa de vegetação. *Fitopatologia Brasileira* 5, 245-255

Christ BJ, Groth JV (1982a) Inheritance of resistance in three cultivars of beans to the bean rust pathogen and the interaction of virulence and resistance genes. *Phytopathology* 72, 771-773

Christ BJ, Groth JV (1982b) Inheritance of virulence to three bean cultivars in three isolates of the bean rust pathogen. *Phytopathology* 72, 767-770

Corrêa RX, Costa MR, Good-God PI, Ragagnin VA, Faleiro FG, Moreira MA, Barros EG (2000) Sequence characterized amplified regions linked to rust resistance genes in the common bean. *Crop Science* 40, 804-807

Edington BR, Shanahan PE, Rijkenberg FHJ (1994) Breeding for partial resistance in dry beans (*Phaseolus vulgaris*) to bean rust (*Uromyces appendiculatus*). *Annals of Applied Biology* 124, 341-350

Faleiro FG, Ragagnin VA, Mesquita AGG, Vinhadelli WS, Paula-Junior TJ, Moreira MA, Barros EG (1998) Diversidade genética de isolados de *Uromyces appendiculatus* utilizando marcadores moleculares RAPD. *Fitopatologia Brasileira* 23, 386-390

Faleiro FG, Vinhadelli WS, Ragagnin VA, Zambolim L, Paula-Junior TJ, Moreira MA, Barros EG (1999a) Identificação de raças fisiologicas de *Uromyces appendiculatus* no estado de Minas Gerais, Brasil. *Fitopatologia Brasileira* 24, 166-169

Faleiro FG, Zambolim L, Vinhadelli WS, Ragagnin VA, Paula-Junior TJ, Moreira MA, Barros EG (1999b) Sistema simplificado para nomenclatura e classificação de raças fisiológicas de *Uromyces appendiculatus*. *Fitopatologia Brasileira* 24, 540-545

Faleiro FG, Vinhadelli WS, Ragagnin VA, Corrêa R, Moreira MA, Barros EG (2000a) RAPD markers linked to a block of genes conferring rust resistance to the common bean. *Genetic and Molecular Biology* 23, 399-402

Faleiro FG, Ragagnin VA, Corrêa R, Vinhadelli WS, Moreira MA, Barros EG (2000b) Ligação gênica da resistência à ferrugem e à antracnose na variedade de feijão Ouro Negro. *Revista Ceres* 47, 375-382

Faleiro FG, Ragagnin VA, Schuster I, Corrêa R, Good-God PI, Brommonshenkel S, Moreira MA, Barros EG (2003) Mapeamento de genes de resistência do feijoeiro à ferrugem, antracnose e mancha-angular usando marcadores RAPD. *Fitopatologia Brasileira* 28, 59-66

Faleiro FG, Ragagnin VA, Moreira MA, Barros EG (2004) Use of molecular markers to accelerate the breeding of common bean lines resistant to rust and anthracnose. *Euphytica* 183, 213-218

Finke ML, Coyne DP, Steadman JR (1986) The inheritance and association of resistance to rust, common bacterial blight, plant habit and foliar abnormalities in *Phaseolus vulgaris* L. *Euphytica* 35, 969-982

Flor HH (1971) Current status of gene-for-gene concept. *Annual Review of Phytopathology* 9, 275-296

Freyre R, Skroch PW, Geffroy V, Adam-Blondon AF, Shirmohamadali A, Johnson WC, Llaca V, Nodari RO, Pereira PA, Tsai SM, Tohme J, Dron M, Nienhuis J, Vallejos CE, 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, 847-856

Freytag GF, Debouck DG (2002) Taxonomy, distribution, and ecology of the genus *Phaseolus* (Leguminosae-Papilionoideae) in North America, Mexico and Central America. Botanical Research Institute of Texas, Ft. Worth, 300 pp

Geffroy V, Sicard D, Oliveira JCF, Sevignac M, Cohen S, Gepts P, Neema C, Langin T, Dron M (1999) Identification of an ancestral resistance gene cluster involved in the coevolution process between *Phaseolus vulgaris* and its fungal pathogen *Colletotrichum lindemuthianum*. *Molecular Plant-Microbe Interaction* 12, 774-784

Gepts P, Osborn TC, Rashka K, Bliss FA (1986) Phaseolin-protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris*): evidence for multiple centers of domestication. *Economic Botanic* 40, 451-468

Grafton KF, Weiser GC, Littlefield LJ, Stavely JR (1985) Inheritance of resistance to two races of leaf rust in dry edible bean. *Crop Science* 25, 537-539

Groth JV, McCain JW, Roelfs AP (1995) Virulence and isoenzyme diversity of sexual versus asexual collections of *Uromyces appendiculatus* (bean rust fungus). *Heredity* 75, 234-242

Haley SD, Miklas PN, Stavely JR, Byrum J, Kelly JD (1993) Identification of RAPD markers linked to a major rust resistance gene block in common bean. *Theoretical and Applied Genetics* 86, 505-512

Haley SD, Afanador LK, Miklas PN, Stavely JR, Kelly JD (1994) Heterogeneous inbred populations are useful as sources of near-isogenic lines for RAPD marker localization. *Theoretical and Applied Genetics* 88, 337-342

Hangen L, Bennink MR (2003) Consumption of black beans and navy beans (*Phaseolus vulgaris*) reduced azoxymethane-induced colon cancer in rats. *Nutrition and Cancer* 44, 60-65

Huang N, Angeles ER, Domingo J, Magpantay G, Singh S, Zhang G, Kumaravadiel N, Bennett J, Khush GS (1997) Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theoretical and Applied Genetics* 95, 313-320

Jochua CN, Steadman JR, Amane MIV, Fenton JG (2004) Pathotype variation and sources of resistance to the common bean rust pathogen in Southern Mozambique. *Annual Report of the Bean Improvement Cooperative* 47, 113-114

Johnson E, Miklas PN, Stavely JR, Martinez-Cruzado JC (1995) Coupling- and repulsion-phase RAPDs for marker-assisted selection of PI 181996 rust resistance in common bean. *Theoretical and Applied Genetics* 90, 659-664

Johnson R (1984) A critical analysis of durable resistance. *Annual Review of Phytopatology* 22, 309-330

Jung G, Coyne DP, Skroch P, Nienhuis J, Arnaud-Santana E, Bokosi J, Ariyarathne H, Steadman J, Beaver J, Kaeppler S (1996) Molecular markers associated with plant architecture and resistance to common blight, web blight, and rust in common beans. *Journal of the American Society for Horticultural Science* 121, 794-803

Jung G, Coyne DP, Bokosi J, Steadman J, Nienhuis J (1998) Mapping genes for specific and adult plant resistance to rust and abaxial leaf pubescence and their genetic relationship using random amplified polymorphic DNA (RAPD) markers in common bean. *Journal of the American Society for Horticultural Science* 123, 859-863

Kelly JD, Afanador L, Haley SS (1995) Pyramiding genes resistance to bean common mosaic virus. *Euphytica* 82, 207-212

Kelly JD, Stavely JR, Miklas PN (1996) Proposed symbols for rust resistance genes. *Annual Report of the Bean Improvement Cooperative* 39, 25-31

Kelly JD, Gepts P, Miklas PN, Coyne DP (2003) Tagging and mapping of genes and QTL and molecular-marker assisted selection for traits of economic importance in bean and cowpea. *Field Crops Research* 82, 135-154

Kelly JD, Vallejo VA (2004) A comprehensive review of the major genes conditioning resistance to anthracnose in common bean. *HortScience* 39, 1196-1207

Liebenberg MM, Pretorius ZA (2004) Proposal for designation of a rust resistance gene in the large-seeded cultivar Kranskop. *Annual Report of the Bean Improvement Cooperative* 47, 255-256

Liebenberg MM, Mienie CMS, Pretorius AZ (2006) The occurrence of rust resistance gene *Ur-13* in common bean cultivars and lines. *Euphytica* 150, 365-386

Liebenberg MM, Madubanya LA, Mienie CMS (2008) A new application for SCAR marker $SAE19_{890}$. *Annual Report of the Bean Improvement Cooperative* 51, 90-91

Linde DC, Groth JV, Roelfs AP (1990a) The genetic basis of isozyme variation in the bean rust fungus (*Uromyces appendiculatus*). *Journal of Heredity* 81, 134-138

Linde DC, Groth JV, Roelfs AP (1990b) Comparison of isozyme and virulence diversity patterns in the bean rust fungus *Uromyces appendiculatus*. *Phytopathology* 80, 141-147

Lindgren DT, Escridge KM, Steadman JR, Schaaf DM (1995) A model for dry bean yield loss due to rust. *HortTechnology* 5, 35-37

Lu TH, Groth JV (1988) Isozymes detection and variation in *Uromyces appendiculatus*. *Canadian Journal of Botany* 66, 885-890

Maclean DJ, Braithwaite KS, Irwin JAG, Manners JM, Groth JV (1995) Random amplified polymorphic DNA reveals relationships among diverse genotypes in Australian and American collections of *Uromyces appendiculatus*. *Phytopathology* 85, 757-765

McCain JW, Groth JV, Roelfs AP (1992) Inter and intrapopulation isozymes variation in collections from sexually reproducing populations of the bean rust fungus, *Uromyces appendiculatus*. *Mycologia* 84, 329-340

McClean P, Myers J (1990) Pedigrees of dry bean cultivars, lines and PIs. *Annual Report of the Bean Improvement Cooperative* 33, xxv-xxx

Melloto M, Kelly JD (1998) SCAR markers linked to major disease resistance genes in common bean. *Annual Report of the Bean Improvement Cooperative* 41, 64-65

Michelmore R (1995) Molecular approaches to manipulation of diseases resistance genes. *Annual Review of Phytopathology* 15, 393-427

Mienie CMS, Naidoo R, Liebenberg MM (2004) Conversion of the RAPD marker for *Ur-4* to a co-dominant SCAR marker $SA14_{1079/800}$. *Annual Report of the Bean Improvement Cooperative* 47, 261-262

Mienie CMS, Liebenberg MM, Pretorius ZA, Miklas PN (2005) SCAR markers linked to the common bean rust resistance gene *Ur-13. Theoretical and Applied Genetics* 111, 972-979

Miklas PN, Stavely JR, Kelly JD (1993) Identification and potential use of a molecular marker for rust resistance in common bean. *Theoretical and Applied Genetics* 85, 745-749

Miklas PN, Stone V, Daly MJ, Stavely JR, Steadman JR, Bassett MJ, Delorme R, Beaver JS (2000) Bacterial, fungal, and viral disease resistance loci mapped in a recombinant inbred common bean population (Dorado/XAN 176). *Journal of the American Society for Horticultural Science* 125, 476-481

Miklas PN, Pastor-Corrales MA, Jung G, Coyne DP, Kelly JD, McClean PE, Gepts P (2002) Comprehensive linkage map of bean rust resistance genes. *Annual Report of the Bean Improvement Cooperative* 45, 125-129

Miklas PN, Kelly JD, Beebe SE, Blair MW (2006) Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. *Euphytica* 147, 105-131

Mmbaga MT, Steadman JR, Stavely JR (1996) The use of host resistance in disease management of rust in common bean. *Integrated Pest Management Reviews* 1, 191-200

Mora-Nuñes OA, Vieira C, Zambolim L (1992) Variedades diferenciadoras de feijão para identificação de raças fisiológicas de *Uromyces phaseoli* var. *typica* Arth. *Revista Ceres* 39, 391-404

Nelson RR (1979) The evolution of parasitic fitness. In: Horsfall JG, Cowling EB (Eds) Plant Disease, an Advanced Treatise, New York Academic Press, New York, pp 23-46

Nemchinova YP, Stavely JR (1998) Development of SCAR primers for the *Ur-3* rust resistance gene in common bean. *Phytopathology* 88, S67

Openshaw SJ, Jarboe SG, Beavis WD (1994) Marker-assisted selection in backcross breeding. In: Lower R (Ed) ASHS/CSSA Joint Plant Breeding Symposium on Analysis of Molecular Marker Data, Oregon State University, Corvallis, pp 41-43

Pachico D (1989) Trends in world common bean production. In: Schwartz HF, Pastor-Corrales MA (Eds) Bean Production Problems in the Tropics (2nd Edn) Centro Internacional de Agricultura Tropical Press, Cali, pp 1-8

Park SO, Coyne DP, Steadman JR (1999a) Molecular markers linked to the *Ur-7* gene conferring specific resistance to rust in common bean. *Annual Report of the Bean Improvement Cooperative* 42, 31-32

Park SO, Coyne DP, Bokosi JM, Steadman JR (1999b) Molecular markers linked to genes for specific rust resistance and indeterminate growth habit in common bean. *Euphytica* 105, 133-141

Park SO, Crosby KM, Coyne DP, Steadman JR (2003a) Development of a SCAR marker linked to the *Ur-6* gene for specific rust resistance in common bean. *Annual Report of the Bean Improvement Cooperative* 46, 189-190

Park SO, Coyne DP, Steadman JR, Skroch PW (2003b) Mapping of the *Ur-7* gene for specific resistance to rust in common bean. *Crop Science* 43, 1470-1476

Park SO, Coyne DP, Steadman JR, Crosby KM, Brick MA (2004) RAPD and SCAR markers linked to the *Ur-6* Andean gene controlling specific rust resistance in common bean. *Crop Science* 44, 1799-1807

Park SO, Steadman JR, Coyne DP, Crosby M (2008) Development of a coupling-phase SCAR marker linked to the *Ur-7* rust resistance gene and its occurrence in diverse common bean lines. *Crop Science* 48, 357-363

Pastor-Corrales MA (2001) The reaction of 19 bean rust differential cultivars to 94 races of *Uromyces appendiculatus* and the implication for the development of rust resistance cultivars. *Annual Report of the Bean Improvement Cooperative* 44, 103-104

Pastor-Corrales MA, Stavely JR (2002) Using races of the common bean rust pathogen to detect resistance genes in *Phaseolus vulgaris*. *Annual Report of the Bean Improvement Cooperative* 45, 78-79

Pastor-Corrales MA (2005) Inheritance of resistance in PI260418 an Andean bean resistant to most races of the bean rust pathogen. *Annual Report of the Bean Improvement Cooperative* 48, 134-135

Pastor-Corrales MA, Pereira APA, Lewers K, Brondani RV, Buso GC, Ferreira MA, Martins WS (2008) Identification of SSR markers linked to rust resistance in Andean common bean PI 260418. *Annual Report of the Bean Improvement Cooperative* 51, 46-47

Pedersen WL, Leath S (1988) Pyramiding major genes for resistance to maintain residual effects. *Annual Review of Phytopatology* 26, 369-378

Pedrosa A, Vallejos CE, Bachmair A, Schweizer D (2003) Integration of common bean (*Phaseolus vulgaris* L.) linkage and chromosomal maps. *Theoretical and Applied Genetics* 106, 205-212

Pedrosa-Harand A, de Almeida CCS, Mosiolek M, Blair MW, Schweizer D, Guerra M (2006) Extensive ribosomal DNA amplification during Andean common bean (*Phaseolus vulgaris* L.) evolution. *Theoretical and Applied Genetics* 112, 924-933

Pedrosa-Harand A, Porch T, Gepts P (2008) Standard nomenclature for common bean chromosomes and linkage groups. *Annual Report of the Bean Improvement Cooperative* 51, 106-107

Queiroz VT, Sousa CS, Souza TLPO, Costa MR, Sanglard DA, Ragagnin VA, Barros EG, Moreira MA (2004) SCAR marker linked to the common bean rust resistance gene *Ur-11*. *Annual Report of the Bean Improvement Cooperative* 47, 271-272

Ragagnin VA, Souza TLPO, Sanglard DA, Arruda KMA, Costa MR, Alzate-Marin AL, Carneiro JES, Moreira MA, Barros EG (2009) Development and agronomic performance of common bean lines simultaneously resistant to anthracnose, angular leaf spot and rust. *Plant Breeding* 128, 156-163.

Rasmussen JB, Grafton KF, Gross PL, Donohue CM (2002) Genetics of rust resistance in Compuesto Negro Chilmaltenango (CNC). *Annual Report of the Bean Improvement Cooperative* 45, 94-95

Sandlin CM, Steadman JR, Araya CM, Coyne DP (1999) Isolates of *Uromyces appendiculatus* with specific virulence to landraces of *Phaseolus vulgaris* of Andean origin. *Plant Disease* 83, 108-113

Santos SC, Rios GP (2000) Identificação de raças fisiológicas de *Uromyces appendiculatus* nos Estados de Goiás, Rio Grande do Sul e Santa Catarina. *Fitopatologia Brasileira* 25, 607-611

Sayler RJ, Ewing JD, McClean PE (1995) Monogenic and epistatic resistance to bean rust infection in common bean. *Physiological and Molecular Plant Pathology* 47, 173-184

Schafer JF, Roelfs AP (1985) Estimated relation between numbers of urediniospores of *Piccinia graminis* f. sp. *tritici* and rates of occurrence of virulence. *Phytopathology* 75, 749-750

Singh SP, Gepts P, Debouck DG (1991a) Races of common bean (*Phaseolus vulgaris*, Fabaceae). *Economic Botany* 45, 379-396

Singh SP, Nodari R, Gepts P (1991b) Genetic diversity in cultivated common bean: I. Allozymes. *Crop Science* 31, 19-23

Singh S, Sidhu JS, Huang N, Vikal Y, Li Z, Brar DS, Dhaliwal HS, Khush GS (2001) Pyramiding three bacterial blight resistance genes (*xa5*, *xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR106. *Theoretical and Applied Genetics* 102, 1011- 1015

Souza TLPO, Alzate-Marin AL, Moreira MA, Barros EG (2002) Use of Belmidak RR-3 as a source for rust resistance in Central Brazil. *Annual Report of the Bean Improvement Cooperative* 45, 140-141

Souza TLPO, Alzate-Marin AL, Moreira MA, Barros EG (2005a) Análise da variabilidade patogênica de *Uromyces appendiculatus* em algumas regiões brasileiras. *Fitopatologia Brasileira* 30, 143-149

Souza TLPO, Sanglard D, Ragagnin VA, Alzate-Marin AL, Moreira MA, Barros EG (2005b) Development of "carioca-type" common bean lines resistant to rust with the aid of molecular markers. *Annual Report of the Bean Improvement Cooperative* 48, 138-139

Souza TLPO, Ragagnin VA, Ribeiro LF, Sanglard D, Moreira MA, Barros EG (2005c) Alternative inoculation method for evaluating common bean reaction to *Uromyces appendiculatus*. *Annual Report of the Bean Improvement Cooperative* 48, 136-137

Souza TLPO, Ragagnin VA, Melo CLP, Arruda KMA, Carneiro JES, Moreira MA, Barros EG (2005d) Phenotypic and molecular characterization of cultivar BRSMG-Talismã regarding the principal common bean pathogens. *Crop Breeding and Applied Biotechnology* 5, 247-252

Souza TLPO, Ragagnin VA, Sanglard D, Moreira MA, Barros EG (2007a) Identification of races of selected isolates of *Uromyces appendiculatus* from Minas Gerais (Brazil) based on the new international classification system. *Fitopatologia Brasileira* 32, 104-109

Souza TLPO, Alzate-Marin AL, Dessaune SN, Nunes ES, Queiroz VT, Moreira MA, Barros EG (2007b) Inheritance study and validation of SCAR molecular marker for rust resistance in common bean. *Crop Breeding and Applied Biotechnology* 7, 11-15

Souza TLPO, Dessaune SN, Sanglard DA, Moreira MA, Barros EG (2007c) Rust resistance gene present in common bean cultivar Ouro Negro (*Ur-ON*) does not correspond to *Ur-3*$^{+}$. *Annual Report of the Bean Improvement Cooperative* 50, 119-120

Souza TLPO, Alzate-Marin AL, Faleiro FG, Barros EG (2008) Pathosystem common bean–*Uromyces appendiculatus*: host resistance, pathogen specialization, and breeding for rust resistance. *Pest Technology* 2, 56-69

Souza TLPO, Dessaune SN, Ragagnin VA, Carneiro JES, Moreira MA, Barros EG (2009) Rust resistance gene pyramiding in the common bean assisted by molecular markers. *Plant and Animal Genome XVII Conference*. Available online: http://www.intl-pag.org/17/abstracts/P03f_PAGXVII_185.html

Stavely JR (1984a) Pathogenic specialization in *Uromyces phaseoli* in the United States and rust resistance in beans. *Plant Disease* 68, 95-99

Stavely JR (1984b) Genetics of resistance to *Uromyces phaseoli* in a *Phaseolus vulgaris* line resistant to must races of the pathogen. *Phytopathology* 74, 339-344

Stavely JR (1998) Recombination of two major dominant rust resistance genes that are tightly linked in repulsion. *Annual Report of the Bean Improvement Cooperative* 41, 17-18

Stavely JR, Freytag GF, Steadman JR, Schwartz HF (1983) The 1983 Bean Rust Workshop. *Annual Report of the Bean Improvement Cooperative* 26, iv-vi

Stavely JR, Grafton KF (1985) Genetics of resistance to eight races of *Uromyces appendiculatus* in *Phaseolus vulgaris* cultivar Mexico 235. *Phytopathology* 75, 1310

Stavely JR, Pastor-Corrales MA (1989) Rust. In: Schwartz HF, Pastor-Corrales MA (Eds) Bean Production Problems in the Tropics (2nd Edn), Centro Internacional de Agricultura Tropical Press, Cali, pp 159-194

Steadman JR, Pastor-Corrales MA, Beaver JS (2002) An overview of the 3rd Bean Rust and 2nd Bean Common Bacterial Blight International Workshops, March 4-8, 2002, Pietermaritzburg, South Africa. *Annual Report of the Bean Improvement Cooperative* 45, 120-125

Thrall PH, Burdon JJ (2003) Evolution of virulence in a plant host-pathogen metapopulation. *Science* 299, 1735-1737

Toenniessen GH, O´Toole JC, Devries J (2003) Advances in plant biotechnology and its adoption in developing countries. *Current Opinion in Plant Biology* 6, 191-198

Vieira C, Borém A, Ramalho MAP, Carneiro JES (2005) Melhoramento do feijão. In: Borém A (ed.) Melhoramento de Espécies Cultivadas, Universidade Federal de Viçosa Press, pp 301-391

Webster DMN, Ainsworth PM (1988) Inheritance and stability of a small pustule reaction of snap beans to *Uromyces appendiculatus*. *Journal of the American Society for Horticultural Science* 113, 938-940

Wortmann CS, Kirkby RA, Eledu CKA, Allen DJ (1998) Atlas of Common Bean (*Phaseolus vulgaris* L.) Production in Africa, Centro Internacional de Agricultura Tropical Press, Cali, 17 pp

Wright EM, Awale HE, Kelly JD (2008) Use of TRAP markers to map resistance to a new race of common bean rust in Michigan. *Annual Report of the Bean Improvement Cooperative* 51, 210-211

Yoshimura S, Yoshimura A, Iwata N, McCouch SR, Abenes ML, Baraoidan MR, Mew TW, Nelson RJ (1995) Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. *Molecular Breeding* 1, 375-387

Zaiter HZ, Coyne DP, Steadman JR (1989) Inheritance of resistance to a rust isolate in beans. *Annual Report of the Bean Improvement Cooperative* 32, 126-127

Characterization of the Rust Resistance Gene Present in the Common Bean Cultivar 'Ouro Negro', the Main Rust Resistance Source Used in Brazil

Abstract: Identification and characterization of new resistance sources are basic steps for plant breeding programs aiming to develop modern cultivars with effective resistance to pathogens. The Mesoamerican black seeded common bean cultivar 'Ouro Negro' is the main rust resistance (RR) source used in Brazil. It is resistant to *U. appendiculatus* isolates from different bean growing areas of the country. However, the RR gene present in 'Ouro Negro', temporally named as *Ur-ON*, has not been fully characterized yet. The main goal of the present work was to characterize the gene *Ur-ON*. We have compared the RR spectrum presented by 'Ouro Negro' with those of other bean lines harboring known RR genes when inoculated with nine selected races of *U. appendiculatus*. In addition, all bean lines were screened with molecular markers linked to *Ur-ON* aiming to identify additional evidence for the presence of alleles for this locus in the screened RR sources. Finally, we tested the allelic relationships of *Ur-ON* with already characterized RR genes from lines resistant to at least one race of the pathogen. We also accomplished allelism tests between 'Ouro Negro' and 'CNC' and 'CSW 643', important RR sources in Brazil harboring unnamed RR genes. The results showed that the major dominant gene conditioning RR in 'Ouro Negro' is positioned at a locus distinct from those with which it was compared. We propose that this gene – or complex gene locus – is unique and be designated *Ur-14*.

Key words: Allelism test – molecular markers – resistance genes – *Uromyces appendiculatus* – *Phaseolus vulgaris*

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is the main source of vegetable protein, minerals, and vitamins for a large fraction of the Brazilians. The economical, nutritional, and social value of the bean crop to millions of people in many other countries of Latin America, and Eastern and Southern Africa is unquestionable (Wortmann et al. 1998; Broughton et al. 2003).

Rust is a bean fungal disease incited by *Uromyces appendiculatus* F. Strauss (sin. *U. phaseoli* G. Winter). Although this fungus is distributed worldwide, it effectively causes major production problems in humid tropical and subtropical areas and periodic severe epidemics in humid temperate regions (Souza et al. 2008). Severe bean rust epidemics have been reported in Latin America, Australia, China, the United States, and some areas of Europe (Stavely and Pastor-Corrales 1989). The major losses in Brazil occur in south, southeast and central areas, including the states of Paraná, Rio Grande do Sul, Santa Catarina, Minas Gerais, São Paulo, and Goiás (Souza et al. 2005a). Recommended disease management practices for bean rust control include crop rotation, planting within recommended dates, growing resistant cultivars, and timely spraying of fungicides (Mmbaga et al. 1996). Chemical products are hardly used due to the need of specific technical knowledge and also because they increase the production costs. Besides, chemical products are often an ecotoxicological concern. The use of resistant cultivars is an efficient, safe, and inexpensive alternative accessible to bean growers for the effective rust control.

Thirteen dominant rust resistance (RR) genes were identified to date (*Ur-1*–to–*Ur-13*). These genes are named according to the nomenclature proposed by Kelly et al. (1996). In addition to them, other important unnamed genes have been identified, such as those present in the common bean lines 'BAC6', 'CNC', 'CSW 643', 'Dorado', 'Ouro Negro', and 'PI 260418' (reviewed in Souza et al. 2008). The appropriate characterization of RR genes from different origins (Mesoamerican and Andean) is essential for the future

of the common bean breeding aiming resistance to *U. appendiculatus*, because it should broaden the spectra of the genes currently used (Liebenberg et al. 2006). Besides, new sources of resistance must always be identified and characterized because of the high virulence diversity and variability present in the pathogen population (Araya et al. 2004; Liebenberg et al. 2006; Souza et al. 2007). The Mesoamerican cultivar 'Ouro Negro' was developed by CIAT (Cali, Colombia) and initially named 'Honduras 35', in 1991 this bean line was introduced in Brazil as cultivar 'Ouro Negro' (Araújo et al. 1991). This black seeded common bean line showed resistance to several isolates of *U. appendiculatus* in Brazil (Faleiro et al. 1999, 2000a; Souza et al. 2007) and in the USA (Alzate-Marin et al. 2004). In addition, it is resistant to races 73, 81, and 89 of *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib., the causal agent of bean anthracnose (Alzate-Marin et al. 2003), and resistant to races 63-63 and 63-23 of *Pseudocercospora griseola* (Sacc.) Crous & U. Braun, the causal agent of angular leaf spot (Sanglard et al. 2009).

Previous works conducted by our research group showed that 'Ouro Negro' possesses single resistance genes to rust and anthracnose located 12.3 cM apart on the same linkage group (Faleiro et al. 2000b). The independence of the anthracnose resistance gene in 'Ouro Negro' has been proven and it was designated *Co-10* (Alzate-Marin et al. 2003). Information is lacking on the independence of the RR gene in this cultivar, which has been temporary named as *Ur-OuroNegro* or *Ur-ON*. It is the principal RR gene used in Brazil for breeding purposes (Faleiro et al. 2004; Souza et al. 2008; Ragagnin et al. 2009).

The main goal of the present work was to characterize the Mesoamerican RR gene *Ur-ON*. We have done a comparative analysis of the resistance spectra presented by 'Ouro Negro' and by bean lines harboring known RR genes which were inoculated with selected Brazilian races of *U. appendiculatus*. These races are used by our breeding program at the Universidade Federal de Viçosa - UFV, Minas Gerais, Brazil, to select genotypes resistant to the pathogen. In addition, all RR sources have been

screened with molecular markers reported as linked to *Ur-ON* aiming to identify additional evidence for the presence/absence of resistance alleles for this locus in the tested bean lines. Finally, when possible, we also studied the allelic relationships of *Ur-ON* with already characterized RR genes and with the unnamed genes present in 'CNC' and 'CSW 643', important RR sources to be explored in Brazil (Souza et al. 2005a).

2. Materials and Methods

2.1. Genetic material and crosses

Seeds from cultivars 'Ouro Negro', 'US Pinto 111' (susceptible control), 'AxS 37', 'CNC', 'CSW 643', and the RR sources listed in Table 1 were provided by the Active Germplasm Bank of the BIOAGRO/UFV (Viçosa, MG, Brazil).

The crosses were performed in the greenhouse. F_1 seeds were sowed and the corresponding F_1 plants generated the F_2 and F_3 generations. 'Ouro Negro' was used as the male parent, except in the cross with 'PI181996'. To identify the true hybrids, all F_1 plants were analyzed morphologically (flower color). The F_1 plants derived from the crosses between 'Ouro Negro' and other black seeded cultivars ('PI181996', 'Mexico 309', and 'CNC') were also analyzed with RAPD markers according to Alzate-Marin et al. (1996). The presence in a F_1 plant of a PCR product which was present only in the male parent confirmed that this F_1 plant was indeed a hybrid.

2.2. *U. appendiculatus* races and inoculation

The selected Brazilian *U. appendiculatus* mono-pustule isolates used in this work were characterized and classified into physiological races by Souza et al. (2007). They are maintained in the fungal collection of the BIOAGRO/UFV Common Bean Breeding Program. To increase and keep the viability of the inoculum, the spores of the pathogen stored under low humidity at 4°C and protected from light were first inoculated on leaves of the susceptible cultivar 'US Pinto 111'. Newly generated spores were used in the inoculation assays.

Table 1. Rust resistance genes identified in common bean (*Phaseolus vulgaris* L.).

Gene	Origin[a]	Cultivar/Source	LG[b]	Note[c]
Ur-1	MA	B1627 (Gallaroy Genotype I)	?	Single dominant gene identified by Ballantyne (1978). 'Gallaroy' is derived by '643' x 'Sanilac'. *Ur-1=Ur-A.* Obsolete resistance gene supplanted for the majority of the *U. appendiculatus* races. Seed stocks for lines with this gene no longer exist.
Ur-2	MA	B2090 (Gallaroy Genotype II)	?	Single dominant gene identified by Ballantyne (1978). 'Gallaroy' is derived by '643' x 'Sanilac'. *Ur-2=Ur-B.* Obsolete resistance gene supplanted for the majority of the *U. appendiculatus* races. Seed stocks for lines with this gene no longer exist.
$Ur\text{-}2^2$	MA	AxS 37	?	Single dominant gene identified by Ballantyne (1978). Allele or closely linked to *Ur-2*. $Ur\text{-}2^2$*=Ur-E.* Important rust resistance source in Brazil (Souza et al. 2005). According to Ballantyne (1978), 'AxS 37' also harbors another resistance gene (*Ur-F*).
Ur-3	MA	Aurora	B11	Tightly linked gene complex or single dominant gene identified by Ballantyne (1978). *Ur-3=Ur-M*. 'Aurora' is a Mesoamerican differential cultivar for bean rust (Steadman et al. 2002).
$Ur\text{-}3^+$	MA	Mexico 235 and Ecuador 299	B11	Allele of the gene *Ur-3* but with different reaction profiles across a differential set of *U. appendiculatus* races (Kelly et al. 1996). 'Mexico 235' is a Mesoamerican differential cultivar for bean rust (Steadman et al. 2002).
Ur-4	A	Early Gallatin and Brown Beauty	B6	Single dominant gene identified by Ballantyne (1978). *Ur-4=Ur-C=*Up_2. 'Early Gallatin' is an Andean differential cultivar for bean rust (Steadman et al. 2002).
Ur-5	MA	Mexico 309	B4	Block of single dominant genes tightly linked in coupling identified by Stavely (1984). *Ur-5=Ur-B190*. 'Mexico 309' is a Mesoamerican differential cultivar for bean rust (Steadman et al. 2002). Important rust resistance source in Brazil (Souza et al. 2005b).
Ur-6	A	Golden Gate Wax	B11	Single dominant gene described by Ballantyne (1978) and further characterized by Grafton et al. (1985). *Ur-6=Ur-G=*Ur_a. 'Golden Gate Wax' is an Andean differential cultivar for bean rust (Steadman et al. 2002).
$Ur\text{-}6^+$	A/MA	Pinto Olathe	B11	Allele of the gene *Ur-6*. $Ur\text{-}6^+=Ur_a{}^+=Ur_c$. Andean gene in a Mesoamerican background (Kelly et al. 1996).
Ur-7	MA	Great Northern 1140	B11	Single dominant gene identified by Augustin et al. (1972). *Ur-7=*R_{B11}. 'Great Northern 1140' is a Mesoamerican differential cultivar for bean rust (Steadman et al. 2002).
Ur-8	A	U.S. #3	?	Single dominant gene identified by Christ and Groth (1982a, b). *Ur-8=Ur-US#3=*Up_1. Obsolete resistance gene supplanted for the majority of the *U. appendiculatus* races. This gene has been ignored for most bean breeders.

Table 1. Continuation...

Gene	Origin[a]	Cultivar/Source	LG[b]	Note[c]
Ur-9	A	PC-50	B1	Single dominant gene identified by Finke et al. (1986). *Ur-9=Ur$_p$*. 'PC-50' is an Andean differential cultivar for bean rust (Steadman et al. 2002).
Ur-10	A	Resisto	?	Single dominant gene identified by Webster and Ainsworth (1988) that conditions a resistance reaction of "slow rusting". *Ur-10=Ur-Resisto=URPRI*. *Ur-10* was not fully characterized and it has not been tagged nor mapped and, therefore, it is not being explored by the bean breeders.
Ur-11	MA	PI181996	B11	Block of tightly linked dominant genes identified by Stavely (1998). *Ur-11=Ur-3^2*. Tightly linked with *Ur-3*. 'PI181996' is a Mesoamerican differential cultivar for bean rust (Steadman et al. 2002). Important rust resistance source in Brazil (Souza et al. 2005b).
Ur-12	A	PC-50	B7	Single dominant gene identified by Jung et al. (1998). Conditions adult plant resistance (APR), expressed at the fourth trifoliate stage.
Ur-13	A(?)	Redlands Pioneer	B8	Single dominant gene identified by Lienberg and Pretorius (2004). *Ur-13=Ur-Red*. 'Redlands Pioneer' is an Andean differential cultivar for bean rust (Steadman et al. 2002). Despite that, the gene *Ur-13* appears to be of Mesoamerican origin (Liebenberg et al. 2006).

[a] Andean (A), and Mesoamerican (MA).

[b] Linkage groups (LG) designated as B1-to-B11 in the common bean core map (reviewed in Miklas et al. 2006) which correspond to the *P. vulgaris* chromosomes 1-to-11, respectively (Pedrosa et al. 2003; Pedrosa-Harand et al. 2006, 2008).

[c] Additional online references: Bean Improvement Cooperative - BIC, List of *Phaseolus vulgaris* L. Genes, Version 2009: http://www.css.msu.edu/bic/PDF/Bean_Genes_List_2009.pdf; and USDA/ARS National Genetic Resources Program, Germplasm Resources Information Network - GRIN: http://www.ars-grin.gov/npgs/index.html.

Inoculation was carried out when the primary leaves of the bean plants reached approximately 2/3 of their full development, about 10 days after sowing under greenhouse conditions (20 ± 5°C), as described by Souza et al. (2007). In addition to the F_2 and F_3 plants used for allelism tests, about ten plants from each RR source and control cultivar were inoculated with the pathogen for the comparative analysis of their resistance spectra. The inoculum concentration was 2.0×10^4 uredospores/mL distilled water containing Tween-20 (0.05%, v:v). The inoculum solution was sprayed on both leaf surfaces using a manual atomizer (De Vilbiss n° 15) adapted to an electric compressor. After inoculation the plants were transferred to a mist chamber (20 ± 1°C and relative humidity > 95%) where they were kept for approximately 48 h under a 12-hour light regime. In order to avoid contamination, plants inoculated with different isolates were kept in separate compartments of the mist chamber. After this period the plants were transferred to a greenhouse (20 ± 5°C), where they were kept until symptom evaluation, about 15 days after the inoculation.

2.3. Disease evaluation

Rust symptoms were determined approximately 15 days after inoculation, when about 50 % of the pustules had sporulated. The disease evaluation was carried out based on a six-degree scale proposed by Stavely et al. (1983): 1- no pustules (immunity); 2 - necrotic spots without sporulation; 3 - pustules undergoing sporulation with a diameter of < 300 μm; 4 - pustules undergoing sporulation with a diameter ranging from 300 μm to 499 μm; 5 - pustules undergoing sporulation with a diameter ranging from 500 μm to 800 μm; and 6 - pustules undergoing sporulation with a diameter of > 800 μm.

The lesions on both surfaces of the inoculated leaves were evaluated visually by at least two researchers. The common bean cultivars or individual plants from the segregating populations that predominantly presented degree 3 or lower were classified as resistant, whereas those with predominant degree 4 or higher were considered susceptible.

2.4. DNA marker analyses

Information about the SCAR and RAPD markers linked to the gene *Ur-ON* used in the molecular screening of RR sources is depicted in Table 2. Fresh leaves that had been detached from the plants before pathogen inoculation were used for DNA extraction according to Doyle and Doyle (1990). DNA amplification by the RAPD technique was according to Faleiro et al. (2000a). Amplification reactions using SCAR markers were as described by Côrrea et al. (2000). The amplified products were visualized under UV light after electrophoresis on 1.2% agarose gels containing ethidium bromide (0.2 μg/mL), immersed in a 1X sodium boric acid (SB) buffer (10 mM sodium hydroxide, pH adjusted to 8.5 with boric acid).

2.5. Chi-square analyses

The phenotypic frequencies (proportion of resistant and susceptible plants) observed in the segregating population used for allelism studies were tested for goodness-of-fit to theoretical ratios by the Chi-square (χ^2) test.

Table 2. DNA markers linked to the rust resistance gene present in the common bean cultivar 'Ouro Negro'.

Marker	Marker type	Primer sequence (5'→3')	Product size (bp)	Distance (cM)	Linkage phase	AT (^{o}C)[a]	Reference
OPX11	RAPD	GGAGCCTCAG	630	5.8 ± 1.6	Coupling	36	Faleiro et al. (2000a)
SBA08	SCAR	F-CCACAGCCGACGGAGGAG R-GCCATGTTTTTTGTCCCC	530	4.3 ± 1.2	Coupling	65	Corrêa et al. (2000)
SF10	SCAR	F-GGAAGCTTGGTGAGCAAGGA R-GGAAGCTTGGCTATGATGGT	1,072	6.0 ± 1.3	Coupling	65	Corrêa et al. (2000)

[a] AT = annealing temperature.

3. Results

3.1. Reaction of RR sources to selected races of *U. appendiculatus*

Out of the 17 RR sources tested against the pathogen (Table 3), seven were susceptible to all nine selected races of *U. appendiculatus*, so was the control cultivar 'US Pinto 111'. Among the RR sources there are the common bean lines harboring the Andean genes *Ur-4* ('Early Gallatin'), *Ur-6* ('Golden Gate Wax'), *Ur-6*$^{+}$ ('Pinto Olathe'; Andean gene in a Mesoamerican background, see Table 1), *Ur-8* ('U.S. #3'), and *Ur-10* ('Resisto'), and the Mesoamerican genes *Ur-3* ('Aurora') and *Ur-7* ('GN 1140'). The resistance spectra presented by these lines indicate that *Ur-ON* is independent from the genes *Ur-3*, *Ur-4*, *Ur-6*, *Ur-6*$^{+}$, *Ur-7*, *Ur-8*, and *Ur-10*. The other sources were resistant to at least one race of the pathogen (Table 3). For this reason they were crossed with 'Ouro Negro' aiming to study the allelic relationship between their RR genes and *Ur-ON*. Like 'Ouro Negro', the Mesoamerican cultivars 'AxS 37' (*Ur-2*^{2}), 'PI181996' (*Ur-11*), and 'CSW 643' (*Ur-?*), and the Andean cultivar 'Brown Beauty' (*Ur-4*), were resistant to all tested *U. appendiculatus* races (Table 3). These results suggest that allelism tests between these cultivars and 'Ouro Negro' are greatly needed.

3.2. Molecular screening with DNA markers linked to the RR gene present in 'Ouro Negro'

Control cultivar 'US Pinto 111', as well as cultivars 'Aurora' (*Ur-3*), 'Ecuador 299' (*Ur-3*$^{+}$), 'Early Gallatin' (*Ur-4*), 'Brown Beauty' (*Ur-4*), 'Pinto Olathe' (*Ur-6*$^{+}$), 'GN 1140' (*Ur-7*), 'Resisto' (*Ur-10*), 'PI181996' (*Ur-11*), and 'Redlands Pioneer' (*Ur-13*) did not present the DNA markers linked to *Ur-ON* (Table 3). These results suggest that the RR gene present in 'Ouro Negro' does not have allelic relationship with genes *Ur-3*, *Ur-3*$^{+}$, *Ur-4*, *Ur-6*, *Ur-7*, *Ur-10*, *Ur-11*, and *Ur-13*.

Table 3. Differential reaction of common bean rust resistance (RR) sources to selected races of *Uromyces appendiculatus* and presence/absence of DNA markers linked to the RR gene of 'Ouro Negro' (*Ur-ON*) in these lines.

Cultivar	Gene	*U. appendiculatus* race[a]									DNA marker[b]		
		21-3	29-3	29-15	53-3	53-7	53-19	61-3	63-3	63-19	OPX11	SBA08	SF10
AxS 37	*Ur-2*^{2}	R	R	R	R	R	-	R	R	R	0	1	1
Aurora	*Ur-3*	S	S	S	S	S	S	S	S	S	0	0	0
Ecuador 299	*Ur-3*$^{+}$	R	R	S	R	R	-	R	R	S	0	0	0
Mexico 235	*Ur-3*$^{+}$	R	R	S	R	R	R	R	R	R	0	1	0
Early Gallatin	*Ur-4*	S	S	S	S	S	S	S	S	S	0	0	0
Brown Beauty	*Ur-4*	R	R	R	R	R	-	R	R	R	0	0	0
Mexico 309	*Ur-5*	R	R	S	R	S	R	R	R	R	0	1	1
Golden Gate Wax	*Ur-6*	S	S	S	S	S	S	S	S	S	0	0	1
Pinto Olathe	*Ur-6*$^{+}$	S	S	S	S	S	-	S	S	S	0	0	0
GN 1140	*Ur-7*	S	S	S	S	S	S	S	S	S	0	0	0
U.S. #3	*Ur-8*	-	S	S	S	S	-	S	S	S	0	0	1
PC-50	*Ur-9*	R	S	S	R	R	R	S	S	S	0	0	1
Resisto	*Ur-10*	S	S	S	S	S	S	S	S	S	0	0	0
PI181996	*Ur-11*	R	R	R	R	R	R	R	R	R	0	0	0
Redlands Pioneer	*Ur-13*	R	R	R	R	R	R	R	S	S	0	0	0
CNC	*Ur-?*	R	R	R	R	R	S	R	R	S	0	0	1
CSW 643	*Ur-?*	R	R	R	R	R	-	R	R	R	0	1	0
US Pinto 111[c]	-	S	S	S	S	S	S	S	S	S	0	0	0
Ouro Negro	*Ur-ON*	R	R	R	R	R	R	R	R	R	1	1	1

[a] Resistant (R) or susceptible (S) reaction; not available (-).
[b] Presence (1) or absence (0) of DNA marker.
[c] Susceptible control cultivar.

Among all cultivars tested, only ‘Ouro Negro’ presented the marker OPX11 (Table 3), indicating that this is a specific marker for the gene *Ur-ON*. As shown in Table 3, marker SBA08 was present in only four of the 17 screened RR sources; the Mesoamerican cultivars ‘AxS 37’, ‘Mexico 235’, ‘Mexico 309’, and ‘CSW 643’. Marker SF10 was present in six cultivars: ‘AxS 37’, ‘Mexico 309’, ‘Golden Gate Wax’, ‘U.S. #3’, ‘PC-50’, and ‘CNC’. Only ‘AxS 37’ and ‘Mexico 309’ presented both SBA08 and SF10 markers.

3.3. Allelism tests

In the allelism tests with the F_2 populations derived from crosses between ‘Ouro Negro’ and ‘Ecuador 299’ (*Ur-3*$^+$), ‘Mexico 235’ (*Ur-3*$^+$), ‘Brown Beauty’ (*Ur-4*), ‘Mexico 309’ (*Ur-5*), ‘BelMiDak RR-3’ (*Ur-11*), ‘PI181996’ (*Ur-11*), and ‘CSW 643’ (*Ur-?*), the segregation ratios of RR were of 15 resistant to 1 susceptible plant (15R:1S) (Table 4). These results show that two independent dominant genes governed RR in each population and that the resistance gene present in ‘Ouro Negro’ segregated independently of the resistance loci *Ur-3*$^+$, *Ur-4*, *Ur-5*, *Ur-11*, and the unnamed locus (*Ur-?*) of ‘CSW 643’.

The segregation ratio of RR in the F_3 population derived from the cross between ‘Ouro Negro’ and ‘Redlands Pioneer’ (*Ur-13*) was 55R:9S (Table 4), indicating that two independent dominant genes governed resistance in this population.

In the F_2 population derived from the cross between ‘Ouro Negro’ and ‘CNC’ the segregation ratio was 63R:1S (Table 4), indicating that three independent dominant genes conditioned resistance in this cross. In the F_2 population derived from the crosses between ‘Ouro Negro’ and ‘AxS 37’ (*Ur-2*^{2}), and ‘Ouro Negro’ and ‘PC-50’ (*Ur-9*), the segregation ratios were 57R:7S (Table 4), indicating that three independent dominant genes determined resistance in these crosses and two of the genes are complementary. In this case *Ur-ON* could be one of the complementary genes.

Table 4. Crosses and races of *Uromyces appendiculatus* used for the characterization of the rust resistance gene present in the common bean cultivar 'Ouro Negro'.

Cross	Tested locus	Studied population	Race	Reaction of the parent-cross[a]	No. of plants	Expected ratio (R:S)	Observed ratio (R:S)	χ^2	*P*(%)[b]
US Pinto 111 × Ouro Negro[c]	*Ur-ON*	F_2	Mixture[d]	S × R	214	3:1	151:63	2.2492	13.37
US Pinto 111 × Ouro Negro[c]	*Ur-ON*	F_2	Multiple[d]	S × R	303	3:1	224:79	0.1859	66.63
US Pinto 111 × Ouro Negro[c]	*Ur-ON*	$F_{2:3}$	Multiple[d]	S × R	303	1:2:1	66:155:82	1.8514	39.62
Ruda × Ouro Negro[c]	*Ur-ON*	RILs	61-3	S × R	152	1:1	80:72	0.4210	51.64
Golden Gate Wax × Ouro Negro	*Ur-ON*	F_2	29-3	S × R	217	3:1	165:52	0.1244	72.43
AxS 37 × Ouro Negro	*Ur-2*^{2} × *Ur-ON*	F_2	63-3	R × R	125	57:7	114:11	0.5862	44.38
Ecuador 299 × Ouro Negro	*Ur-3*$^+$ × *Ur-ON*	F_2	21-3	R × R	142	15:1	134:8	0.0920	76.16
Mexico 235 × Ouro Negro	*Ur-3*$^+$ × *Ur-ON*	F_2	63-3	R × R	81	15:1	75:6	0.1851	66.69
Brown Beauty × Ouro Negro	*Ur-4* × *Ur-ON*	F_2	63-3	R × R	128	15:1	119:9	0.1333	71.50
Mexico 309 × Ouro Negro[e]	*Ur-5* × *Ur-ON*	F_2	29-3	R × R	208	15:1	193:15	0.3282	56.67
PC-50 × Ouro Negro	*Ur-9* × *Ur-ON*	F_2	21-3	R × R	297	57:7	263:34	0.0793	77.81
BelMiDak RR3 × Ouro Negro[e]	*Ur-11* × *Ur-ON*	F_2	29-3	R × R	64	15:1	60:4	0.0000	100.00
Ouro Negro × PI181996	*Ur-11* × *Ur-ON*	F_2	29-3	R × R	49	15:1	46:3	0.0013	97.06
Redlands Pioneer × Ouro Negro	*Ur-13* × *Ur-ON*	F_3	29-15	R × R	335	55:9	285:50	0.2063	64.96
CNC × Ouro Negro	*Ur-?* × *Ur-ON*	F_2	63-19	R × R	163	63:1	160:3	0.0818	77.47
CSW 643 × Ouro Negro	*Ur-?* × *Ur-ON*	F_2	63-19	R × R	177	15:1	166:11	0.0003	98.45

[a] Resistant (R) or susceptible (S) reaction.
[b] Percent probability (*P*) of the Chi-square (χ^2) test; α=5%.
[c] Inheritance studies previously accomplished by our research group (Corrêa et al. 2000; Faleiro et al. 2000a, 2003).
[d] Races 29-3, 53-3, 61-3, and 63-19.
[e] Allelism testes previously accomplished by our research group (Alzate-Marin et al. 2004).

4. Discussion

Inheritance studies previously developed by our research group and additional data reported by the present work show that a major dominant gene – or complex gene locus – confers resistance to rust in 'Ouro Negro' (Table 4). The temporary symbol *Ur-ON* was assigned to this gene because it had not been fully characterized. Allelism studies reported by Alzate-Marin et al. (2004) showed that *Ur-ON* does not correspond to genes *Ur-5* ('Mexico 309') or *Ur-11* ('BelMiDak RR-3') (Table 4). *Ur-ON* is also different from the Mesoamerican dominant genes *Ur-1* ('B1627') and *Ur-2* ('B2090') identified by Ballantyne (1978). These are obsolete RR genes supplanted by the majority of the *U. appendiculatus* races. Seed stocks for the lines 'B1627' and 'B2090' that harbor these genes no longer exist. In addition, *Ur-ON* does not correspond to the gene *Ur-12* ('PC-50') that conditions adult plant resistance expressed at the fourth bean trifoliate stage (Jung et al. 1998), since *Ur-ON* confers seedling rust resistance (Corrêa et al. 2000; Faleiro et al. 2000a, 2003).

In the present work we further characterized *Ur-ON* in relation to other RR genes previously identified (*Ur-2*^{2}, *Ur-3*, *Ur-3*$^+$, *Ur-4*, *Ur-5*, *Ur-6*, *Ur-6*$^+$, *Ur-7*, *Ur-8*, *Ur-9*, *Ur-10*, *Ur-11*, and *Ur-13*; see Table 1) and the genes present in the bean lines 'CNC' and 'CSW 643', important RR sources to be explored in Brazil (Souza et al. 2005a). We did a comparative analysis of the resistance spectra presented by 'Ouro Negro' and by other 17 RR sources when inoculated with nine selected Brazilian races of *U. appendiculatus*. These races are currently used by our common bean breeding program to select resistant bean lines. The resistance spectra analysis indicated that *Ur-ON* is independent from the genes *Ur-3*, *Ur-4*, *Ur-6*, *Ur-6*$^+$, *Ur-7*, *Ur-8*, and *Ur-10* because unlike *Ur-ON* these genes did not confer resistance to any of the tested *U. appendiculatus* races (Table 3).

In addition, we also screened all RR sources with molecular markers reported as linked to *Ur-ON* (Table 2) aiming to identify additional evidence for the presence/absence of resistance alleles for the locus *Ur-ON* in the

screened bean lines. The results suggested that *Ur-ON* does not have allelic relationship with the genes *Ur-3*, *Ur-3*$^{+}$, *Ur-4*, *Ur-6*, *Ur-7*, *Ur-10*, *Ur-11*, and *Ur-13* because the bean lines harboring these genes did not present any of the DNA markers (Table 3). In this assay marker OPX11 showed to be specific for *Ur-ON*. This marker can be used to select *Ur-ON* during its pyramiding with any of the RR genes listed in Table 3. Previous attempts to convert OPX11 into a SCAR marker were unsuccessful (Côrrea et al. (2000)), but we are currently pursuing this objective. SCAR markers have the advantage to be highly reproducible and can be used in a multiplex PCR analysis since they identify specific loci.

Finally, we also studied the allelic relationship of *Ur-ON* with some of the already characterized RR genes and with the unnamed genes present in 'CNC' and 'CSW 643'. The allelism tests demonstrated that 'Ouro Negro' carries one dominant gene segregating independently of *Ur-3*, *Ur-3*$^{+}$, *Ur-4*, *Ur-5*, *Ur-11*, and *Ur-13*, and of the resistance genes present in 'CNC' and 'CSW 643' (Table 4).

It was verified that the RR gene present in the common bean cultivar 'Ouro Negro', temporarilly named as *Ur-ON*, is distinct from the other RR genes already characterized. In addition, *Ur-ON* does not have allelic relationships with the resistance genes present in 'CNC' and 'CSW 643'. Based on the evidences for independence of *Ur-ON* reported in the present work, we propose that this gene is unique and be named as *Ur-14*. We have already submitted our proposal to the Genetics Committee of the Bean Improvement Cooperative - BIC (http://www.css.msu.edu/bic/) to be officially analyzed.

Acknowledgments

Financial support to this work was provided by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais); Brazilian Government. The first author was supported by a PhD fellowship from CNPq.

References

Alzate-Marin AL, Baía GS, Martins Filho S, Paula Junior TJ, Sediyama CS, Barros EG, Moreira MA (1996) Use of RAPD-cPCR to identify true hybrid plants from crosses between closely related progenitors. Rev Bras Genet 19:621–623

Alzate-Marin AL, Costa MR, Arruda KM, Barros EG, Moreira MA (2003) Characterization of the anthracnose resistance gene present in Ouro Negro (Honduras 35) common bean cultivar. Euphytica 133:165–169

Alzate-Marin AL, Souza TLPO, Ragagnin VA, Moreira MA, Barros EG (2004) Allelism tests between the rust resistance gene present in common bean cultivar Ouro Negro and genes *Ur-5* and *Ur-11*. J. Phytopathol. 152:60–64

Araújo GAA, Vieira C, Souza Filho BF (1991) 'Ouro Negro': nova variedade de feijão preto para os estados de Minas Gerais e Rio de Janeiro. EPAMIG (Boletim Técnico No. 1), Viçosa, MG.

Araya CM, Alleyne AT, Steadman JR, Eskridge KM, Coyne DP (2004) Phenotypic and genotypic characterization of *Uromyces appendiculatus* from *Phaseolus vulgaris* in the Americas. Plant Dis 88:830–836

Augustin E, Coyne DP, Schuster ML (1972) Inheritance of resistance in *Phaseolus vulgaris* to *Uromyces phaseoli typica* Brazilian rust race B11 and of plant habit. J. Am Soc Hortic Sci 97:526–529

Ballantyne BJ (1978) The genetic basis of resistance to rust, caused by *Uromyces appendiculatus* in beans (*Phaseolus vulgaris*). PhD thesis, University of Sydney, Sydney, 262 pp. Available online: http://www.css.msu.edu/bic/PDF/Ballantyne%20Thesis%20Bean%20Rust.pdf

Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J (2003) Beans (*Phaseolus* spp.) - model food legumes. Plant and Soil 252:55–128

Christ BJ, Groth JV (1982a) Inheritance of resistance in three cultivars of beans to the bean rust pathogen and the interaction of virulence and resistance genes. Phytopathology 72:771–773

Christ BJ, Groth JV (1982b) Inheritance of virulence to three bean cultivars in three isolates of the bean rust pathogen. Phytopathology 72:767–770

Corrêa RX, Costa MR, Good-God PI, Ragagnin VA, Faleiro FG, Moreira MA, Barros EG (2000) Sequence characterized amplified regions linked to rust resistance genes in the common bean. Crop Sci 40:804–807

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Faleiro FG, Vinhadelli WS, Ragagnin VA, Zambolim L, Paula Junior TJ, Moreira MA, Barros EG (1999) Identificação de raças fisiologicas de *Uromyces appendiculatus* no estado de Minas Gerais, Brasil. Fitopatol Bras 24:166–169

Faleiro FG, Vinhadelli WS, Ragagnin VA, Corrêa RX, Moreira MA, Barros EG (2000a) RAPD markers linked to a block of genes conferring rust resistance to the common bean. Genet Mol Biol 23:399–402

Faleiro FG, Ragagnin VA, Corrêa RX, Vinhadelli WS, Moreira MA, Barros EG (2000b) Ligação gênica da resistência à ferrugem e à antracnose na variedade de feijão Ouro Negro. Rev Ceres 47:375–382

Faleiro FG, Ragagnin VA, Schuster I, Corrêa RX, Good-God PI, Brommonshenkel S, Moreira MA, Barros EG (2003) Mapeamento de genes de resistência do feijoeiro à ferrugem, antracnose e mancha-angular usando marcadores RAPD. Fitopatol Bras 28:59–66

Faleiro FG, Ragagnin VA, Moreira MA, Barros EG (2004) Use of molecular markers to accelerate the breeding of common bean lines resistant to rust and anthracnose. Euphytica 183:213–218

Finke ML, Coyne DP, Steadman JR (1986) The inheritance and association of resistance to rust, common bacterial blight, plant habit and foliar abnormalities in *Phaseolus vulgaris* L. Euphytica 35:969–982

Grafton KF, Weiser GC, Littlefield LJ, Stavely JR (1985) Inheritance of resistance to two races of leaf rust in dry edible bean. Crop Sci 25:537–539

Jung G, Coyne DP, Bokosi J, Steadman J, Nienhuis J (1998) Mapping genes for specific and adult plant resistance to rust and abaxial leaf pubescence and their genetic relationship using random amplified polymorphic DNA (RAPD) markers in common bean. J. Am Soc Hortic Sci 123:859–863

Kelly JD, Stavely JR, Miklas PN (1996) Proposed symbols for rust resistance genes. Annu Rep Bean Improv Coop 39:25–31

Liebenberg MM, Mienie CMS, Pretorius AZ (2006) The occurrence of rust resistance gene *Ur-13* in common bean cultivars and lines. Euphytica 150:365–386

Liebenberg MM, Pretorius ZA (2004) Proposal for designation of a rust resistance gene in the large-seeded cultivar Kranskop. Annu Rep Bean Improv Coop 47:255–256

Miklas PN, Kelly JD, Beebe SE, Blair MW (2006) Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. Euphytica 147:105–131

Mmbaga MT, Steadman JR, Stavely JR (1996) The use of host resistance in disease management of rust in common bean. Integrated Pest Manag Rev 1:191–200

Pastor-Corrales MA (2005) Inheritance of resistance in PI260418 an Andean bean resistant to most races of the bean rust pathogen. Annu Rep Bean Improv Coop 48:134–135

Pedrosa A, Vallejos CE, Bachmair A, Schweizer D (2003) Integration of common bean (*Phaseolus vulgaris* L.) linkage and chromosomal maps. Theor Appl Genet 106:205–212

Pedrosa-Harand A, de Almeida CCS, Mosiolek M, Blair MW, Schweizer D, Guerra M (2006) Extensive ribosomal DNA amplification during Andean common bean (*Phaseolus vulgaris* L.) evolution. Theor Appl Genet 112:924–933

Pedrosa-Harand A, Porch T, Gepts P (2008) Standard nomenclature for common bean chromosomes and linkage groups. Annu Rep Bean Improv Coop 51:106–107

Ragagnin VA, Souza TLPO, Sanglard DA, Arruda KMA, Costa MR, Alzate-Marin AL, Carneiro JES, Moreira MA, Barros EG (2009) Development and agronomic performance of common bean lines simultaneously resistant to anthracnose, angular leaf spot and rust. Plant Breeding 128:156–163

Sanglard DA, Ribeiro CAG, Balbi BP, Barros EG, Moreira MA (2009) Reaction of common bean cultivars and elite lines to isolates of *Pseudocercospora griseola*. Annu Rep Bean Improv Coop 52:64–65

Souza TLPO, Alzate-Marin AL, Moreira MA, Barros EG (2005a) Análise da variabilidade patogênica de *Uromyces appendiculatus* em algumas regiões brasileiras. Fitopatol Bras 30:143–149

Souza TLPO, Sanglard D, Ragagnin VA, Alzate-Marin AL, Moreira MA, Barros EG (2005b) Development of “carioca-type” common bean lines resistant to rust with the aid of molecular markers. Annu Rep Bean Improv Coop 48:138–139

Souza TLPO, Ragagnin VA, Sanglard D, Moreira MA, Barros EG (2007) Identification of races of selected isolates of *Uromyces appendiculatus* from Minas Gerais (Brazil) based on the new international classification system. Fitopatol Bras 32:104–109

Souza TLPO, Alzate-Marin AL, Faleiro FG, Barros EG (2008) Pathosystem common bean–*Uromyces appendiculatus*: host resistance, pathogen specialization, and breeding for rust resistance. Pest Technology 2:56–69

Stavely JR (1984) Genetics of resistance to *Uromyces phaseoli* in a *Phaseolus vulgaris* line resistant to must races of the pathogen. Phytopathology 74:339–344

Stavely JR (1998) Recombination of two major dominant rust resistance genes that are tightly linked in repulsion. Annu Rep Bean Improv Coop 41:17–18

Stavely JR, Freytag GF, Steadman JR, Schwartz HF (1983) The 1983 Bean Rust Workshop. Annu Rep Bean Improv Coop 26:iv–vi

Stavely JR, Pastor-Corrales MA (1989) Rust. In: Schwartz HF, Pastor-Corrales MA (Eds) Bean production problems in the tropics. CIAT, Cali, pp 159–194

Steadman JR, Pastor-Corrales MA, Beaver JS (2002) An overview of the 3rd Bean Rust and 2nd Bean Common Bacterial Blight International Workshops, March 4-8, 2002, Pietermaritzburg, South Africa. Annu Rep Bean Improv Coop 45:120–125

Webster DMN, Ainsworth PM (1988) Inheritance and stability of a small pustule reaction of snap beans to *Uromyces appendiculatus*. J. Am Soc Hortic Sci 113:938–940

Wortmann CS, Kirkby RA, Eledu CKA, Allen DJ (1998) Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa. CIAT, Cali, 17 pp

DNA Marker-Assisted Breeding Aiming Rust Resistance Gene Pyramiding in the Common Bean

Abstract: The agronomic performance of the common bean (*Phaseolus vulgaris* L.), the world most important grain legume for direct human consumption, is affected by several diseases. Among them is rust, which is incited by the fungus *Uromyces appendiculatus*. In our breeding program we used a gene pyramiding approach assisted by DNA markers aiming to develop bean lines with durable and ample resistance to this pathogen. The rust resistance genes *Ur-5* (from 'Mexico 309'), *Ur-11* (from 'BelMiDak RR-3'), and *Ur-ON* (from a carioca-type line 'Vi-4899') were combined in the carioca-type bean cultivar 'Rudá'. Initially, two different backcross programs were conducted separately to produce carioca-type progenies harboring individually the genes *Ur-5* and *Ur-11*. Molecular fingerprinting analysis was used to select plants genetically closer to 'Rudá' in the backcross cycles to accelerate the recurrent-background recovery. The progenies obtained were intercrossed and then crossed with 'Vi-4899' (*Ur-ON*), using a tri-cross design. The resulting plants were screened with the molecular markers $SI19_{460C}$ (*Ur-5*), $SAE19_{890R}$ (*Ur-11*) and $OPX11_{550C}$ (*Ur-ON*) to identify those containing all the alleles of interest. The selected plants were selfed to obtain the next generations. The selection based on DNA markers was conducted up to the $F_{4:5}$ generation. We were able to select $F_{4:7}$ progenies showing all the DNA markers associated to the genes of interest and resistant to all *U. appendiculatus* isolates tested. They were also resistant under field conditions. Yield evaluations show that these selected lines are as productive as the recurrent parent 'Rudá' and other high performing cultivars currently grown in Brazil.

Key words: *Uromyces appendiculatus* – *Phaseolus vulgaris* – disease resistance – marker-assisted selection – agronomic performance – plant breeding

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an economically, nutritionally, and socially important crop, especially in developing countries of Latin America, Eastern and Southern Africa. It is grown and consumed worldwide in distinct areas and different seasons, mainly by subsistence level farmers with low-technology input but also by farmers that use high input technologies (Pachico 1989; Wortmann et al. 1998; Broughton et al. 2003). Dry bean cultivars were grown on approximately 27 million hectares in more than 120 countries in 2007, but their yield is quite low compared to other important grain legumes such as soybean and peas (http://faostat.fao.org). One of the several factors contributing to this situation is the high number of destructive diseases that attack *P. vulgaris*. Among them is the common bean rust, incited by the basidomycete fungus *Uromyces appendiculatus* F. Strauss (syn. *U. phaseoli* G. Winter), which can cause great yield losses. This fungus is a highly variable and is among the most pathogenically variable of all plant pathogens (Stavely and Pastor-Corrales 1989).

Bean rust is distributed around the world, but it effectively causes major production problems in humid tropical and subtropical areas. Severe epidemics have been reported in Australia, China, the United States, and some areas of Europe. Major losses have occurred in Burundi, Ethiopia, Kenya, Malawi, Rwanda, South Africa, Tanzania, Uganda, and Zimbabwe. In Latin America, the bean rust is also a serious problem, major losses occurred in Argentina, Bolivia, Brazil, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, Guatemala, Haiti, Honduras, Jamaica, Mexico, Nicaragua, and Peru (Stavely and Pastor-Corrales 1989; Souza et al. 2008). In Brazil, the disease causes major losses in south, southeast and central areas,

including the states of Paraná, Rio Grande do Sul, Santa Catarina, Minas Gerais, São Paulo, and Goiás (Souza et al. 2005). Rust losses worldwide measured in greenhouse and field conditions can vary from 18 to 100% (Stavely and Pastor-Corrales 1989; Staples 2000). Yield losses higher than 68% were detected in Minas Gerais state in southeastern Brazil (Vieira et al. 2005).

No single control or disease management measure can be recommended for rust. Management of this disease has relied primarily on three strategies: application of fungicides, host resistance, and various cultural practices. The use of resistant cultivars is certainly the main component of the integrated bean rust management. The pyramiding of different race-specific resistance genes is an important strategy for developing broad and durable resistance to *U. appendiculatus* (Souza et al. 2008; Raganin et al. 2009). This approach using only conventional breeding methods has not been effective mainly due to the difficulties in selecting genotypes harboring different resistance genes. For this reason, molecular markers are often used to aid the pyramiding process. The use of molecular markers not only eliminates the need for multiple and sequential inoculations but also the confounding effect of potential epistatic interactions among the different resistance genes present in the same genetic background (Michelmore 1995; Bigirimana and Höfte 2001; Singh et al. 2001; Toenniessen et al. 2003). However, for each resistance allele a specific marker or markers need to be identified. Several RAPD markers associated with genes conferring resistance to rust in common bean have been identified and some of them were converted into SCAR markers to increase their reproducibility (Souza et al. 2008).

Molecular markers can also be used to accelerate the recovery of the recurrent parent's genome in backcross breeding programs. Simulation studies and real data indicate that only three or four backcrosses are necessary to recover the recurrent parent's genome when molecular markers are used (Openshaw et al. 1994; Faleiro et al. 2004; Ragagnin et al. 2009). Separate backcross programs assisted by molecular marker fingerprinting can be used

for the individual introgression of resistance genes in commercial cultivars. This strategy can be the initial step for pyramiding rust resistance alleles.

The main goal of the present work was to develop high yielding carioca-type common bean lines with broad and durable resistance to *U. appendiculatus*. Carioca seeded beans are the most consumed in Brazil. Backcross programs were conducted separately to produce genetic materials harboring individual rust resistance alleles. DNA fingerprinting was used to accelerate the recurrent-background recovery. Molecular marker-assisted selection was used to combine the distinct rust resistance genes in the carioca-type genetic background. The agronomic performance of the advanced lines obtained was evaluated in two growing seasons and the presence of the resistance alleles was confirmed by inoculations with the rust pathogen.

2. Material and Methods

2.1. Plant genetic material

The Mesoamerican carioca-type common bean cultivar 'Rudá' was used as recurrent parent in all backcrosses. It has been highly recommended for many growing regions in Brazil, but it is susceptible to rust. 'Mexico 309' and 'BelMiDak RR-3' (derived from 'PI181996') were used as donor parents. They are Mesoamerican bean lines harboring genes *Ur-5* and *Ur-11*, respectively, that confer ample resistance to *U. appendiculatus* in Brazil (Souza et al. 2005, 2007a) and in other parts of the world (Stavely 2000; Steadman et al. 2002). The common bean line 'Vi-4899', derived from the cross 'Rudá' × 'Ouro Negro' by molecular marker-assisted backcrosses (Faleiro et al. 2004), was also used as donor parent in the gene pyramiding process. This carioca seeded line recently released in Brazil as the cultivar 'BRSMG Pioneiro' (Moreira et al. 2006) harbors the gene *Ur-ON* from 'Ouro Negro'. It is resistant to several isolates of *U. appendiculatus* and *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib., the causal agent of bean anthracnose (Faleiro et al. 2004).

2.2. Crosses

All crosses were conducted in the greenhouse. Two separate backcross (BC) programs were conducted until BC_3 generations using 'Rudá' as recurrent parent and cultivars 'Mexico 309' or 'BelMiDak RR-3' as donor parents. After each BC the plants were screened with the rust pathogen according to item 2.3 and the genetic distances between the resistant plants and the recurrent parent were determined with RAPD markers. The plants closest to 'Rudá' were used for the next BC cycle. The BC_3F_1 plants selected based on these criteria were selfed to obtain the BC_3F_2 population that was inoculated with *U. appendiculatus* and screened with molecular markers linked to the genes of interest (Table 1). The resistant BC_3F_2 plants presenting the DNA markers were selfed to obtain $BC_3F_{2:3}$ progenies which were submitted to progeny tests based on inoculation with the pathogen.

$BC_3F_{2:3}$ progenies homozygous for resistance to rust were selected for the pyramiding process (Figure 1). This process was done using a tri-cross design consisted basically of two steps: step 1 - pairwise crosses among the $BC_3F_{2:3}$ resistant progenies to obtain single-hybrids; step 2 - crosses of the single-hybrids with the common bean line 'Vi-4899' to obtain the triple-hybrids with the three resistance genes of interest. The triple-hybrid plants were screened with the markers SI19 (gene *Ur-5*) and OPX11 (gene *Ur-ON*) to confirm the cross. Plants harboring both markers were selfed to obtain the subsequent generations. The F_2, F_3, F_4 and $F_{4:5}$ plants derived from the triple-hybrids were also selected with molecular markers specifically linked to each one of the rust resistance genes, which were identified in a preliminary genotyping test of the parental cultivars. $F_{4:5}$ seeds from the selected F_4 plants were multiplied and the resulting plants were used for progeny tests based on screening with molecular markers to identify homozygous families for the rust resistance loci *Ur-5*, *Ur-11*, and *Ur-ON* (Figure 2).

Table 1. RAPD (OP) and SCAR (S) markers linked to resistance genes to *Uromyces appendiculatus*.

Marker	Primer sequence (5' → 3')	Gene	Source	Distance (cM)[a]	AT (^{o}C)[b]	Reference
$OPX11_{550}$	GGAGCCTCAG	*Ur-ON*	Ouro Negro	5.8 (C)	35	Faleiro et al. (2000)
$OPF10_{970}$	GGAAGCTTGG	*Ur-5*	B-190	2.1 (C)	35	Haley et al. (1993)
$OPAC20_{490}$	ACGGAAGTGG	*Ur-11*	PI181986	NR (C)	35	Johnson et al. (1995)
$SF10_{1.050}$	F-GGAAGCTTGGTGAGCAAGGA R-GGAAGCTTGGCTATGATGGT	*Ur-ON*	Ouro Negro	4.3 (C)	65	Corrêa et al. (2000)
$SBA08_{560}$	F-CCACAGCCGACGGAGGAG R-GCCATGTTTTTTGTCCCC	*Ur-ON*	Ouro Negro	6.0 (C)	65	Corrêa et al. (2000)
$SI19_{460}$	F-AATGCGGGAGTTCAATAGAAAAACC	*Ur-5*	B-190	NR (C)	53	Melotto & Kelly (1998)
	R-AATGCGGGAGATATTAAAAGGAAAG		Mexico 309	3.3 (C)	50	Souza et al. (2007b)
$SAE19_{890}$	F-CAGTCCCTGACAACATAACACC R-CAGTCCCTAAAGTAGTTTGTCCCTA	*Ur-11*	BelMiDak RR-3	1.0 (R)	58	Queiroz et al. (2004)

[a] NR = no recombinants. Linkage phase of molecular marker: coupling (C) or repulsion (R).
[b] AT = annealing temperature.

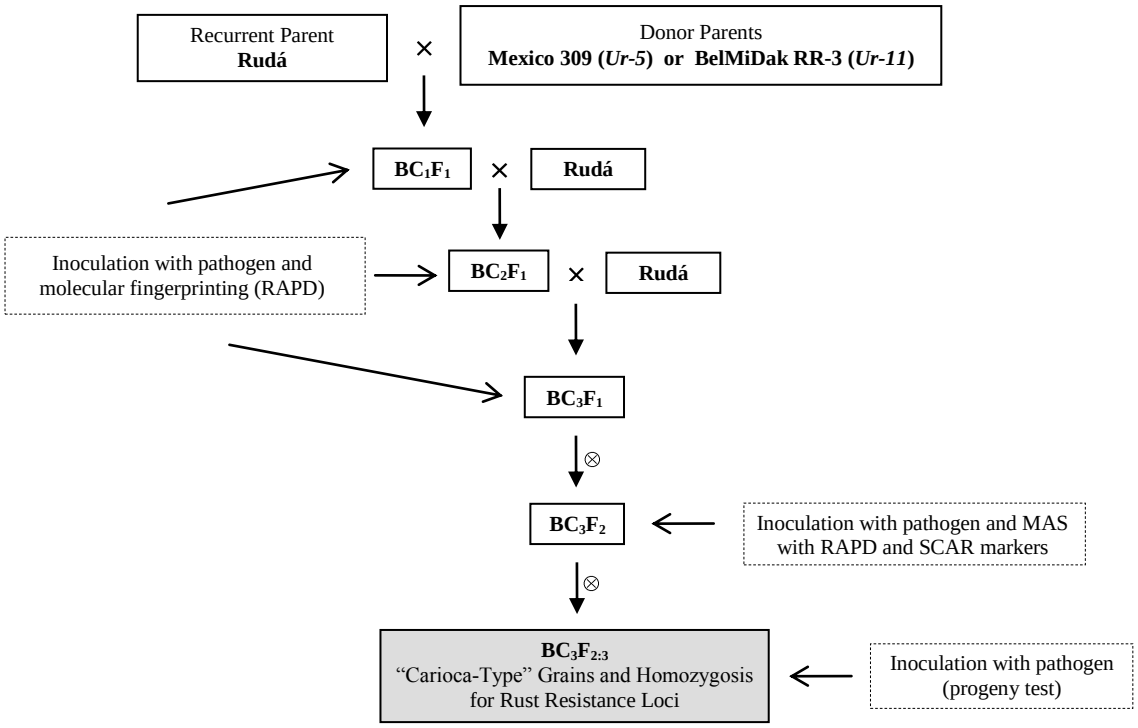

Figure 1. Diagram representing the breeding strategy used to develop carioca-type common bean progenies harboring individually different rust resistance genes in two separate backcross (BC) programs.

$F_{4:6}$ seeds from the selected families were sowed in the field for yield determination. The corresponding $F_{4:7}$ families were also evaluated for grain yield, reaction to rust, and grain aspect in another growing season. These $F_{4:7}$ lines (pyramid lines) were also tested for resistance to specific isolates of *U. appendiculatus* in greenhouse inoculations and screened with DNA markers (Figure 2).

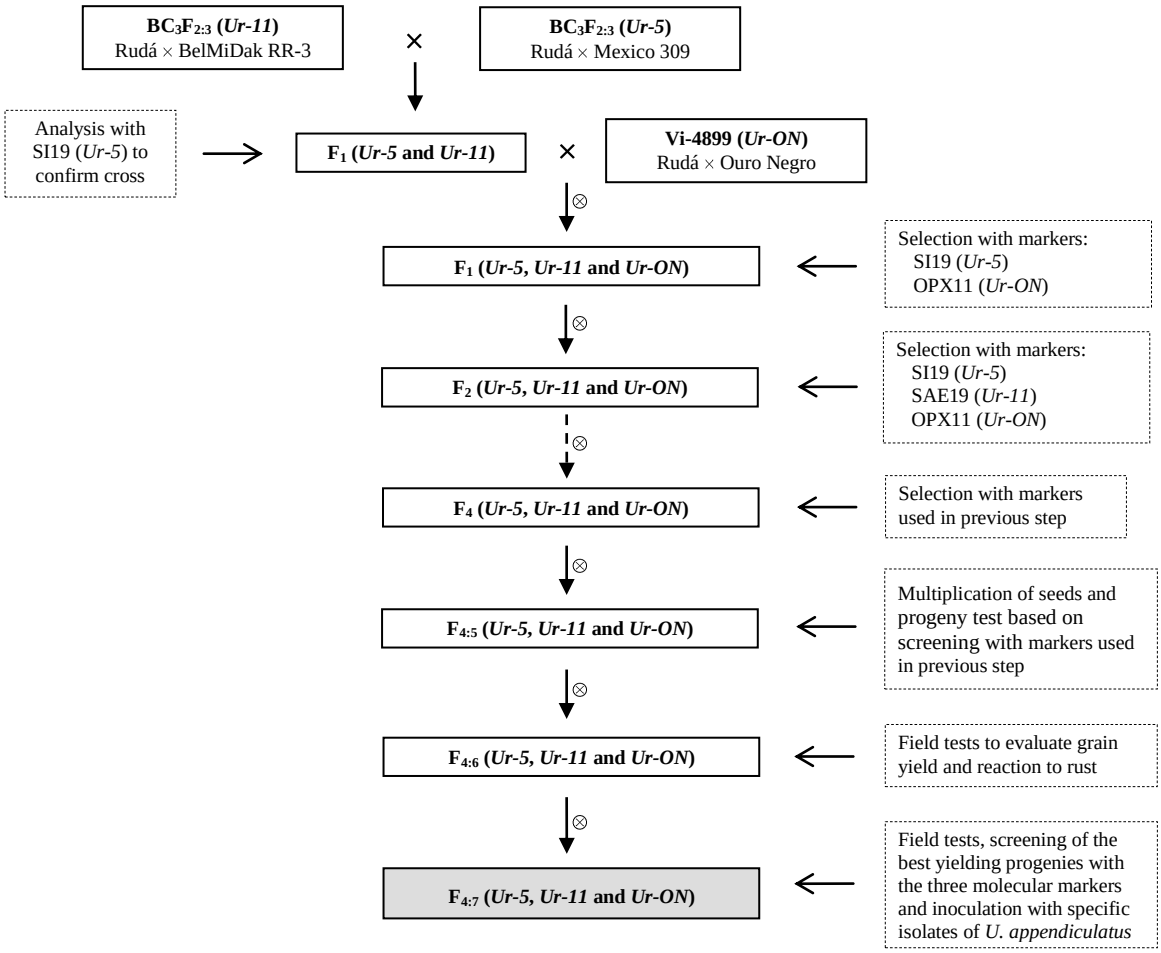

Figure 2. Diagram representing the breeding strategy used to develop carioca-type common bean advanced lines (pyramid lines) harboring simultaneously three distinct rust resistance genes.

2.3. Pathogen inoculation and disease evaluation

Brazilian *U. appendiculatus* mono-pustule isolates used in this work were characterized and classified into physiological races by Souza et al. (2007a). They are maintained in the fungal collection of the BIOAGRO/UFV Common Bean Breeding Program. Artificial inoculations were carried out when the primary leaves of the bean plants reached approximately 2/3 of their full development, about 10 days after sowing under greenhouse conditions (20 ± 5°C), as described previously (Souza et al. 2007a). Each one of the BC_nF_1 and BC_3F_2 plants and 12 plants each of the tested progenies ($BC_3F_{2:3}$ families and $F_{4:7}$ pyramid lines) and of a control cultivar were inoculated (Figures 1 and 2). The inoculum concentration was 2.0×10^4 uredospores/mL distilled water

containing Tween-20 (0.05%, v:v). The inoculum solution was sprayed on both leaf surfaces using a manual atomizer (De Vilbiss n^{o} 15) adapted to an electric compressor. After inoculation the plants were transferred to a mist chamber (20 ± 1°C and relative humidity > 95%) where they were kept for approximately 48 h under a 12-hour light regime. In order to avoid contamination, plants inoculated with different isolates were kept in separate compartments of the mist chamber. After this period the plants were transferred to a greenhouse (20 ± 5°C), where they were kept until symptom evaluation, about 15 days after inoculation.

Six grades of rust reaction were considered in the evaluation of the disease symptoms based on the scale proposed by Stavely et al. (1983): 1- no pustules (immunity); 2 - necrotic spots without sporulation; 3 - pustules undergoing sporulation with a diameter of < 300 μm; 4 - pustules undergoing sporulation with a diameter ranging from 300 μm to 499 μm; 5 - pustules undergoing sporulation with a diameter ranging from 500 μm to 800 μm; and 6 - pustules undergoing sporulation with a diameter of > 800 μm. Reaction grades to the pathogen were determined visually by at least two researchers. Plants with grades 1 and 2 were considered resistant (R), those with grade 3 were considered to be moderately resistance (MR), plants presenting grade 4 were considered moderately susceptible (MS), and those with grade 5 or 6 were considered to be susceptible (S).

2.4. DNA marker analysis

Leaf DNA was extracted according to Doyle and Doyle (1990). In each backcross cycle a screening was done to identify polymorphic RAPD primers (Operon Technologies, Alameda, CA) to be used in the molecular fingerprinting assays. The primers that revealed at least one polymorphic DNA band among the analyzed plants were selected. DNA amplification by RAPD technique was according to Faleiro et al. (2000). The polymorphic and monomorphic DNA bands were used to build a matrix based on the presence (1) or absence (0) of bands. The genetic similarity values were calculated by

the Euclidian method for binary data and used to cluster the plants by the nearest neighbor method with the aid of the program GENES (Cruz 2006).

For the molecular marker-assisted selection, including the progeny test to identify $F_{4:5}$ pyramid families homozygous for the rust resistance loci, RAPD and SCAR markers specifically linked to each one of the resistance genes were used. In the assays with the SCAR technique, the DNA amplification reactions were as described by Côrrea et al. (2000). The annealing temperatures for the markers are described in Table 1.

The amplified products were visualized under UV light after electrophoresis on 1.2% agarose gels containing ethidium bromide (0.2 μg/mL), immersed in a 1X sodium boric acid (SB) buffer (10 mM sodium hydroxide, pH adjusted to 8.5 with boric acid).

2.5. Agronomic performance

Two field experiments were conducted at the Universidade Federal de Viçosa Experimental Station in Coimbra, MG, Brazil, to evaluate the pyramid lines: one in the Autumn of 2007 ($F_{4:6}$ progenies) and other one in the Winter/Spring of 2007 ($F_{4:7}$ progenies). In the first experiment the only trait evaluated was grain production. Besides this trait, reaction to rust and grain aspect were also evaluated in the second experiment. Both experiments included 16 pyramid progenies identified as homozygous for all rust resistance loci by the progeny test based on molecular markers (Figure 2), 75 carioca-type progenies harboring one or two rust resistance genes derived from the backcross and pyramid programs, and nine reference cultivars: 'Rudá', 'Vi-4899', 'Majestoso', 'Pérola', 'Talismã', and 'VC-3' (carioca seeded beans high yielding and widely grown in Brazil); 'Ouro Negro' and 'Mexico 309' (black seeded beans resistant to rust); and 'Vermelhinho' (red seeded bean susceptible to rust).

In all experiments a randomized triple 10×10 lattice design was used. Each plot consisted of two rows each 2.0 m long, spaced by 0.5 m, with 15 seeds per meter. The equivalent of 350 Kg/ha of the formula 8-28-16 (N, P_2O_5

and K_2O, respectively) was used as fertilizer during sowing, and 150 Kg/ha ammonium sulfate was applied 25 days after emergence. Harvest of the whole plot was done manually. The grain yield was determined for each plot and expressed as g/2m^2 (g/plot). Reaction to rust was determined in the field using the six-grade scale proposed by Stavely et al. (1983). The grain aspect was evaluated visually using a five-grade scale as reported by Marques-Júnior et al. (1997). In this scale grade 1 represents the wanted grain aspect (carioca-type) and grade 5, the extreme opposite. At least two separate researchers evaluated the reactions to rust and grain aspect. The Tukey test was used to evaluate the significance of the differences among the mean values obtained for each trait.

3. Results

3.1. Development of pyramid lines assisted by molecular markers

BC_3F_1 plants derived from the separate backcross programs using 'Mexico 309' (gene *Ur-5*) and 'BelMiDak RR-3' (gene *Ur-11*) as donor parents were inoculated with a mixture of the *U. appendiculatus* races 29-3, 61-3, 63-3, and 63-19. The relative genetic similarities between the recurrent genitor 'Rudá' and the resistant BC_3F_1 plants varied from 80.8 to 98.1% ('Rudá' × 'Mexico 309') and from 82.1 to 98.2% ('Rudá' × 'BelMiDak RR-3'). The total number of bands and the percentage of polymorphic DNA bands used to calculate these genetic similarity values were 39 and 48.7%, and 39 and 41.0%, respectively (Table 2).

The resistant BC_3F_1 plants which were genetically closest to 'Rudá' were conducted up to the $BC_3F_{2:3}$ generation (Figure 1). The carioca seeded $BC_3F_{2:3}$ families homozygous for the rust resistance loci were identified by inoculation with the *U. appendiculatus* races 21-3 ('Rudá' × 'Mexico 309') and 29-15 ('Rudá' × 'BelMiDak RR-3'). The selected $BC_3F_{2:3}$ families were crossed pairwise to start the pyramiding process. The F_1 plants obtained were crossed with the carioca-type common bean line 'Vi-4899' (gene *Ur-ON*),

derived from the cross 'Rudá' × 'Ouro Negro' by molecular marker-assisted backcrosses (Faleiro et al. 2004). The triple-hybrid plants that presented the markers SI19 and OPX11 were selfed and 189 F_2 seeds were obtained. DNA obtained from the F_2 plants was amplified with the molecular markers identified as specifically linked to each one of the rust resistance genes (Table 3), and eight F_2 plants were selected. These plants were selfed to obtain the next generations. For the selection of plants carrying all resistance genes to *U. appendiculatus* in subsequent generations, the presence of specific RAPD and SCAR markers (Table 3) was used as selection criterion (Figure 2). Following this methodology it was possible to obtain 16 $F_{4:5}$ families phenotypic and genotypically similar to the recurrent cultivar 'Rudá' and non-segregating for the DNA markers associated to the rust resistance genes.

Table 2. Parameters used to estimate the relative genetic similarity between the recurrent genitor 'Rudá' and BC_2F_1 and BC_3F_1 plants resistant to rust.

Parameter	Cross/Generation			
	Rudá × Mexico 309 (*Ur-5*)		Rudá × BelMiDak RR-3 (*Ur-11*)	
	BC_2F_1	BC_3F_1	BC_2F_1	BC_3F_1
Proportion of resistant plants	6/22	7/33	5/18	7/22
Number of RAPD primers randomly selected	13	13	13	13
Total number of evaluated DNA bands[a]	93	80	69	95
Number of polymorphic bands	35	39	36	39
Percentage of polymorphic bands	37.6%	48.7%	52.2%	41.0%
Expected genetic similarity[b]	87.5%	93.7%	87.5%	93.7%
Average genetic similarity	89.4%	90.2%	86.1%	90.7%
Range of genetic similarity	86.8–94.3%	80.8–98.1%	75.8–97.0%	82.1–98.2%

[a] PCR discrete products easily visualized.
[b] Estimator: $[1 – (0.5)^{n+1}] \times 100$, where 'n' is the number of backcross cycles.

Table 3. Genotyping of the common bean genetic material to be used for rust resistance gene pyramiding aiming to identify molecular markers specifically linked to each one of the resistant loci.

Genetic material	Resistance gene	Molecular marker (Resistance gene)[a]						
		$OPX11_{550}$ (*Ur-ON*)[b]	$OPF10_{970}$ (*Ur-5*)	$OPAC20_{490}$ (*Ur-11*)	$SF10_{1.050}$ (*Ur-ON*)	$SBA08_{560}$ (*Ur-ON*)	$SI19_{460}$ (*Ur-5*)[b]	$SAE19_{890}$ (*Ur-11*)[b]
$BC_3F_{2:3}$ plants (Rudá × Mexico 309)	*Ur-5*	0	1	1	1	1	1	1
$BC_3F_{2:3}$ plants (Rudá × BelMiDak RR-3)	*Ur-11*	0	0	1	0	0	0	1
Vi-4899 (Rudá × Ouro Negro)	*Ur-ON*	1	1	1	1	1	0	1
Rudá	-	0	0	0	0	0	0	1
Mexico 309	*Ur-5*	0	1	1	1	1	1	1
BelMiDak RR-3	*Ur-11*	0	0	0	0	0	0	0
Ouro Negro	*Ur-ON*	1	1	1	1	1	0	1

[a] Presence (1) or absence (0) of DNA marker. No expected results (false positives) are highlighted in grey.
[b] Markers selected for monitoring the introgression of the respective rust resistance genes into cultivar 'Rudá'.

3.2. Agronomic performance evaluation

The 16 selected $F_{4:5}$ pyramid families were multiplied in the field to obtain enough seeds for evaluation of agronomic traits. The corresponding $F_{4:6}$ families and 75 carioca-type progenies harboring one or two rust resistance genes derived from the backcross and pyramiding programs, in addition to nine reference cultivars, were tested for grain yield during the Autumn of 2007. The resulting 16 $F_{4:7}$ pyramid lines and the other 84 treatments were also evaluated for grain production, reaction to rust, and grain aspect during the Winter/Spring of 2007. The *F*-statistic ($P < 0.01$) indicated that there was genetic variability for the three evaluated traits among the analyzed genetic material (data not shown). The mean values for grain production of the $F_{4:7}$ pyramid lines did not differ statistically in relation to 'Rudá' and to other high yielding carioca seeded reference cultivars widely grown in Brazil. These lines were also resistant under field conditions and presented grain aspect similar to that of the recurrent parent 'Rudá' (Table 4).

3.3. Rust resistance test

The 16 $F_{4:7}$ pyramid lines and reference cultivars were inoculated with five specific *U. appendiculatus* races and screened with the DNA markers linked to the rust resistance genes listed in Table 3. The pyramid lines presented all molecular markers. Nine lines out of the 16 pyramid lines were resistant (R) to all *U. appendiculatus* races tested. The resistance spectra of these nine selected lines indicate that they posses the resistance genes *Ur-5*, *Ur-11*, and *Ur-ON* in homozygosis (Table 5).

Table 4. Agronomic performance of advanced pyramid lines and reference cultivars in relation to grain production (Autumn and Winter/Spring of 2007), reaction to rust and grain aspect (Winter/Spring of 2007), Coimbra, MG, Brazil.

Pyramid line/Reference cultivar	Grain production (g/2m^2)			Reaction to rust	Grain aspect
	Autumn 2007	Winter/spring 2007	Joint analysis	(Scale 1-to-6)	(Scale 1-to-5)
TL-005	870.39 ab	665.94 abcde	768.16 ab	3.33 ab	2.80 cdef
TL-006	906.98 ab	799.86 abc	853.42 a	3.00 a	2.93 cdef
TL-009	927.78 ab	626.82 Bcde	777.30 ab	2.67 a	3.13 defg
TL-011	819.05 ab	711.10 Abcd	765.08 ab	3.33 ab	2.83 cdef
TL-012	928.98 ab	741.37 Abcd	835.17 a	3.00 a	3.00 cdef
TL-015	883.53 ab	749.76 Abcd	816.65 a	3.00 a	3.03 cdefg
TL-016	847.52 ab	759.17 Abcd	803.35 a	2.81 a	2.70 bcde
TL-026	783.47 ab	740.05 Abcd	761.76 ab	3.00 a	2.73 cde
TL-031	955.72 a	780.22 abc	867.97 a	3.00 a	3.00 cdef
TL-032	888.08 ab	762.50 Abcd	825.29 a	2.83 a	2.83 cdef
TL-034	905.43 ab	752.36 Abcd	828.89 a	3.00 a	2.97 cdef
TL-035	778.49 ab	761.24 Abcd	769.87 ab	3.00 a	3.07 cdefg
TL-037	889.84 ab	638.69 Bcde	764.26 ab	3.17 ab	3.07 cdefg
TL-038	930.52 ab	759.69 Abcd	845.11 a	3.17 ab	2.83 cdef
TL-039	863.25 ab	680.46 abcde	771.86 ab	3.33 ab	3.23 defg
TL-041	868.40 ab	710.47 Abcd	789.43 ab	3.33 ab	2.87 cdef
Rudá	847.62 ab	666.24 abcde	756.93 ab	4.33 b	3.20 defg
Vi-4899	873.31 ab	818.21 abc	845.76 a	2.67 a	2.90 cdef
Majestoso	764.80 ab	458.90 Ef	611.85 ab	3.33 ab	2.47 bc
Pérola	817.90 ab	615.93 Cdef	716.92 ab	4.00 ab	2.07 ab
Talismã	762.29 ab	392.09 F	577.19 ab	5.10 bc	2.50 bcd
VC-3	803.21 ab	873.85 A	838.53 a	3.33 ab	1.50 a
Ouro Negro	750.56 ab	612.24 Cdef	681.40 ab	3.00 a	5.00 h
México 309	678.83 b	551.18 Def	615.00 ab	5.57 c	5.00 h
Vermelhinho	556.02 b	344.40 F	450.21 c	6.00 c	5.00 h
Mean	838.14	719.67	766.64	3.37	3.10
CV (%)	15.32	10.70	12.27	13.99	8.59

Means followed by the same letter do not differ by the Tukey test at 5% probability.

Table 5. Rust resistance phenotypic and molecular characterization of $F_{4:7}$ pyramid lines and reference cultivars.

Pyramid line/Reference cultivar	Race of *Uromyces appendiculatus*[a]					DNA marker (Resistance gene)[b]		
	21-3	29-3	29-15	53-3	53-7	$SI19_{460}$ (*Ur-5*)	$SAE19_{890}$ (*Ur-11*)	$OPX11_{550}$ (*Ur-ON*)
TL-005	R	R	MR	R	MR	+	-	+
TL-006	R	R	R	R	R	+	-	+
TL-009	R	R	R	R	R	+	-	+
TL-011	R	R	R	R	MR	+	-	+
TL-012	R	R	R	R	MR	+	-	+
TL-015	R	R	R	R	R	+	-	+
TL-016	R	R	R	R	R	+	-	+
TL-026	R	R	R	R	R	+	-	+
TL-031	R	R	R	R	MR	+	-	+
TL-032	R	R	R	R	R	+	-	+
TL-034	R	R	R	R	R	+	-	+
TL-035	R	R	R	R	R	+	-	+
TL-037	R	R	R	R	R	+	-	+
TL-038	R	R	R	R	MR	+	-	+
TL-039	R	R	MR	R	R	+	-	+
TL-041	R	R	MR	R	R	+	-	+
Mexico 309 (*Ur-5*)	R	R	S	MR	S	+	+	-
BelMiDak RR-3 (*Ur-11*)	MR	R	R	MR	R	-	-	-
PI181996 (*Ur-11*)	MR	R	R	MR	R	-	-	-
Ouro Negro (*Ur-ON*)	MR	R	MR	R	MR	-	+	+
Rudá	S	MR	S	S	S	-	+	-
US Pinto 111	S	S	S	S	S	-	+	-

[a] Rust reaction: resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S).
[b] Presence (1) or absence (0) of DNA marker.
Pyramid lines selected as harboring simultaneously three distinct rust resistance genes are highlighted in grey.

4. Discussion

In this work we report a gene pyramiding approach assisted by DNA markers to develop carioca-type common bean lines harboring simultaneously the rust resistance genes *Ur*-5 – from 'Mexico 309', *Ur-11* – from 'BelMiDak RR-3', and *Ur-ON* – from 'Vi-4899', a carioca seeded line derived from 'Ouro Negro' (Faleiro et al. 2004). Carioca seeded beans are the most consumed in Brazil. The use of gene pyramiding as breeding strategy aimed to develop high yielding lines with wide and potentially durable resistance to the rust pathogen, the fungus *U. appendiculatus*.

The concept of molecular marker-assisted breeding was successfully used in this work. The use of two separate backcross programs assisted by RAPD fingerprinting allowed the individual introgression of the rust resistance genes *Ur-5* and *Ur-11* in cultivar 'Rudá' after only three backcross cycles. In addition, molecular markers specifically linked to the three resistance genes were identified and/or validated during the breeding process (Figure 1; Tables 1 and 3). These markers were effectively used to aid the pyramiding process and the development of advanced lines harboring simultaneously all resistance genes in homozygosis. The efficiency of the markers was confirmed by inoculation with specific races of the rust pathogen at the end of the breeding process (Table 5). Yield evaluations of the advanced lines demonstrated that no grain yield penalty in relation to the recurrent progenitor 'Rudá' was observed after the pyramiding process. The carioca seeded pyramid lines were also resistant under field conditions (Table 4).

The rust resistance gene pyramid of the lines developed in this work is now being transferred to modern carioca-type cultivars and to other bean types which are also consumed in Brazil, e.g., black and red seeded beans. In addition, the pyramid lines will be further evaluated in different bean growing regions by a Brazilian Bean Assay Network for productivity and disease resistance. If the superior agronomic performance of these lines is confirmed

they could be recommended as new cultivars. New rust resistance sources are also being tested and added to our breeding program to widen the basis of the present resistance gene pyramid. To be effective, the gene pyramiding strategy must be a continuous effort (Ragagnin et al. 2009).

Although gene pyramiding is not fully accepted by the scientific community as an efficient breeding strategy to develop wide and potentially durable resistance to pathogens, there are experimental evidence demonstrating that it confers more effective resistance than that conferred by the sum of the resistance present in the parental lines (Yoshimura et al. 1995; Huang et al. 1997; Singh et al. 2001). According to Schafer and Roelfs (1985), the probability that a pathogen will overcome a gene pyramid of more than two genes is extremely low. In order for this to happen, independent mutations in the pathogen genome must occur and they should be combined in the same genetic background, or they could occur simultaneously or sequentially in the genome of a specific isolate of the pathogen. Thrall and Burdon (2003) also report epidemiology data that support the use of gene pyramiding as an effective strategy for disease control. According to these authors, there is an inverse correlation between pathogen fitness and the number of avirulence genes present in its genome. By studying the pathosystem *Melampsora lini-Linum marginale* in Australia, these authors observed that natural pathogen populations which were able to infect a greater number of host populations were less aggressive than pathogen populations which were able to infect a lower number of host populations. The authors concluded that inactivation of several avirulence genes in the pathogen compromises its adaptability. In other words, it indicates that gene pyramiding can potentially keep the disease below an economical damage level and also prevent its fast dissemination.

Acknowledgments

This work was supported by grants from “Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq” and “Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG” (Brazilian government). CNPq provided a PhD fellowship to T. L. P. O. de Souza.

References

Bigirimana J, Höfte M (2001) Bean anthracnose: inoculation methods and influence of plant stage on resistance of *Phaseolus vulgaris* cultivars. Journal of Phytopathology 149, 403-408

Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, Vanderleyden J (2003) Beans (*Phaseolus* spp.) - model food legumes. Plant and Soil 252, 55-128

Corrêa RX, Costa MR, Good-God PI, Ragagnin VA, Faleiro FG, Moreira MA, Barros EG (2000) Sequence characterized amplified regions linked to rust resistance genes in the common bean. Crop Science 40, 804-807

Cruz CD (2006) GENES Vol. I - Estatística experimental e matrizes. Universidade Federal de Viçosa, Viçosa, 285 pp

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12, 13-15

Faleiro FG, Vinhadelli WS, Ragagnin VA, Corrêa R, Moreira MA, Barros EG (2000) RAPD markers linked to a block of genes conferring rust resistance to the common bean. Genetic and Molecular Biology 23, 399-402

Faleiro FG, Ragagnin VA, Moreira MA, Barros EG (2004) Use of molecular markers to accelerate the breeding of common bean lines resistant to rust and anthracnose. Euphytica 183, 213-218

Haley SD, Miklas PN, Stavely JR, Byrum J, Kelly JD (1993) Identification of RAPD markers linked to a major rust resistance gene block in common bean. Theoretical and Applied Genetics 86, 505-512

Huang N, Angeles ER, Domingo J, Magpantay G, Singh S, Zhang G, Kumaravadiel N, Bennett J, Khush GS (1997) Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theoretical and Applied Genetics 95, 313-320

Johnson E, Miklas PN, Stavely JR, Martinez-Cruzado JC (1995) Coupling- and repulsion-phase RAPDs for marker-assisted selection of PI 181996 rust resistance in common bean. Theoretical and Applied Genetics 90, 659-664

Marques-Júnior OG, Ramalho MAP, Ferreira DF, Santos JB (1997) Viabilidade do emprego de notas na avaliação de alguns caracteres do feijoeiro (*Phaseolus vulgaris* L.). Revista Ceres 44, 411-420

Melloto M, Kelly JD (1998) SCAR markers linked to major disease resistance genes in common bean. Annual Report of the Bean Improvement Cooperative 41, 64-65

Michelmore R (1995) Molecular approaches to manipulation of diseases resistance genes. Annual Review of Phytopathology 15, 393-427

Moreira MA, Barros EG, Carneiro JES, Faleiro FG, Faria LC, Carneiro GES, Del-Peloso MJ, Paula-Júnior TJ, Abreu AFB, Ramalho MAP, Melo LC, Santos JB, Rava CA, Costa JGC, Sartorato A, Faria JC (2006) BRSMG Pioneiro: new carioca common bean cultivar resistant to anthracnose and rust, for the southern of Brazil. Annual Report of the Bean Improvement Cooperative 49, 279-280

Openshaw SJ, Jarboe SG, Beavis WD (1994) Marker-assisted selection in backcross breeding. In: Lower R (Ed) ASHS/CSSA Joint Plant Breeding Symposium on Analysis of Molecular Marker Data. Oregon State University, Corvallis, pp 41-43

Pachico D (1989) Trends in world common bean production. In: Schwartz HF, Pastor-Corrales MA (Eds) Bean Production Problems in the Tropics (2nd Edn). CIAT, Cali, pp 1-8

Queiroz VT, Sousa CS, Souza TLPO, Costa MR, Sanglard DA, Ragagnin VA, Barros EG, Moreira MA (2004) SCAR marker linked to the common bean rust resistance gene *Ur-11*. Annual Report of the Bean Improvement Cooperative 47, 271-272

Ragagnin VA, Souza TLPO, Sanglard DA, Arruda KMA, Costa MR, Alzate-Marin AL, Carneiro JES, Moreira MA, Barros EG (2009) Development and agronomic performance of common bean lines simultaneously resistant to anthracnose, angular leaf spot and rust. Plant Breeding 128, 156-163

Schafer JF, Roelfs AP (1985) Estimated relation between numbers of urediniospores of *Piccinia graminis* f. sp. *tritici* and rates of occurrence of virulence. Phytopathology 75, 749-750

Singh S, Sidhu JS, Huang N, Vikal Y, Li Z, Brar DS, Dhaliwal HS, Khush GS (2001) Pyramiding three bacterial blight resistance genes (*xa5*, *xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR106. Theoretical and Applied Genetics 102, 1011- 1015

Souza TLPO, Alzate-Marin AL, Moreira MA, Barros EG (2005) Análise da variabilidade patogênica de *Uromyces appendiculatus* em algumas regiões brasileiras. Fitopatologia Brasileira 30, 143-149

Souza TLPO, Ragagnin VA, Sanglard D, Moreira MA, Barros EG (2007a) Identification of races of selected isolates of *Uromyces appendiculatus* from Minas Gerais (Brazil) based on the new international classification system. Fitopatologia Brasileira 32, 104-109

Souza TLPO, Alzate-Marin AL, Dessaune SN, Nunes ES, Queiroz VT, Moreira MA, Barros EG (2007b) Inheritance study and validation of SCAR molecular marker for rust resistance in common bean. Crop Breeding and Applied Biotechnology 7, 11-15

Souza TLPO, Alzate-Marin AL, Faleiro FG, Barros EG (2008) Pathosystem common bean–*Uromyces appendiculatus*: host resistance, pathogen specialization, and breeding for rust resistance. Pest Technology 2, 56-69

Staples RC (2000) Research on the rust fungi during the twentieth century. Annual Review of Plant Pathology 38, 49-69

Stavely JR (2000) Pyramiding rust and viral resistance genes using traditional and marker techniques in common bean. Annual Report of the Bean Improvement Cooperative 44, 1-4

Stavely JR, Freytag GF, Steadman JR, Schwartz HF (1983) The 1983 Bean Rust Workshop. Annual Report of the Bean Improvement Cooperative 26, iv-vi

Stavely JR, Pastor-Corrales MA (1989) Rust. In: Schwartz HF, Pastor-Corrales MA (Eds) Bean production problems in the tropics. CIAT, Cali, pp 159-194

Steadman JR, Pastor-Corrales MA, Beaver JS (2002) An overview of the 3rd Bean Rust and 2nd Bean Common Bacterial Blight International Workshops, March 4-8, 2002, Pietermaritzburg, South Africa. Annual Report of the Bean Improvement Cooperative 45, 120-125

Thrall PH, Burdon JJ (2003) Evolution of virulence in a plant host-pathogen metapopulation. Science 299, 1735-1737

Toenniessen GH, O´Toole JC, Devries J (2003) Advances in plant biotechnology and its adoption in developing countries. Current Opinion in Plant Biology 6, 191-198

Vieira C, Borém A, Ramalho MAP, Carneiro JES (2005) Melhoramento do feijão. In: Borém A (Ed) Melhoramento de espécies cultivadas (2nd Edn). Universidade Federal de Viçosa, Viçosa, pp 301-391

Wortmann CS, Kirkby RA, Eledu CKA, Allen DJ (1998) Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa. CIAT, Cali, 17 pp

Yoshimura S, Yoshimura A, Iwata N, McCouch SR, Abenes ML, Baraoidan MR, Mew TW, Nelson RJ (1995) Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. Molecular Breeding 1, 375-387

Livros Grátis

(http://www.livrosgratis.com.br)

Milhares de Livros para Download:

Baixar livros de Literatura
Baixar livros de Literatura de Cordel
Baixar livros de Literatura Infantil
Baixar livros de Matemática
Baixar livros de Medicina
Baixar livros de Medicina Veterinária
Baixar livros de Meio Ambiente
Baixar livros de Meteorologia
Baixar Monografias e TCC
Baixar livros Multidisciplinar
Baixar livros de Música
Baixar livros de Psicologia
Baixar livros de Química
Baixar livros de Saúde Coletiva
Baixar livros de Serviço Social
Baixar livros de Sociologia
Baixar livros de Teologia
Baixar livros de Trabalho
Baixar livros de Turismo