ANA LETICIA ROCHA MONTEIRO

QUANTITATIVE RESISTANCE OF *Phaseolus vulgaris* TO COMMON BACTERIAL BLIGHT: SOURCES, INHERITANCE AND GENOMIC ASSOCIATION

Thesis presented to the Plant Pathology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae.*

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“*Utopía está en el horizonte. Me acerco dos pasos, ella se aleja dos pasos. Camino diez pasos y el horizonte se corre diez pasos más allá. Por mucho que yo camine, nunca la alcanzaré. ¿Para que sirve la utopía? Para eso sirve: para caminar*.”
Eduardo Galeano

To my parents, Cezar and Lusivane,
To my sisters Camila and Caroline
To my nieces Ana Luiza and Maria Cecília
To all my family and friends who made me who I am
I dedicate

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BIOGRAPHY

ANA LETICIA ROCHA MONTEIRO, daughter of Lusivane da Rocha Monteiro and Cezar Henrique Silva Monteiro, was born in São Luis - MA on June 26th, 1991. In September 2008, she joined the Universidade Estadual de Maranhão (UEMA), São Luis - MA, to study Agronomy, graduating on August 9th, 2013. During her graduation, she received two scientific initiation studentships at the Plant Pathology Laboratory to conduct research under the guidance of Dr. Alice Rodrigues. In August 2013, she began her Master's studies in the Graduate Program in Plant Pathology at Universidade Federal Rural de Pernambuco (UFRPE), Recife - PE under the advisory of Prof. Sônia Oliveira, having defended her dissertation on March 24th, 2015. In March 2015, she joined the PhD Program in Phytopathology at UFV, and defended her thesis under the guidance of Prof. Jorge Luis Badel and co-advisory of Prof. José Eustáquio de Souza Carneiro and Pedro Crescêncio Souza Carneiro.

SUMMARY

ABSTRACT

MONTEIRO, Ana Leticia Rocha, D.Sc., Universidade Federal de Viçosa, February, 2019. **Quantitative Resistance of *Phaseolus vulgaris* to Common Bacterial Blight: Sources, Inheritance and Genomic Association.** Advisor: Jorge Luis Badel. Co-Advisors: José Eustáquio de Souza Carneiro, Pedro Crescêncio Souza Carneiro and Fabrício de Ávila Rodrigues.

Common Bacterial Blight (CBB), caused by the Gram-negative bacterium *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) is one of the major diseases that affect common bean production worldwide. To understand diverse aspects of the mechanisms behind resistance of common bean to CBB, this study first (Chapter 1) demonstrated that the use of scissors and multiple needle inoculation methods, that introduce the bacterium directly into the intercellular spaces, result in disease symptoms considered more severe than spray inoculation and, because it resembles natural infection, the latter was chosen to conduct further experiments. Of nine genotypes tested, BRS Radiante and IAPAR 16 exhibited high resistance against seven *Xap* isolates from different origins. The resistance of these varieties was associated with restriction of bacterial multiplication *in planta* and was not necessarily related to stomatal density. In the second step (Chapter 2), hybrids of genotype combinations in a partial diallel scheme (3 x 5) were generated to identify the most promising for resistance to CBB, based on estimates of general combining ability (GCA) and specific combining ability (SCA). Because of the lack of reliable morphological markers to distinguish hybrids from parents for several of these combinations, a toolkit of SCAR (Sequence Characterized Amplified Region) markers was assembled. Based on GCA values, it was concluded that variety BRS Radiante greatly contributes to enhance common bean resistance to CBB whereas nine hybrid combinations were shown to be promising to increase resistance to CBB according to SCA values. Then, hypotheses for resistance inheritance in the BRS Radiante × Carioca MG combination were tested by chi-square statistics. The resistance inheritance of BRS Radiante to CBB was best explained by a 9:7 ratio, suggesting that it is conditioned by two complementary dominant genes. In addition, a maximum likelihood analysis indicated the effect of a main dominant gene with additive effect and polygenes involved in CBB resistance. In the third step (Chapter 3) of this work, BC_1F_1 and BC_2F_1 plants derived from the combination BRS Radiante (parental donor) × Carioca MG (recurrent parent) were obtained. The backcrossed plants obtained were genotyped with SCAR markers and phenotyped for resistance to CBB. Two BC_1F_1 and BC_2F_1 plants were highly resistant to CBB, indicating that they are excellent candidates to advance them to the next generations. Finally (Chapter 4), it was aimed to classify 103 common bean varieties for resistance to CBB based on disease severity and area under the disease progress curve (AUDPC). Twenty-nine varieties with high levels of horizontal resistance to CBB associated with lower AUDPC values were identified. Then, an exploratory Genome-Wide Association Study (GWAS) was conducted with 80 of these varieties using 384 Single Nucleotide Polymorphism (SNP) markers. Genes coding for proteins whose putative functions have previously been associated with plant resistance to diseases, such as serine/threonine kinases, glutamine synthetases, lectin-domain proteins, among others, were identified. The main contributions of this study to the scientific community are: knowledge on common bean genotypes highly resistant to CBB; knowledge on generation of genotype combinations appropriate to obtain resistance against CBB; biological material useful to undertake studies on the genetic and molecular mechanisms underlying common bean resistance against CBB; candidate genes that may be involved in the resistance response of common bean to *Xap*; and molecular tools useful in the identification of common bean hybrid plants. The knowledge and biological material generated in this work set the stage for additional studies on the common bean-*X. axonopodis* pv. *phaseoli* interaction.

RESUMO

MONTEIRO, Ana Leticia Rocha, D.Sc., Universidade Federal de Viçosa, fevereiro de 2019. **Resistência Quantitativa de *Phaseolus vulgaris* ao Crestamento Bacteriano Comum: Fontes, Herança e Associação Genômica.** Orientador: Jorge Luis Badel. Coorientadores: José Eustáquio de Souza Carneiro, Pedro Crescêncio Souza Carneiro e Fabrício de Ávila Rodrigues.

O Crestamento Bacteriano Comum (CBC), causado pela bactéria Gram-negativa *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) é uma das principais doenças que prejudicam a produção do feijão em todo o mundo. Para entender sobre os diversos mecanismos subjacentes à resistência do feijoeiro ao CBC, este estudo (Capítulo 1) primeiro demonstrou que métodos de inoculação com tesouras e agulhas múltiplas, que introduzem a bactéria diretamente nos espaços intercelulares, resultam em sintomas considerados mais severos da doença do que a inoculação por pulverização, a qual, por se assemelhar mais à infecção natural, foi escolhida para conduzir experimentos adicionais. Dentre nove genótipos testados, BRS Radiante e IAPAR 16 mostraram alta resistência contra sete isolados de *Xap* de diferentes origens. A resistência dessas variedades esteve associada a restrição da multiplicação bacteriana *in planta* mas não necessariamente à densidade estomática. Na segunda fase (Capítulo 2), geraram-se híbridos de combinações genotípicas em um esquema de dialelo parcial (3 x 5) para identificar cruzamentos promissores quanto à resistência ao CBC, baseado em estimativas de capacidade geral de combinação (CGC) e capacidade específica de combinação (CEC). Devido à falta de marcadores morfológicos confiáveis para distinguir os híbridos dos parentais para algumas combinações, um grupo de marcadores SCAR (*Sequence Characterized Amplified Region*) foi utilizado. Baseado nos valores de CGC, concluiu-se que a variedade BRS Radiante contribui no acréscimo da resistência ao CBC enquanto que nove combinações de híbridos se mostraram promissoras para aumentar a resistência ao CBC segundo valores de CEC. Em seguida, testou-se hipóteses para a herança da resistência ao CBC na combinação BRS Radiante × Carioca MG mediante teste do qui-quadrado. A herança da resistência da variedade BRS Radiante ao CBC explicou-se por uma proporção de segregação 9:7, sugerindo que é condicionada por dois genes dominantes complementares. Além disso, a análise de máxima verossimilhança indicou o efeito de um gene principal dominante com efeito aditivo e poligenes envolvidos na resistência ao CBC. Na terceira fase (Capítulo 3) deste trabalho, geraram-se plantas F_1RC_1 e F_1RC_2 derivadas da combinação BRS Radiante (parental doador) × Carioca MG (parental recorrente). As plantas retrocruzadas obtidas foram genotipadas com marcadores SCAR e fenotipadas para resistência ao CBC. Duas plantas F_1RC_1 e F_1RC_2 se mostraram altamente resistentes, indicando que são candidatas para avança-las às próximas gerações. Por último (Capítulo 4), objetivou-se classificar 103 variedades de feijoeiro comum para resistência ao CBC com base na severidade da doença e área abaixo da curva de progresso da doença (AACPD). Identificaram-se vinte nove variedades com altos níveis de resistência horizontal ao CBC associada a baixos valores de AACPD. Depois, realizou-se um estudo exploratório de associação genômica ampla (GWAS) com 80 dessas variedades usando 384 marcadores baseados em polimorfismo de nucleotídeo único (SNP). Identificaram-se genes que codificam para proteínas cujas funções têm sido associadas com resistência das plantas a doenças, tais como serina/treonina quinases, glutamina sintetases, proteínas com domínio de lectina, entre outras. As principais contribuições deste estudo à comunidade científica são: conhecimento sobre genótipos de feijoeiro comum altamente resistentes ao CBC; conhecimento e geração de combinações de genótipos apropriados para obter resistência ao CBC; material biológico útil para desenvolver estudos sobre os mecanismos genéticos e moleculares envolvidos na resistência do feijoeiro comum a *Xap*; e ferramentas moleculares uteis na identificação de plantas híbridas de feijoeiro comum. O conhecimento e material biológico gerado neste trabalho fornece a base para estudos adicionais sobre a interação feijoeiro comum- *X. axonopodis* pv. *phaseoli.*

GENERAL INTRODUCTION

Brazilians have common bean (*Phaseolus vulgaris* L.) as one of the most traditional foods in their diet. Brazil is the second largest common bean producer in the world after India; Myanmar ranks in third place (FAOSTAT, 2019). In the 2017/2018 period, the Brazilian production of this legume achieved 3.3 million tons in an estimated area of around 3,244,300 hectares (CONAB, 2019).

The Common Bacterial Blight (CBB) disease can cause reductions in field production from 10% to 45% depending on the environmental conditions and the level of resistance of the common bean variety (Fininsa, 2003). The disease is caused by the Gram-negative bacterium *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) [(Smith) Vauterin *et al.* (synonym *X. campestris* pv. *phaseoli* Smith (Dye)). Common Bacterial Blight limits the production of common bean in several producing areas around the world (Miklas *et al.*, 2017).

The symptoms of CBB mainly affect the leaves and pods of common bean plants. On leaves, initially water-soaked spots appear, which are more evident on the underside. These symptoms later evolve to form irregular necrotic lesions surrounded by chlorotic haloes. The necrosis can be quite intense and may cause defoliation. On pods water-soaked spots appear, which can become darker, red, and lightly depressed. (Saettler, 1989). The bacteria penetrate through wounds and natural openings, mainly by the stomata (Akhavan *et al.*, 2013). Disease development is favored by temperatures between 28-32 °C; the most severe symptoms are observed when temperatures are close to 30 °C and the humidity is high (Yu *et al.*, 1998). The use of varieties with high levels of resistance is one of the main alternatives to minimize losses in production and make it possible to obtain seeds free of the pathogen (Singh & Muñoz, 1999).

Identification of sources of resistance is important for common bean breeding programs. After their identification, it is necessary to cross those sources of resistance with varieties that possess desirable agronomic characteristics for the purpose of introgressing the resistance genes into varieties that can be released to the farmers. (Kelly & Miklas, 1998). The resistance should desirably be of broad-spectrum. Hence, it is necessary that the common bean genotypes expressing high genetic resistance to CBB be challenged with several isolates from different geographic origins (Bar *et al.*, 2008). Also, unless the screening of sources of resistance are conducted in the field under the pressure of the native pathogen population, appropriate inoculation methods (preferably resembling natural infection) should be used (Silva *et al.*, 2009; Scandiani *et al.*, 2011).

Previous studies have shown that the resistance of *P. vulgaris* to CBB is of horizontal type, also called quantitative (Coyne & Schuster, 1983; Singh & Muñoz, 1999; Ferreira *et al.*, 2003; Tryphone *et al.*, 2012). As opposed to qualitative resistance, in which the phenotypes are associated with presence or absence of disease symptoms, quantitative resistance is characterized by different levels of disease in diverse plant genotypes. Because of that, the differentiation between resistance levels based on severity of symptoms is not always easy, and thus, it is important to determine the progress of the disease over time, most commonly through an estimation of the area under the disease progress curve (AUDPC) (Andrivon *et al.*, 2006; Andrade *et al.*, 2017).

In order to make good use of the sources of resistance identified, a better knowledge about the genetics and inheritance of resistance is important, which helps determine the most appropriate approaches for gene introgression (Chataika *et al.*, 2011; Tryphone *et al.*, 2012). Therefore, after the selection of varieties with high levels of resistance to CBB, the contribution to resistance of particular genotypes could be assessed by conducting diallelic analysis, for which the most commonly used method is that of Griffing (1956). This method provides the so-called General Combining Ability (GCA) estimates; In the case of disease resistance, a negative GCA value for a particular genotype suggests that it contributes positively to that particular trait. The Griffing (1956) method also allows estimates of Specific Combining Ability (SCA) for combinations (crosses) between genotypes, which provides indications as to whether a particular cross has the potential to enhance the character under study, in this case resistance to CBB. Based on this approach, the most promising combination could be selected to obtain improved genotypes or to conduct molecular and genetic studies (Ramalho *et al.*, 1993; Jung *et al.*, 2007).

To gain insights into the inheritance of resistance, crosses between resistant and susceptible genotypes to generate segregant populations are made. These segregating populations are essential to estimate the genetic contribution of the selected varieties (Bertan *et al.*, 2005). To ascertain hybridization between the parents, it is necessary to distinguish hybrids from the parents. Phenotype-based assessment is only reliable if phenotypic traits are highly contrasting between parents. When morphological characters are not enough for such a distinction, the use of molecular markers can facilitate confirmation of hybridization in the candidate hybrid plants (Sharma *et al.*, 2018). Amongst the markers capable of distinguishing hybrids are the Sequence Characterized Amplified Region (SCAR) markers, which are considered good tools due to their low cost, simplicity and easy implementation (Yu *et al.*, 2000). SCAR markers are derived from Random Amplification of Polymorphic DNA (RAPD) markers. After observation of RAPD tags, the fragment is sequenced and specific primers of approximately 24 base pairs are designed. Thus, SCAR primers are targeted to specific regions of the genome (Zaccaro *et al.*, 2007; Borém & Caixeta, 2016).

Traditionally, the identification of genes conferring disease resistance have been accomplished by positional mapping and cloning (Puliti *et al.*, 2007). Nonetheless, with the advent of new generation sequencing technologies, alternative approaches can be utilized to speed up the process and identify candidate genes (Silva *et al.*, 2012). Two different alternatives have recently been used for this purpose: comparative transcriptomics and Genome-Wide Association Studies (GWAS) (Martin *et al.*, 2013; Turchetto-Zolet *et al.*, 2017). In the former approach, differences in gene expression between resistant and susceptible genotypes upon disease pressure are determined by massive RNA sequencing (RNAseq) (Hewezi & Baum, 2012; Silva *et al.*, 2012). Nonetheless, this approach is more efficient in identifying *bona fide* candidate genes if differences in the genetic background unrelated to resistance between the genotypes being compared is minimized, and backcrossing could serve this purpose (Lorencetti *et al.*, 2006). Studies of comparative transcriptomics also allow to gain some insights into the molecular mechanisms governing resistance (Jain *et al.*, 2016; Meyer *et al.*, 2017; Todd *et al.*, 2017; Ma *et al.*, 2019).

Information on genes potentially associated with plant resistance to disease has also been accessed through GWAS studies (Perseguini *et al.*, 2016; Resende *et al.*, 2018). This approach takes advantage of the natural variation in the population to identify SNP (single nucleotide polymorphisms) that could be associated with resistance (Wang *et al.*, 2012; Perseguini *et al.*, 2016; Mourad *et al.*, 2018). SNPs are polymorphisms of a single nucleotide in a specific position in the genome that result from nucleotide substitutions. They are an abundant form of variation in eukaryotic genomes, and so, they provide a rich source of DNA polymorphisms (Borém & Caixeta, 2016). This type of molecular marker allows a broad and efficient genome coverage that provides a high-resolution power to GWAS studies (Elshire *et al.*, 2011).

Thus, the main objectives of this work were: (i) to evaluate different methods for *Xap* inoculation on common bean plants and to select the most appropriate for germplasm screening; (ii) to identify common bean genotypes expressing high levels of resistance to CBB and to determine if their resistances are related to restriction of bacterial growth in plant tissue or to stomatal density with the purpose of using them in studies on the genetics and molecular mechanisms underlying resistance; (iii) to estimate the contribution to CBB resistance of the highly resistant varieties identified and the potential of their combinations with susceptible genotypes to enhance CBB resistance through a partial diallel analysis; (iv) to determine the type of resistance acting in a BRS Radiante × Carioca MG cross through inheritance and maximum likelihood function tests; (v) to assemble a set of SCAR markers capable of differentiating parents from hybrids in those genotype combinations; (vi) to generate backcross plants appropriate to undertake comparative transcriptomic studies on resistance to CBB; and (vii) to identify genes potentially associated with common bean resistance to CBB through GWAS. The results are presented in four chapters, each addressing specific and related aspects of the work.

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Chapter 1 - Research Article

Sources of broad-spectrum quantitative resistance in *Phaseolus vulgaris* against Common Bacterial Blight

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ABSTRACT

Common bean (*Phaseolus vulgaris*) is one of the most consumed agricultural products in the world. Its production is affected by Common Bacterial Blight (CBB) caused by the Gram-negative bacterium *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*). Plant genetic resistance to CBB is one of the most desirable forms of disease control since it does not adversely affect the environment and is of low cost for farmers. The objective of this work was to identify common bean genotypes with high-level, broad-spectrum, quantitative resistance against *Xap* suitable to undertake studies aimed at understanding the genetic and molecular bases of this type of resistance. To this aim, we first tested the scissors, multiple-needle and spray inoculation methods on the common bean genotypes Ouro Negro and Diamante Negro and found that while the first two classified both genotypes as susceptible, the latter classified them as moderately resistant and was selected for further experimentation because it better resembles natural infection. Spray inoculation of nine selected common bean genotypes with a *Xap* isolate showed that BRS Radiante and IAPAR 16 were highly resistant. Inoculation of these two genotypes with six additional *Xap* isolates of different origins demonstrated their broad-spectrum resistance and revealed pathogenic differences among isolates. Quantification of bacterial populations in foliar tissue of these resistant genotypes also indicated lower numbers when compared with susceptible varieties. In addition, quantification of stomatal density of varieties IAPAR 16 and BRS Radiante by obtaining enamel printings of the abaxial leaf surfaces showed that they have significantly higher densities than variety IAPAR 81 and no difference with respect to Carioca MG, both considered susceptible. Overall, the results of this study demonstrate that the resistance to CBB expressed in varieties IAPAR 16 and BRS Radiante is of broad-spectrum, associated with restriction of bacterial multiplication *in planta* and not necessarily related to stomatal density. These two resistant varieties are potential sources of plant genetic resistance for breeding programs and good candidates to undertake studies aimed at understanding the genetic and molecular mechanisms underlying quantitative resistance to CBB.

KEYWORDS: bacterial multiplication, *Phaseolus vulgaris*, stomatal density, *Xanthomonas axonopodis* pv. *phaseoli*.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important legume crops worldwide because of its high nutritional content, and consequently, its commercial value (Popovic *et al.*, 2012). One of the most serious diseases affecting its productivity is Common Bacterial Blight (CBB) caused by the Gram-negative bacterium *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Vauterin (Nunes *et al.*, 2008). It has been estimated that CBB causes yield reductions between 10 and 45%, depending on the environmental conditions and the susceptibility of the host genotype (Tar'an *et al.*, 2001; Gillard *et al.*, 2005).

The pathogen penetrates through natural openings (primarily stomata) and wounds and spreads systemically throughout the plant affecting different types of tissue. The initial symptoms of CBB appear on the leaves in the form of water-soaked lesions, more readily visible on the abaxial side. The spots increase in size and coalesce, forming large necrotic areas surrounded by chlorotic haloes that advance with the progress of the disease, resulting in premature fall of the leaves. The bacterium can colonize the stem causing cankers. Infected pods show water-soaked lesions that may become brown and infected seeds can develop extensive discoloration (Saettler, 1989).

As for many other bacterial plant diseases, chemical control of CBB is difficult because of the limited number of available products and their low inefficacy as curative methods. Hence, to control CBB, it is recommended to use certified seeds (Mutlu *et al.*, 2005; Darrasse *et al.*, 2007), plant resistant varieties (Tar'an *et al.*, 2001), practice crop rotation with non-hosts and deep tillage to incorporate crop residues into the soil (Paula & Zambolim, 2006). The use of plant genetic resistance is the preferred alternative since it provides good disease control, is easily adopted because of its low cost to the farmers and is ecologically safe, reducing or even avoiding the indiscriminate use of pesticides (Costa & Rava, 2006).

A few studies reported on the identification of resistance to CBB in common bean germplasm, but the number, degree of action and interaction of the genes involved were shown to be very variable. Genotypes of common bean resistant to CBB were identified in studies carried out in Brazil. High resistance to CBB was identified in the genotypes BRS Radiante in the field (Costa *et al.*, 2008, Maringoni, 1998), and in IAPAR 16 and Diamante Negro under greenhouse conditions (Silva *et al.*, 2000). On the other hand, other genotypes, including Ouro Negro, IPR Chopim, BRS Campeiro, IAPAR 81, Manteigão Fosco 11 and Carioca MG were classified as either moderately susceptible or highly susceptible (Maringoni, 1998; Kobayasti *et al.*, 1999; Silva *et al.*, 2000; Costa *et al.*, 2008). Genetic breeding aiming resistance to CBB is complex because of the genetic diversity of the pathogen and its co-evolution with the host (Coyne & Schuster, 1983; Rodrigues *et al.*, 1999; Ferreira *et al.*, 2003; Santos *et al.*, 2003). Most reports have shown that resistance is mainly quantitative (Singh & Muñoz, 1999; Tryphone *et al.*, 2012), with exception of a single study in which qualitative resistance was found (Zapata *et al.*, 2011).

Plants are constantly challenged by a large diversity of microbes, but they have several defense mechanisms to prevent penetration, multiplication and infection by phytopathogens. Among the structural defense mechanisms utilized by plants against bacteria are the cuticle, stomates and trichomes (Melotto *et al.*, 2008; Arnaud & Hwang, 2015). Stomates are present in large numbers on both abaxial and adaxial leaf sides depending on the plant species and are the main port of entry into the host tissue for foliar bacterial pathogens. Nonetheless, stomates also play a key role in plant defense and resistance (Melotto *et al.*, 2006, 2008). For instance, it has been proven that stomatal density (number of stomates per leaf area) may be related to plant resistance against bacterial pathogens (Ramos & Volin, 1987; Nicolas *et al.*, 2018).

Inducible plant defense responses also occur in the stomates. In the first tier of the plant immune response, pathogen-associated molecular patterns (PAMPs) recognition by membrane-associated receptor-like kinases (RLK) activates signal cascades leading to the so-called PAMP-triggered immunity (PTI). For example, recognition of the flagellum (a PAMP) by its receptor FLS2 (Flagellin Sensing 2; an RLK) triggers a signaling that ends in stomatal closure to prevent bacterial entry into the plant tissue (Melotto et al., 2006; 2008). Perception of PAMPs is thought to be related to basal defense (also called partial, quantitative or horizontal resistance) (Arnaud & Hwang, 2015; Kushalappa *et al.*, 2016).

The objective of this work was to identify common bean genotypes exhibiting broad-spectrum and high levels of resistance to CBB suitable to undertake further studies on the molecular basis of quantitative (horizontal) resistance. First, we evaluated different inoculation methods to select the most suitable for assessing the plant responses and then evaluated the reaction of a select group of common bean genotypes upon challenge with pure *Xap* isolates. We quantified the bacterial population in resistant and susceptible varieties to assess a possible correlation between the ability to sustain pathogen populations and disease development. The implication of stomatal density on the resistant phenotype was also investigated.

MATERIALS AND METHODS

Bacterial strains

The *Xap* isolates used in this study either belong to the culture collection of the Laboratory of Molecular Phytobacteriology at the Universidade Federal de Viçosa (LBM-

UFV) or were obtained from the Laboratory of Phytobacteriology at the Universidade Federal de Lavras (Table S1). Resistance to rifampicin in strain *Xap-7* was induced by growing it at 28 °C for 24 h on solid 523 medium (Kado and Heskett, 1970) ammended with successive increasing concentrations of the antibiotic (from 1 to 100 µg ml^{-1}). A strain obtained from a single rifampicin-resistant colony was multiplied and stored in the LBM-UFV collection (Lilian Cação Costa, unpublished). Bacterial isolates were routinely recovered from 30% glycerol stocks at -80 °C by plating on solid 523 medium (Kado & Heskett, 1970) and incubating at 28 °C for 48 h.

Plant material and maintenance

The seeds of the common bean genotypes used in this study were obtained from the Germplasm bank of the Universidade Federal de Viçosa (UFV), Viçosa, MG, Brazil. The genotypes were selected based on previous reports regarding their responses to CBB. Accessions classified as highly resistant or highly susceptible in previous studies and that have phenotypic and agronomic characteristics similar to those of cultivated varieties were selected (Maringoni, 1998; Kobayasti *et al.*, 1999; Silva *et al.*, 2000; Costa *et al.*, 2008).

Pure seeds of the nine common bean varieties Ouro Negro, Diamante Negro, BRS Campeiro, BRS Radiante, IAPAR 81, Carioca MG, Manteigão Fosco 11, IPR Chopim and IAPAR 16 were used in the experiments (Table S2). To accelerate the germination process and favor a homogeneous growth of the seedlings, the seeds were scarified by scrapping the tegument with the aid of sandpaper. Then, the seeds were pre-germinated on germination paper sterilized in an oven at 100 ºC for five hours. After the emission of the radicle (after approx. 3 days), the seeds were transplanted to 2-l pots containing a 1:1 mixture of substrate Tropstrato HT Hortaliças® (Vida Verde, Mogi Mirim, SP, Brazil) and soil. Every 15 days the plants were fertilized with Niphokam® (Fênix-Agro-Pecus Industrial Ltda, Tietê, SP, Brazil) and watered according to the daily requirement. During warmer climate, they were watered twice a day. The plants were maintained in a greenhouse or a growth chamber with controlled temperature (25 ºC) at the Department of Plant Pathology (DPP) of the UFV.

Evaluation of inoculation methods

In order to select the most suitable method to evaluate the response of common bean genotypes to inoculation with *Xap*, the accessions Ouro Negro and Diamante Negro were inoculated using three different methods previously reported in the literature: delivering bacterial cells in suspension to the plant by spraying, using multiple-needles or using scissors (Andrus, 1948; Schoonhoven & Pastor-Corrales, 1987; Rava, 1984). For all methods, the third fully expanded trifoliate of each plant (stage V3) was inoculated and the same inoculum preparation was used. Cultures of the *Xap-7* strain were grown on solid 523 medium (Kado & Heskett, 1970) at 28 ºC for 24-48 h. Bacterial cells were resuspended in 10 mM $MgCl_2$ and the OD_{600} adjusted to 0.1. At least five plants of each genotype were treated with 10 mM $MgCl_2$ to serve as controls.

To inoculate using multiple needles, a 7-pin-needle inoculator was immersed in the bacterial suspension and the selected trifoliate were pricked with it (Andrus, 1948). Disease symptoms were recorded at 15 days after inoculation (dai) using a 1 to 5 rating scale based on necrotic, water-soaked, and chlorotic leaf areas, in which 1 is no disease symptoms, 2 is 1% to 5%; 3 is >5% to 25%; 4 is >25% to 50%; and 5 is > 50% of the inoculated area with symptoms, according to Arnaud-Santana *et al.* (1993).

For the scissors inoculation method, the chosen leaves were clip inoculated with scissors dipped in the bacterial suspension (Rava, 1984). Two cuts perpendicular to the central vein were made in each leaflet, at an approximate 5-cm distance from one another. Disease evaluations were performed using a rating scale ranging from 1 (absence of symptoms) to 5 (chlorosis and/or necrosis in more than 50% of the area comprised between the two cuts), adapted from Fernández-Isaula (1982).

Inoculation by spraying was performed by applying the bacterial suspension onto both leaf surfaces with an atomizer Jet Master 1/3 HP (Schulz S.A., Joinville, SC, Brazil) until run-off point. The severity of the disease was evaluated using a diagrammatic 1-9 scale proposed by Schoonhoven & Pastor-Corrales (1987). The plants were maintained in a mist chamber at 25 °C for 24 h before and after inoculation to promote stomatal aperture and facilitate bacterial penetration.

Inoculated plants using the three different methods were maintained in a greenhouse in a completely randomized design in a 3×2 factorial (three inoculation methods and two common bean genotypes) and three repetitions. The experimental unit consisted of two plants per pot (both of which were independently evaluated). For each inoculation method, the severity means of the two varieties were compared by t-test ($P \leq 0.05$), and classified as resistant (R), moderately resistant (MR), moderately susceptible (MS) or susceptible (S) based on the appropriate disease rating scale.

Screening for resistance to Common Bacterial Blight

Plants of varieties Ouro Preto, Diamante Negro, BRS Campeiro, BRS Radiante, IAPAR 81, Carioca MG, Manteigão Fosco 11, IPR Chopim and IAPAR 16 were planted in September, 2016 and the whole experiment repeated in March, 2017. At the V3 phenological stage, they were spray inoculated with a suspension of the rifampicin-resistant strain *Xap-7*. Inoculum preparation and plant inoculation were performed as indicated above. Seven plants of each common bean genotype, were treated with 10 mM $MgCl_2$ to serve as controls. Inoculated plants were kept in the greenhouse using a completely randomized design, in which the experimental unit consisted of two plants per pot (both of which were evaluated), with six repetitions per genotype. At 15 dai, the two most affected trifoliates of each plant were selected and scanned at 600 dpi resolution with an HP scanner model PSC 1500 series (Hewlett-Packard, Palo Alto, CA, USA) to obtain images for disease severity evaluation. The images were processed using the QUANT software version 1.0.1 (Vale *et al.,* 2003) to quantify the leaf area containing lesions. The data were subjected to analysis of variance and the means were compared by the Scott-Knott test ($P \leq 0.05$).

Assessment of the resistance spectrum

The spectrum of resistance of varieties IAPAR 16, BRS Radiante (highly resistant to CBB) and Carioca MG (susceptible to CBB) was assessed by spray inoculating them with seven *Xap* isolates from diverse origins. Each genotype was independently inoculated with each isolate. Inoculum preparation and plant inoculation were conducted as described above. The experiment was set up in a completely randomized block design, in which each block comprised one repetition of all plants inoculated with the same isolate and each experimental unit consisted of two plants per pot (both of which were evaluated), with five repetitions per treatment. Disease evaluation was performed using the QUANT software (Vale *et al.,* 2003) as indicated above. The data were subjected to analysis of variance and the means were compared by the Scott-Knott test ($P \leq 0.05$).

Quantification of *in planta* bacterial populations

Three 0.7-cm diameter discs were taken using a cork-borer from the same leaves that were used for disease severity assessment, such that the location of the sampled area was standardized for all treatments. The discs were macerated in 1 ml of 10 mM $MgCl_2$ solution inside 2-ml microfuge tubes and serial dilutions from 10^{-1} to 10^{-6} of the macerates were prepared. A 20-µl aliquot of each dilution was placed on solid 523 medium amended with rifampicin (100 µg ml^{-1}) and cycloheximide (50 µg ml^{-1}) and the plates incubated at 28 °C. After 24 h, the number of individual colonies on the medium was counted and the number of colonies forming units per square centimeter of plant tissue (CFU cm^{-2}) was calculated. Each dilution was evaluated in triplicate. The data were subjected to analysis of variance and the means were compared by the Tukey test ($P \leq 0.05$).

Determination of stomatal density

In order to determine the stomatal density, the varieties IAPAR 16, BRS Radiante (highly resistant to CBB), Carioca MG and IAPAR 81 (susceptible to CBB) were used. The plants were grown in the greenhouse as indicated above and evaluated when they were at the V3 phenological stage. Six plants of each variety were used in the experiment. Two fully expanded leaves from the trifoliate of each plant were collected. A colorless cellulose acetate enamel film was carefully applied on the abaxial sides of the leaves and let dry for 3 min. Then, the films were gently removed to obtain printings of the leaf surface, which were later analyzed under a light microscope Olympus (CX31, Shinjuku, TYO, Japan) using the 40x objective. Images were captured with the camera of a cell phone J7 Prime (Samsung, Seoul, South Korea) and the stomata counted. The formula D = (number of stomata)/0,272902 mm^2 was used to calculate the stomatal density of each variety. The data were $\log_{10}$ transformed, subjected to ANOVA and the transformed means compared with the Scott-Knott test ($P \leq 0.05$).

Statistical analysis

Statistical analyses of all experiments conducted in this study were applied using the modules of the R software (R Development Core Team, 2018): agricolae (Mendiburu, 2017), easyanova (Arnhold, 2013), ExpDes for analysis of variance (Ferreira *et al.*, 2018), lattice for data visualization (Sarkar, 2008), and ScottKnott, for mean comparations (Jelihovschi *et al.*, 2014).

RESULTS

Selection of the inoculation method

Inoculation of *Xap-7* on Ouro Negro and Diamante Negro resulted in high disease severity as assessed by appropriate rating scales for each method. ANOVA did not indicate significant differences between varieties for each inoculation method (Table 1). Both varieties were classified as MR by the spray inoculation method and as S by the scissors and multiple needles methods. Thus, considering the fact that it better resembles the conditions of natural infection, the spray inoculation method was chosen to conduct further experiments.

Levels of resistance expressed in different common bean genotypes

The first symptoms of CBB appeared on inoculated leaves beginning at 11 dai as water-soaked spots that later developed onto necrotic lesions surrounded by chlorotic haloes. Differences in disease severity among varieties began to be readily noticeable at 15 dai, which allowed the distinction between resistant and susceptible phenotypes. According to the separation of mean percent affected leaf areas, varieties IAPAR 81, Carioca MG, BRS Campeiro, Diamante Negro, Manteigão Fosco 11 e IPR Chopim were classified as susceptible whereas IAPAR 16 and BRS Radiante were classified as highly resistant. The disease severity exhibited by these two genotypes was consistently lower than those of the other genotypes tested in both experiments (Table 2). Variety Ouro Negro grouped along with the resistant genotypes in the first experiment, but in the second, it exhibited a response similar to those of susceptible genotypes (Table 2).

Resistance of bean varieties is effective against isolates from different origins

In order to investigate how broad the resistance to CBB exhibited by some common bean genotypes was, we challenged them with *Xap* isolates of different origins. Inoculation of seven strains of *Xap* on three varieties of common bean revealed significant differences in virulence among the isolates, as demonstrated by the severity of disease symptoms caused on susceptible variety Carioca MG (Table 3). Isolates *Xap-3* and *Xap-2* failed to cause disease symptoms to the same extent as the other isolates on such variety. Importantly, varieties IAPAR 16 and BRS Radiante, classified as resistant in the previous experiments, consistently showed resistance to all isolates tested (Table 3). These results indicate that the resistance expressed by IAPAR 16 and BRS Radiante is of broad spectrum.

Resistant genotypes sustain smaller bacterial populations

The bacterial population sizes recovered from varieties IAPAR 81, Carioca MG and IAPAR 16 at 15 dai were 3.9 x 10^3; 3.8 x 10^3 and 3.7 x 10^3 CFU cm^{-2}, respectively (Figure 1). Even though Carioca MG and IAPAR 81 (classified as susceptible) had higher bacterial titers than IAPAR 16 (considered resistant), no significant differences between these varieties were detected. However, the population size (3.0 x 10^3 CFU cm^{-2}) recovered from the highly resistant variety BRS Radiante was significantly smaller than those of the other two varieties (Figure 1).

Stomatal density is not necessarily associated with disease resistance

The resistant varieties BRS Radiante and IAPAR 16 had the same stomatal density as the susceptible variety Carioca MG. Interestingly, the susceptible variety IAPAR 81 had a significantly lower stomatal density than the other three varieties (Table 4). These results indicate that the resistance of the BRS Radiante and IAPAR 16 varieties to CBB is not necessarily related to stomatal density.

DISCUSSION

In this study, we identified common bean genotypes highly resistant to CBB and suitable to undertake studies aimed at understanding the molecular mechanisms underlying plant genetic resistance. First, we tested three commonly used inoculation methods on two varieties to determine which one was more suitable for germplasm screening. Knowledge on the effectiveness of the inoculation method in reproducing disease symptoms that occur under natural infection is necessary for breeding programs to identify sources of resistance in plant germplasm (Garcia *et al.*, 2017). Evaluation of common bean genotypes for resistance to CBB using spray and multiple needles inoculation has been shown to cause plant responses similar to those observed in the field and not to alter the classification of genotypes (as either susceptible or resistant) when compared to natural infection (Popovic *et al.*, 2012). Similarly, distinction between the scissors and needle inoculation methods was not observed when the response of a larger number of common bean genotypes was evaluated in a different study (Silva *et al.*, 2009). In contrast, in the work described here, the Ouro Negro and Diamante Negro varieties inoculated using multiple needles and scissors were classified as susceptible according to rating scales appropriate for each method, whereas when spray inoculated both varieties were classified as moderately resistant. The higher susceptibility observed on plants inoculated by multiple needles and scissors, may be due to the fact that such methods deliver the bacterial cells directly to the intercellular spaces, overpassing bacterial entry through stomates. Therefore, the spray inoculation method was chosen to conduct further studies because it better resembles natural infection. The results of the experiments described in this study revealed significant differences in the foliar reaction between varieties spray inoculated with pure *Xap* isolates, making it possible to distinguish between resistant and susceptible plant phenotypes.

Screening of common bean genotypes was purposely conducted during two different seasons in order to determine if resistance to *Xap* was affected by environmental conditions. In both experiments, the first CBB symptoms were readily noticeable on the common bean genotypes at 11 dai, indicating that climatic conditions did not strongly influence the development of disease in most varieties. Two varieties (BRS Radiante and IAPAR 16), out of nine evaluated, were consistently highly resistant whereas six (IAPAR 81, Carioca MG, BRS Campeiro, Diamante Negro, Manteigão Fosco 11 and IPR Chopim) were consistently susceptible to *Xap* in both experiments. In contrast, variety Ouro Negro showed a resistance response in the first experiment, but in the second, it was classified as susceptible, suggesting that the genetic resistance present in this variety was highly influenced by environmental factors. This observation is in agreement with the fact that the development of bacterial spot has been reported to be influenced by climatic conditions (Halfeld-vieira *et al.*, 2011).

Varieties IAPAR 16 and BRS Radiante have already been shown to be highly resistant to *Xap* in other studies using different sources of inoculum (Maringoni, 1998; Silva *et al.*, 2009), suggesting that their resistances are of broad spectrum. Consistently, the results of this study demonstrate that these two varieties remained resistant when challenged with all seven *Xap* isolates tested. This information is important for the selection of plant genotypes to be used in breeding programs for disease resistance and for molecular studies aimed at understanding broad-spectrum plant genetic resistance.

Consistent with previous reports, varieties IAPAR 81, Carioca MG, Manteigão Fosco 11 and Ouro Negro were found to be susceptible in the present study (Rava & Romeiro, 1990; Kobayasti *et al.*, 1999; Costa *et al.*, 2008). On the contrary, varieties Diamante Negro, IPR Chopim and BRS Campeiro were classified as resistant in other studies, but here they exhibited a susceptible response (Silva *et al.*, 2000; Silva *et al.*, 2009). Although the authors of those studies delivered the pathogen to the plants using scissors, which is more aggressive (since it directly introduces the pathogen to the plant tissue through wounds) than by spraying, based on the results obtained using different methods in this study, it is tempting to speculate that the differences in classification may have resulted from differences in the inoculum source.

It is worth mentioning that no common bean genotype was found completely resistant (immune) to CBB. Instead, it was noticed that the varieties presented quantitative resistance since different levels of disease severity were observed. These results support those of several previous studies indicating that the resistance of common bean to *Xap* is of horizontal and polygenic nature (Singh & Muñoz, 1999; Miklas *et al.*, 2003; Tryphone *et al.*, 2012). Because the resistance observed in variety BRS Radiante and IAPAR 16 seems to be of broad spectrum, they represent excellent candidates to undertake studies aimed at understanding this type of plant resistance. Variety BRS Radiante is of Andean origin and IAPAR 16 is of Mesoamerican origin, which support the notion that sources of quantitative resistance to CBB are found in both gene pools (Souza, 2009; Barili *et al.*, 2015).

The results of experiments inoculating several different *Xap* isolates also revealed significant differences in their ability to cause disease on variety Carioca MG (considered susceptible). Isolates *Xap-2* and *Xap-3* caused little disease on such variety, which emphasizes the importance of testing different pathogen isolates in order to gain a better understanding of the resistance carried on particular genotypes of the host plant (Zamani *et al.*, 2011). Nonetheless, the varieties BRS Radiante and IAPAR 16 showed broad spectrum resistance to isolates from different geographic origins in Brazil such as Minas Gerais (*Xap 1* to *Xap-4* from Lavras and *Xap-5* from Guaxupé) and Brasília (*Xap-7*) as well as to an isolate from the United States of America (*Xap-6*). Amongst the factors that determine success in obtaining effective genetic resistance of common bean to diseases in the field is a good knowledge of the pathogenic variability and genetic structure of the aetiologic agent (Orozco & Araya, 2005). The main reason for overcoming plant genetic resistance is the capacity of the pathogen to evolve and build up a population virulent to formerly resistant varieties (Duche *et al.*, 2015).

Phytopathogenic bacteria that cause foliar spots penetrate their host plants mainly through stomata (Melotto *et al.*, 2006). After penetration, the bacterium must multiply inside the plant tissue to reach a population large enough to cause macroscopically visible disease symptoms. Therefore, symptom development is the result of colonization and multiplication of bacteria *in planta* and, in general, a positive correlation between parasitic fitness and virulence is observed. In other words, the size of the bacterial population recovered from infected plants could be a good indication of the degree of susceptibility or resistance (Wilson *et al.*, 1999; Wang *et al.*, 2017). Consistently, it was found here that the population size recovered from resistant variety BRS Radiante was significantly lower than those of susceptible varieties IAPAR 81 and Carioca MG. Nonetheless, significant differences between resistant variety IAPAR 16 and susceptible varieties were not detected. It is likely that some components of quantitative resistance that restrict pathogen growth present in BRS Radiante are absent in IAPAR 16. It is believed that the growth restriction of the pathogen is the result of the specific recognition of pathogenic strains by means of the products of plant defense genes (Rigault *et al.*, 2017). In agreement to this notion is the observation that mutations in some bacterial genes results in reduced ability of the pathogen to cause disease symptoms but not to multiply *in planta* (Badel *et al.*, 2003).

Stomata play important roles in plant defense against pathogenic bacteria that cause foliar spots (Melotto, *et al.,* 2006; Zeng *et al.*, 2010). Not only has it been demonstrated a correlation between their numbers on the leaf surface and the severity of the disease (Ramos & Volin, 1987), but also their involvement in the first line of plant defense responses against plant pathogenic bacteria. Upon perception of PAMPs, plants rapidly close their stomates to avoid bacterial cell entry into the host tissue (Melotto *et al.,* 2006; Zeng *et al.,* 2010), which is counteracted by the injection of effector proteins into the host cytoplasm by the bacterium using the type III secretion system (Kay & Bonas, 2009; Deslandes & Rivas, 2012)

After identifying the resistant bean varieties BRS Radiante and IAPAR 16, a possible association between stomatal density and the resistance response was investigated in this study. Stomatal density was evaluated on the lower leaf surface of these resistant varieties in comparison with susceptible Carioca MG and IAPAR 81, taking into account that larger numbers of *Xap* cells and stomata are found on this side of bean leaves (Karavina *et al.*, 2011). Even though the varieties used in our study showed different levels of resistance to CBB, the resistant varieties showed stomatal density similar to that of the susceptible ones.

The results of this study suggest that the resistance of the BRS Radiante and IAPAR 16 varieties cannot be explained by a lower stomatal density. However, the possibility that the size of the stomatal pore is smaller or that stomata closure upon PAMP perception occurs more rapidly in resistant varieties BRS Radiante and IAPAR 16 cannot be ruled out and remains to be investigated. It has been shown that stomatal density is important for plant resistance in other plant-bacteria interactions, such as lettuce - *Xanthomonas campestris* pv. *vitians* (Nicolas *et al.*, 2018), but for other interactions stomatal opening is more important. For instance, tomato varieties susceptible and resistant to *Xanthomonas perforans* showed differences in the timing of stomatal opening but not in stomatal density (Wang *et al.*, 2017). In addition to stomatal opening, another feature that can contribute to resistance of plants to bacteria is stomatal structure, whose importance was demonstrated for citrus resistance to *Xanthomonas axonopodis* pv. *citri* in early studies (McLean, 1921). Overall, this study shows that high levels of broad-spectrum quantitative resistance are present in BRS Radiante and IAPAR 16, which may be related to restriction of bacterial multiplication *in planta* and that are not necessarily correlated with lower foliar stomatal densities. These varieties are good sources of plant genetic resistance to be incorporated in breeding programs and good candidates to undertake studies aimed at understanding the molecular mechanisms underlying quantitative resistance since the reduction of disease symptoms development on them seems not to occur through differences at the port of bacterial entry into the host tissue.

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TABLES

Table 1. Disease severity on two common bean varieties after inoculation of *Xanthomonas axonopodis* pv. *phaseoli Xap-7* by spraying, using scissors or multiple needles.

	Inoculation method*		
Variety	Spraying	Scissors	Multiple-needles
Ouro Negro	4.8 (MR)**	4.3 (S)	5.0 (S)
Diamante Negro	4.9 (MR)	4.7 (S)	3.9 (S)

* No significant differences between varieties for each inoculation method according to the t-test ($P \leq 0.05$).

** MR, moderately resistant; S, susceptible.

Table 2. Disease severity on common bean varieties spray inoculated with *Xanthomonas axonopodis* pv. *phaseoli Xap-7* in two independent experiments.

	Severity (% affected area)*		Classification**
Variety	First experiment	Second experiment	
IAPAR 81	10.7 a	19.8 a	S
Ouro Negro	3.9 b	14.1 a	S
Carioca MG	12.1 a	14.1 a	S
BRS Campeiro	7.7 a	13.1 a	S
Diamante Negro	7.6 a	12.6 a	S
Manteigão Fosco 11	8.3 a	12.2 a	S
IPR Chopim	6.6 a	11.5 a	S
IAPAR 16	3.4 b	7.8 b	R
BRS Radiante	1.3 b	2.6 b	R

* Within each column, means followed by the same letter are not significantly different according to the Scott-Knott test ($P \leq 0.05$).

** R, resistant; S, susceptible.

Table 3. Response of three common bean varieties to inoculation with several different isolates of *Xanthomonas axonopodis* pv. *phaseoli.*

Isolate	Varieties*, **		
	IAPAR 16 (R)	Carioca MG (S)	BRS Radiante (R)
*Xap-1****	1.9 a B	11.7 a A	1.39 a B
Xap-2	0.54 a A	3.07 b A	0.35 a A
Xap-3	0.29 a A	1.98 b A	2.88 a A
Xap-4	2.10 a B	11.0 a A	2.09 a B
Xap-5	1.66 a B	11.6 a A	6.55 a B
Xap-6	0.96 a B	14.1 a A	2.16 a B
Xap-7	1.16 a B	12.7 a A	2.21a B

* R, resistant; S, susceptible to CBB.

** Within each column, means followed by the same lowercase latter are not significantly different according to the Scott-Knott test (P ≤ 0.05).

*** Within each row, means followed by the same uppercase letter are not significantly different according to the Scott-Knott test (P ≤ 0.05).

Table 4. Stomatal density of common bean varieties resistant and susceptible to Common Bacterial Blight (CBB).

Variety	Response to CBB*	Stomatal density (No. mm^{-2})**
IAPAR 16	R	215 b
IAPAR 81	S	110 a
Carioca MG	S	187 b
BRS Radiante	R	208 b

* R, resistant; S, susceptible.

** Means followed by the same letter do not differ significantly according to the Scott-Knott test ($P \leq 0.05$).

FIGURES

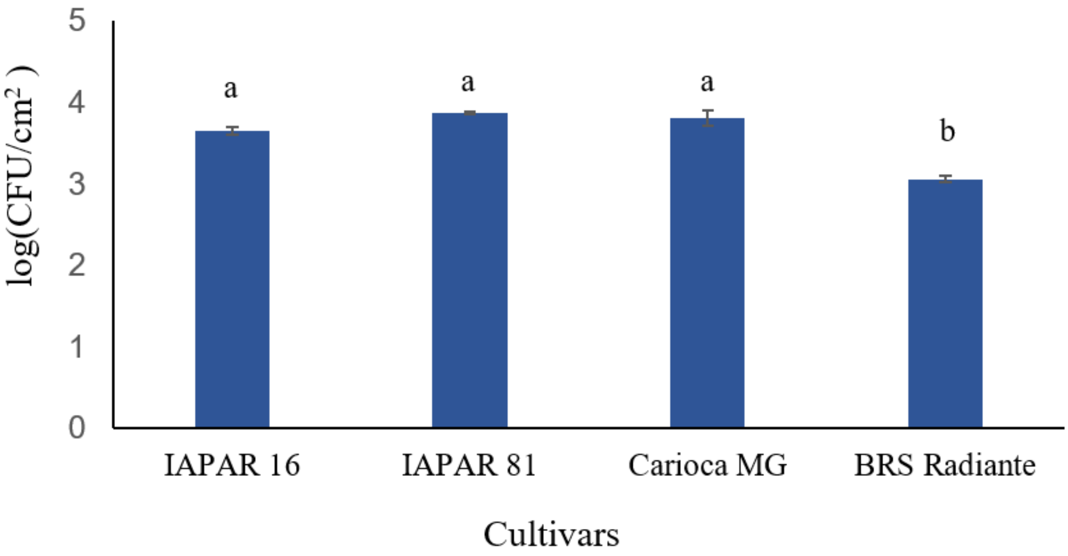

Figure 1. Bacterial populations recovered from bean varieties resistant and susceptible to Common Bacterial Blight at 15 days after inoculation with *Xanthomonas axonopodis* pv. *phaseoli Xap-7*. Bars indicate the means and vertical lines standard errors. Means of columns labeled with the same letter are not significantly different according to the Tukey test ($P \leq 0.05$).

SUPPLEMENTARY MATERIAL

Table S1. *Xanthomonas axonopodis* pv. *phaseoli* strains used in this study, their geographic origins and parts of the plant from which they were isolated.

Strain code	**Geographic origin***	**Plant part**
Xap-1	Lavras, MG, Brazil	Leaf
Xap-2	Lavras, MG, Brazil	Leaf
Xap-3	Lavras, MG, Brazil	Seed
Xap-4	Lavras, MG, Brazil	Leaf
Xap-5	Guaxupé, MG, Brazil	Seed
Xap-6	USA	Leaf
Xap-7	Brasilia, Brazil	Leaf

* MG, Minas Gerais.

Table S2. Agronomic characteristics of the common bean varieties used in this study.

Variety	Commercial group	Growth habit	Flower color	Weight of 100 grains (g)	Cycle (days)
BRS Campeiro	Black	Type II indeterminate	Violet	24.6	75 - 85
BRS Radiante	Manteigão/Rajado	Type I determinate	Pink wings and light violet banner	43.5	80
Carioca MG	Carioca	Type II indeterminate	White	21.0	90
Diamante Negro	Black	Type II indeterminate	Violet	21.3	92
IAPAR 16	Carioca	Indeterminate	White	22.1	90
IAPAR 81	Carioca	Indeterminate	White	25.1	92
IPR Chopim	Black	Type II indeterminate	Violet	23.2	90
Manteigão Fosco 11	Manteigão	Type I determinate	Pink wings and light violet banner	40.0	77
Ouro Negro	Black	Indeterminate	Violet	25.0 – 27.0	92

Chapter 2 - Research Article

Diallelic analysis and inheritance of *Phaseolus vulgaris* resistance to Common Bacterial Blight

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ABSTRACT

Study of the genetic resistance of common bean to Common Bacterial Blight (CBB) is important to achieve a reduction in the production losses caused by the disease. The use of resistant varieties is the most desirable method of disease control since it is environmentally safe and economically viable for the producers. In the present study, eight genotypes of common bean were combined in a partial diallel scheme (3 x 5). To confirm hybridization, a set of 16 SCAR markers were tested, eleven of which were polymorphic for the parents and useful in the identification of hybrids in 15 cross combinations. The hybrid plants along with the parents were used to estimate the general and specific combining ability (GCA and SCA, respectively) by inoculating with a *Xanthomonas axonopodis* pv. *phaseoli* strain. The results showed that variety BRS Radiante increases the resistance character as indicated by a very low mean of the GCA effect. For the SCA effect, nine combinations showed negative values, indicating their potential to increase resistance against CBB. In addition, F_1 plants from a cross between BRS Radiante and Carioca MG were advanced to the F_2 generation and hypotheses of resistance inheritance tested by the chi-square test. Of 160 F_2 plants evaluated, 94 were classified as resistant and 66 as susceptible, which was best explained by a segregation ratio of 9:7 and indicated that the resistance present in BRS Radiante is governed by two complementary dominant genes. Maximum likelihood analysis showed that the BRS Radiante resistance is due to a gene of major effect with contribution of additional polygenes. The results of this study contribute with molecular tools for identification of common bean hybrids, knowledge on the inheritance of resistance against CBB in Brazilian varieties, and selection of superior genotypes for this phenotypic trait.

KEYWORDS: common bean, SCAR, *Xanthomonas axonopodis* pv. *phaseoli,*

INTRODUCTION

Common Bacterial Blight (CBB) caused by the Gram-negative bacterium *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) is one of the major diseases affecting common bean (*Phaseolus vulgaris* L.) in production areas worldwide (Belete & Bastas, 2017). Management of CBB includes the use of pathogen-free seeds and crop rotation, but these cultural practices do not efficiently reduce losses in common bean production. A further complication for disease management is that curative control using chemical compounds in the case of bacterial diseases is not efficient either (Coyne & Schuster, 1983; Paula & Zambolim, 2006). Genetic resistance to CBB is the most effective and practical form of management from the technical and economic standpoints (Miklas *et al.*, 2003).

The incorporation of resistance genes into varieties with desirable agronomic characteristics is one of the main objectives in breeding programs for common bean (Yu *et al.*, 2000). A good understanding of the genetic control involved is important for the development of resistant varieties because it can provide insights into the most appropriate and efficient strategies to be used for gene introgression (Zimmermann *et al.*, 1996). For this, selection of resistant germplasm is the first step in the breeding process, the accuracy of which is essential to the success of the next steps. Several studies on the identification of sources of resistance to CBB in *P. vulgaris* as well as in other related species such as *P. acutifolius* and *P. coccineus* have been reported (Coyne & Schuster, 1983; Miklas *et al.*, 2002; Tryphone *et al.*, 2013; Singh & Miklas, 2015). The resistance present in common bean varieties BRS Radiante, IAPAR 16, and IPR Chopim against CBB has been demonstrated in several of these studies (Maringoni, 1998; Silva *et al.*, 2000; Costa *et al.*, 2008; Silva *et al.*, 2009, Monteiro *et al.*, unpublished).

After germplasm selection, appropriate combinations of resistant and susceptible genotypes must be identified and the inheritance of resistance understood by studying segregating populations derived from their crosses (Bertan *et al.*, 2005). In this regard, most studies on the genetic resistance of common bean to CBB indicate that the inheritance is quantitative and polygenic in nature (Coyne & Schuster, 1983; Silva *et al.*, 1989; Singh & Muñoz, 1999; Kelly *et al.*, 2003; Tryphone *et al.*, 2012; Singh & Miklas, 2015).

An alternative approach to determine the potential of combining parental genotypes is by conducting a diallel analysis, in which the genetic values, combining ability in hybrids that result in potential segregating populations, the type of gene action that controls the characters of interest, and the type of inheritance of the character in the identified parents can be estimated (Jaramillo *et al.*, 2005) The analysis of populations obtained by diallel crosses can be accomplished using several methods, among which the most commonly used is the Griffing method (1956) that provides information on the general combining ability (GCA) and specific combining ability (SCA) (Jung *et al.*, 2007).

Distinctive morphological trait of parental genotypes can be used as descriptors that facilitate hybrid identification, although some of them may be useful only in adult plants. But, when the morphological characteristics of the parents are not easily distinguishable in hybrid plants it is necessary to use additional tools to confirm hybridization (Bastianel *et al.*, 2006). The use of molecular markers is a valuable and accurate tool for identifying hybrids as it is based on DNA sequence polymorphisms between individual genomes. Sequence Characterized Amplified Region (SCAR) are PCR-based markers (Borém & Caixeta, 2016) that have already been used to genotype common bean segregating populations and to identify quantitative trait loci (QTL) and genes linked to resistance to diseases, such as rust (Corrêa *et al.*, 2000; Kelly *et al.*, 2003), anthracnose (Corrêa *et al.*, 2000; Méndez-Vigo *et al.*, 2005), *Beet Curly Top Virus* (BCTV) (Larsen *et al.*, 2010), *Bean Common Mosaic Virus* (BCMV) (Hegay *et al.*, 2013), *Bean Golden Yellow Mosaic Virus* (BGYMV) (Blair *et al.*, 2007), and CBB (Miklas *et al.*, 2000b, 2006; O'Boyle *et al.*, 2007).

Here, the potential for contribution to resistance against CBB was investigated via partial diallel analysis using hybrids derived from crosses between eight common bean varieties, three resistant and five susceptible. Also, the nature and number of genes involved in the resistance expressed in variety BRS Radiante was studied by testing inheritance hypotheses and genetic models by maximum likelihood. In order to confirm hybridization in F_1 and F_2 plants, a molecular toolkit of 11 SCAR markers was assembled.

MATERIALS AND METHODS

Bacterial strain

The *Xap-7* isolate of *Xanthomonas axonopodis* pv. *phaseoli* used in this study belongs to the collection of phytopathogenic bacteria of the Laboratory of Molecular Phytobacteriology at the Universidade Federal de Viçosa (LBM-UFV). For inoculation experiments, it was grown on solid 523 medium (Kado & Heskett, 1970) at 28ºC for 48 h.

Common bean accessions and plant growth

Seeds of eight common bean varieties were used in this study (Table 1). Amongst these varieties, BRS Radiante and IAPAR 16 were previously classified as highly resistant and IPR Chopim as moderately resistant (Silva *et al.*, 2009; Monteiro *et al,*unplubished) and were chosen to compose the group 1 of a partial diallel. Varieties

Diamante Negro, Ouro Negro, IAPAR 81, Carioca MG, and BRS Campeiro were previously classified as highly susceptible to *Xap* and were chosen to compose group 2 of a partial diallel (Silva *et al.*, 2000; Costa *et al.*, 2008; Monteiro *et al,*unplubished). The plants were grown in a 1:1 mixture of soil and substrate Tropstrato HT Hortaliças® (Vida Verde, Mogi, Mirim, SP, Brazil) in 2 l pots. Fertilization was performed every 15 days with Niphokam® (Fênix-Agro-Pecus Industrial Ltda, Tietê, SP, Brazil). Plants were watered according to the daily requirement and kept in a greenhouse at the Department of Plant Pathology (DFP) of the UFV

Obtention of F_1 plants

Plants of groups 1 and 2 were crossed in the form of partial diallels to obtain 15 combinations of plants candidates to hybrids. The crosses to obtain F_1 plants were made during days of mild temperatures, between 07:00 and 08:30 or between 17:00 and 18:00 (solar time). The flowers selected as pollen donors were in the balloon stage and the plants with open flowers on the same day were selected as male parents. Artificial pollination was carried out without emasculation with the aid of a stainless-steel forceps disinfested with 70% ethanol, the flower that received the pollen was closed and carefully labeled with a tag.

DNA extraction and quantification

DNA was extracted with the Wizard Genomic DNA Purification System kit (Promega, Madison, WI, USA) according to the manufacturer's instructions or using the CTAB method (Doyle & Doyle, 1987). For that, cotyledonary leaf tissue was collected and immediately frozen in liquid nitrogen. A total of 50 mg of leaf tissue was macerated in liquid nitrogen and used for DNA extraction. The extracted DNA was quantified using a Nanodrop™ 2000, (Thermo Fisher Scientific, Wilmington, DE, USA) and stored at -20 °C until used. Dilutions of the extracted DNA solution were prepared to obtain a final concentration of 10 ng μl^{-1} for PCR.

SCAR markers amplification

Sixteen primers (Table 2) were used to amplify SCAR markers previously reported in the literature (Corrêa *et al.*, 2000; Méndez de Vigo *et al.*, 2000; Miklas *et al.*, 2000a,b; Mahuku *et al.*, 2004; Mutlu *et al.*, 2005; Blair *et al.*, 2007; O'Boyle *et al.*, 2007; Larsen *et al.*, 2010; Hegay *et al.*, 2013) from DNA samples extracted from the parents and the hybrid candidates. PCR reactions were performed with the GoTaq Master Mix Green 2X (Promega, Madison, WI, USA) in a 25 μl mixture containing 12.5 μl of GoTaq, 1 μl of each primer (10 μM), 1.5 μl of DNA (10 ng μl^{-1}) and 10 μl of nuclease-free water. The PCR reactions were performed on a Peltier PTC-100 Thermocycler (Bio-Rad, Hercules, CA, USA), programmed for preheating at 95 ºC for 5 min, followed by 30 cycles at 94 ºC for 1 min, 50 ºC for 1 min (except for the SCARF10 primers, for which the annealing temperature was 60 °C), 72 °C for 1.5 min and a final step of 72 ºC for 10 min. An electrophoresis in 1.5% agarose gel was carried out to separate the amplification products, which were visualized and photographed using an L-PIX EX gel documentation system (Loccus, São Paulo, SP, Brazil). Presence, absence and size of the bands were used to determine the polymorphism between the parents and to confirm the hybridization in the progenies.

Evaluation of disease severity

Parental, F_1 (in all possible combinations of resistance × susceptibility) and F_2 plants were inoculated when the first trifoliate was fully expanded (V3 stage) by spraying with a suspension of *Xap-7* prepared in 10 mM $MgCl_2$ solution, whose absorbance at 600 nm was adjusted to contain 10^8 colony forming units per milliliter (CFU ml^{-1}). The plants were kept for 24 h before and after inoculation in a mist chamber, and then transferred to the greenhouse, where they remained until the end of the experiment. The development of the symptoms was monitored daily and their severity was evaluated at 15 days after the inoculation (DAI) by the 1-9 rating scale of Schoonhoven & Pastor-Corrales (1987). Plants showing disease note of 3 or lower were considered highly resistant.

Diallel analysis

Three plants of each parent and of each F_1 (in all possible combinations of resistance × susceptibility) were grown, inoculated with *Xap-7*, maintained in the greenhouse, and disease severity rated as indicated above. Disease severity data were subjected to analysis of variance to estimate the means and variances of the populations by the weighted least squares method (Ramalho *et al.*, 1993). The diallel analysis for parental and F_1 plants was performed according to the Geraldi & de Miranda-Filho (1988) model adapted from the Griffing method (1956) using the equation:

$$Yij = \mu + \frac{1}{2}(d_1 + d_2) + \hat{g}i + \hat{g}'j + sij + eij$$

where Yij is the mean of the cross that includes the i^{th} parent of group 1 and the j^{th} parent of group 2; μ is the general mean of the diallel; d_1 and d_2 are the contrasts that include the means of groups 1 and 2 and the general mean; ĝi is the effect of the general combining ability of the i^{th} parent of group 1; ĝ'j is the effect of the general combining ability of the

i^{th} parent of group 2; sij is the effect of specific combining ability; and eij is the mean experimental error. The standard deviation of the estimates of general (ĝi and ĝj) and combination (ŝij) capacity was obtained by the square root of the variances, as in Cruz *et al.* (2012). Statistical analyzes were performed with the Genes program (Cruz, 2013).

Tests for inheritance hypotheses

One hundred and sixty F_2 plants derived from a cross between BRS Radiante and Carioca MG were grown, inoculated with *Xap-7*, maintained in the greenhouse, and disease severity rated as indicated above. Five inoculated plants of each parent were used as control. Plants with disease severity scores of 1 to 3 were classified as resistant (R) and those with scores > 3 were classified as susceptible (S). Using the frequencies of R and S plants in the F_2 generation, hypotheses of Mendelian inheritance of resistance by the chi-square test at 5% probability were tested using the Genes program (Cruz, 2013).

Tests of genetic models using the maximum likelihood function

The methodology was based on maximum likelihood estimators proposed by Silva (2003). The hypotheses tested were of a gene of greater effect and / or presence of polygenes affecting resistance gene expression in common bean. The methodology of Mather and Jinks, (1978) based on means and variances, with the following structure was used:

$P_1 = (\mu - [a] - A,\ \sigma^2)$

$P_2 = (\mu - [a] - A,\ \sigma^2)$

$F_1 = (\mu - [a] - D,\ \sigma^2)$

$$F_2 = \frac{1}{4}\left(\mu + \frac{[d]}{2} - A, \sigma^2 + V_A + V_d\right) + \frac{1}{2}\left(\mu + \frac{[d]}{2} + D, \sigma^2 + V_A + V_d\right) + \frac{1}{4}\left(\mu + \frac{[d]}{2} + D, \sigma^2 + V_A + V_d\right)$$

Where: μ = reference constant; A = additive effect of the major effect gene; D = effect of dominance of the major effect gene; [a] = additive effect of polygenic; [d] = effect polygenic dominance; V_A = additive variance of the polygenics; V_d = variance attributed to polygenic dominance deviations and σ^2 = environmental variance. The likelihood functions allow to compose models with tests of interest with the following hypotheses of the Table 3. The models were tested using Monogen v.0.1 (Silva, 2003). Models 1, 3 and 5 could not be tested due to the small number of backcross populations, making it impossible to estimate the dominance effect associated to polygene effects. The likelihood tests were made using the LR (Likelihood ratio) proposed by Mood *et al.*, (1974).

RESULTS

Identification of common bean hybrids using SCAR markers

Varieties BRS Radiante, BRS Campeiro, Carioca MG, Diamante Negro, IAPAR 16, IAPAR 81, IPR Chopim and Ouro Negro were clearly distinguished by the banding patterns of SCAR markers (Table 4; Fig. S1). Eleven of the 16 SCAR markers tested presented informative bands (Table 4). The lack of informative bands for some primers was due to absence of polymorphic bands, absence of amplification products or occurrence of weak bands with low reproducibility. No DNA amplification was observed when the common bean varieties were tested with SCAR primers BC420, SB12 and

SU91. Of the 346 hybrid candidate plants derived from artificial pollinations, 177 were identified as hybrids using the SCAR markers. It was possible to use at least one SCAR marker to identify hybrids for each of the 15 combinations of crosses (Table 5).

Diallel analysis for resistance to CBB in common bean varieties

The sum of the mean squares in the general combining ability (GCA) and specific combining ability (SCA) were decomposed such that GCA was divided into two groups, group 1 referring to the resistant varieties and group 2 composed of the susceptible varieties. The GCA and SCA mean square values of groups 1 and 2 were significant for the trait of resistance to CBB (Table 6).

Within group 1, variety BRS Radiante showed the lowest GCA value and the smallest disease severity notes whereas in group 2, Diamante Negro and Carioca MG exhibited the most severe disease symptoms whereas Ouro Negro 81, IAPAR 81 and Carioca MG showed the lowest GCA values (Table 7). With respect to the SCA effect, the combinations IPR Chopim × Ouro Negro, IPR Chopim × Diamante Negro, IPR Chopim × BRS Campeiro, IAPAR 16 × Ouro Negro, IAPAR 16 × Diamante Negro, IAPAR 16 × IAPAR 81, IAPAR 16 × Carioca MG, BRS Radiante × IAPAR 81, BRS Radiante × Carioca MG showed negative values, the lowest of which was for BRS Radiante × Carioca MG (Table 8). This combination also showed the lowest mean disease severity values.

Inheritance of the resistance to CBB expressed in BRS Radiante

To study the inheritance to CBB contained in BRS Radiante, F_1 plants derived from its cross with Carioca MG and confirmed as hybrids by molecular marker were advanced to the F_2 population. Of 160 F_2 plants evaluated, 94 were resistant and 66 were susceptible to *Xap-7*. The Mendelian segregation hypotheses tested were ratios corresponding to 3:1, 9:7, 13:3, 15:1 and 63:1 (Table 9). The hypothesis that best explained the proportion of R:S plants observed in the F_2 population was a 9:7 ratio with a probability of 52%.

Tests of genetic models using the maximum likelihood function

The significance of the contrast between models 2 and 9, 4 and 9 and 6 and 9 indicated an influence of the environment on CBB resistance. Significance was also observed for the contrast between models 2 and 4 (Table 10), which test for the presence of a major dominant gene. In addition, additive effects are also considered as a result of the significance of the contrast between models 7 and 8. Furthermore, the significance of the test between models 2 and 8 indicated that the additive effects of the larger genes are important. The contrast of the test between models 2 and 6 was also significant, indicating the existence of a gene of greater effect and polygenes with additive effects for CBB resistance. However, the contrast between models 2 and 7 was not significant, indicating that the additive effects of the polygenes are not too relevant for the resistance of the BRS Radiante variety to CBB.

DISCUSSION

This study set out to investigate the genetics behind the resistance of common bean to CBB by conducting a partial diallelic analysis based on combinations of eight resistance and susceptible varieties as well as by testing hypotheses for the inheritance of the resistance contained in BRS Radiante (a variety that showed the lowest GCA) in the F_2 population derived from its cross with Carioca MG (a combination that exhibited the lowest SCA). For this, first a molecular toolkit based on SCAR markers was developed that allowed to discriminate hybrid from parental plants since, in most cases, the latter were morphologically very similar. SCAR primers that either amplified bands only in the male parent or amplified bands whose sizes differed between the parents were used to identify hybrid plants, with the exception of the SBD5 and Q14 SCAR markers that were co-dominant.

The 16 SCAR markers used in this study were previously used in common bean studies associated with disease resistance (Corrêa *et al.*, 2000; Méndez de Vigo *et al.*, 2000; Miklas *et al.*, 2000a,b; Mahuku *et al.*, 2004; Mutlu *et al.*, 2005; Blair *et al.*, 2007; O'Boyle *et al.*, 2007; Larsen *et al.*, 2010; Hegay *et al.*, 2013). The SCARF10 marker, also referred to as SF10, was previously used in variety Ouro Negro and shown to be linked to resistance against anthracnose (*Colletotrichum lindemuthianum*) and rust (*Uromyces appendiculatus*). SCAR markers A14, I19 and SW13 have also been linked to rust resistance (Miklas *et al.*, 1993, 2000a; Corrêa *et al.*, 2000; Hegay *et al.*, 2013). SCAR markers SAP 6, SU91 and BC420 are linked to the three major quantitative trait loci (QTL) for resistance to CBB in *P. acutifolius* and *P. vulgaris*. The SAP, SU91 and BC420 markers are positioned on chromosome 11, 8 and 6 of the common bean genome, respectively (Yu *et al.*, 2000; Mutlu *et al.*, 2005). With the exception of SCARF10, the SCAR markers here described had not been previously used in the varieties included in the present study. Some of these SCAR markers, such as SCARF10, A14, I19, and SW13, allowed the identification of hybrids in more than one combination of crosses.

Selection of the most promising populations by means of diallelic analysis is based on both GCA and SCA estimates in order to identify populations in which the parents have a high estimate of these capacities. In the present study the significance for GCA of groups 1 and 2, which are composed of resistant and susceptible varieties, respectively, indicates that the varieties present in group 1 contribute genetically to common bean resistance against CBB. Variety BRS Radiante showed the lowest GCA mean indicating that it is a promising parental for resistance to CBB. The significance of the GCA effect for group 2 also indicates that these varieties contribute differently to susceptibility against the disease. Notably, a low SCA mean for BRS Radiante × Carioca MG indicates that this particular combination is a promising choice for resistance against CBB. Diallelic analyses make it possible to identify the parents with the highest frequency of favorable alleles and to estimate the genetics involved in the control of the character. It also points to the most promising segregant populations to obtain superior lineages. In the case of studies on plant disease resistance, the response of interest is a low disease severity along with negative estimates associated with genotypes contributing to the reduction of the expression of that character (Silva *et al.*, 2000).

Varieties IAPAR 16, BRS Radiante (high resistance) and IPR Chopim (moderate resistance) from group 1 showed resistance to CBB (average severity of 2.6, 1.3 and 3.3, respectively). Nonetheless, not all hybrids involving at least one of these parents were resistant to CBB indicating that resistance is not determined by a few fully dominant genes. These results are consistent with the observation that in the F_2 population derived from BRS Radiante × Carioca MG the hypothesis that best explains inheritance of resistance indicates the involvement of two complementary dominant genes.

Silva *et al.* (2000), in a complete diallel analysis without reciprocals on common bean resistance to CBB, performed with three resistant and two susceptible parents along with F_1 hybrids from their combinations, observed that Diamante Negro, CB 733753 and CB 511687-1 contributed genetically to enhance resistance to CBB whereas Rosinha G-

2 and Compuesto Chimaltenango 2 contributed to the susceptibility of the hybrids. Conversely, in the present study variety Diamante Negro was allocated in group 2 (of susceptible varieties), presented medium disease severity (note 6.0), and contributed to the susceptibility of the hybrids. Differences in inoculum source are likely responsible for the discrepancy between the two studies.

When comparing the values of the SCA estimates of BRS Radiante × Ouro Negro and BRS Radiante × Carioca MG, it is noticed that the latter hybrid presented the lowest estimate values whereas the former hybrid had the highest estimate values. From this, it can be inferred that BRS Radiante presents low divergence in relation to Ouro Negro and high divergence in relation to Carioca MG with regard to CBB resistance. Higher SCA values indicate more distinct genotypes with respect to the frequency of genes with dominance (Gomes *et al.*, 2000), *i.e.* BRS Radiante and Carioca MG are dissimilar in dominant genes when compared to the average genotype frequency of the parents present in the diallel study. Consistently, the BRS Radiante × Carioca MG combination showed the lowest mean disease severity score.

The hypotheses tests for resistance inheritance of BRS Radiante against CBB obtained in this work led to the possibility that this phenotypic trait is governed by two dominant complementary genes as explained by a 9:7 segregation ratio (x^2 = 0.40; P = 0.52%). These results demonstrate that the resistance expressed in BRS Radiante is not monogenic and are in agreement with the oligogenic nature of the previously reported common bean resistance to CBB (Coyne & Schuster, 1983; Singh & Muñoz, 1999; Ferreira *et al.*, 2003; Tryphone *et al.*, 2012, Monteiro *et al.*, unpublished). For instance, a segregating F_2 population of a cross between Long 5 (resistant) and Long 4 (susceptible) exhibited pronounced variation and segregation for resistance to CBB, indicating the quantitative inheritance of this character Zhu *et al.*, (2016).

The results of this work contrast with those obtained in some previous inheritance studies. In a segregating population generated from crosses between Vax 4 (resistant) and Kablanketi (susceptible), the inheritance study revealed that resistance is conditioned by a major gene that has partial effects (Tryphone *et al.*, 2012). Also, Zapata *et al.*(2011) found that the segregation pattern of the F_2 population of a cross between PR0313-58 (resistance) and Rosada Nativa (susceptible) was best explained by a single dominant resistance gene. Taken together, these observations support the notion that the inheritance of common bean resistance to CBB is complex (Jung *et al.*, 1996).

Besides the number of genes involved in resistance to CBB, additional testing of genetic inheritance models were set by the maximum likelihood estimators to understand the effects involved in the genetic control. If the contrast between the models is significant, the difference of parameters between them is believed to be relevant. When the opposite occurs, that is, if the contrast between models is not significant, the inheritance of resistance is better explained by the reduced model (Silva, 2003). The significance of the contrast between models 2 and 4 shows the presence of additive and dominance effects of resistance genes. The dominance effect had already been detected in this study by chi-square inheritance hypotheses tests. Moreover, the significance of the contrast between models 4 and 8 indicates the presence of polygenes in addition to a dominant gene of major effect.

The nature of the resistance inheritance in the *Phaseolus vulgaris* - *X. axonopodis* pv. *phaseoli* interaction is highly dependent on the genotype used as susceptible parent as well as on the source of resistance and can be conferred by major and minor effect genes (Singh & Muñoz, 1999). The results of the diallelic analysis and the maximum likelihood estimators performed in the present work support this interpretation.

In conclusion, in this work 11 SCAR markers that were useful to select F_1 hybrids from crosses between BRS Radiante, BRS Campeiro, Carioca MG, Diamante Negro, IAPAR 16, IAPAR 81, IPR Chopim and Ouro Negro were identified. It is shown that there are differences in the contribution to the genetic resistance against CBB between varieties BRS Radiante, IAPAR 16 and Chopim IPR due to significant differences in GCA in a diallelic analysis. Based on SCA values, the F_1 hybrids derived from the crosses BRS Radiante × Carioca MG, IAPAR 16 × IAPAR 81, and IPR Chopim × Ouro Negro have high potential for common bean breeding for CBB resistance. Important insights into the genetic basis of resistance to CBB expressed in BRS Radiante have been obtained through inheritance hypotheses tests, which indicated that it is conditioned by two complementary dominant genes. The maximum likelihood analysis indicated that resistance of common bean to CBB is dominant and governed by a gene of major effect and additional polygenes. Further studies are needed to determine the proportion of polygene effects on CBB resistance. The findings and biological material generated in this work greatly contribute to the purpose of obtaining effective genetic resistance against CBB in common bean.

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TABLES

Table 1. Common bean parental varieties, their origins and classification for resistance to Common Bacterial Blight used in the partial diallel analysis.

Genitors	Origin[1]	Classification[2]
Group 1		
BRS Radiante	EMBRAPA	HR[3]
IAPAR 16	IAPAR	HR[3]
IPR Chopim	IAPAR	MR[4]
Group 2		
Diamante Negro	EMBRAPA	S
Ouro Negro	EMBRAPA	S
Carioca MG	EMBRAPA	S
IAPAR 81	IAPAR	S
BRS Campeiro	EMBRAPA	S

[1] EMBRAPA, Empresa Brasileira de Pesquisa Agropecuária; IAPAR, Instituto Agronômico de Paraná.

[2] HR, highly resistant; MR, moderately resistant; S, susceptible.

[3] Monteiro *et al.*, unpublished;

[4] Silva et al, 2009.

Table 2. SCAR primers used in this study.

Marker	Sequence (5' - 3')	Tagged locus	Disease	Reference
A14	F: CTATCTGCCATTATCAACTCAAAC R: GTGCTGGGAAACATTACCTATT	*Ur-4*	Rust	Haley *et al.* (1993)
AS8	F: GGCTGCCAGTATCTTGTCTAACACC R: GGCTGCCAGTGACGCAATTCTGCAG	*Bct*	Beet curly top virus (BCTV)	Miklas *et al.* (2000a)
AU05	F: GAGCTACCGTCAGTTTACTAA R: GAGCTACCGTGGCTTTTTTCT	QTL (WM6.1)	White mold	Miklas & Delorme (2003)
BC420	F: GCAGGGTTCGAAGACACACTGG R: GCAGGGTTCGCCCAATAACG	Major QTL (XAN 159)	Common Bacterial Blight (CBB)	Mutlu *et al.* (2005)
E-ACA/M-CTT330	F: CTTGTTCTGAGTCATTTACCTTGC R: GAATTCACAGTCCAAACTCTAATC	*Phg-1*	Angular leaf spot	Mahuku *et al.* (2004)
I19	F: AATGCGGGAGATATTAAAAGGAAAG R: AATGCGGGAGTTCAATAGAAAAACC	*Ur-5*	Rust	Miklas *et al.* (2000a)
Q14	F: GGACGCTTCATGACATTGGATGAACAG R: GGACGCTTCACCCTTTGTGGTATTG	QTL BCT6.1 (G122)	BCTV	Larsen *et al.* (2010)
ROC11	F: CCAATTCTCTTTCACTTGTAACC R: GCATGTTCCAGCAAACC	*I*	Bean common mosaic virus (BCMV)	Hegay *et al.* (2013)
SAP6	F: GTCACGTCTCCTTAATAGTA R: GTCACGTCTCAATAGGCAAA	Major QTL (GN#1 sel 27)	CBB	Miklas *et al.* (2000a)
SB12	F: CCTTGACGCACCTCCATG R: TTGACGATGGGTTGGCC	*Co-9*	Anthracnose	Méndez de Vigo *et al.* (2000)
SBD5	F: GTGCGGAGAGGCCATCCATTGGTG R: GTGCGGAGAGTTTCAGTGTTGACA	*bc-12*	BCMV	Blair *et al.* (2007)
SCARF10	F: GGAAGCTTGGTGAGCAAGGA R: GGAAGCTTGGCTATGATGGT	Not determined	Rust	Corrêa *et al.* (2000)
SG6	F: GTGCCTAACCGAGTTATCTAGAGT R: GTGCCTAACCCTCCTAAATGACCT	*Bc-3*	BCMV	Hegay *et al.* (2013)
SR2	F: CACAGCTGCCCTAACAAAAT R: CACAGCTGCCACAGGTGGGA	*Bgm-1*	Bean golden yellow mosaic virus (BGYMV)	Blair *et al.* (2007)
SU91	F: CCACATCGGTTAACATGAGT R: CCACATCGGTGTCAACGTGA	CBB	Major QTL (XAN 159)	O'Boyle *et al.* (2007)
SW13	F: CACAGCGACATTAATTTTCCTTTC R: CACAGCGACAGGAGGAGCTTATTA	*I*	BCMV	Hegay *et al.* (2013)

Table 3. Genetic models tested for resistance of common bean to Common Bacterial Blight.

Genetic inheritance models	Genetic parameters
1 = major gene with additive effect and dominance polygenes with additive and dominance effect	$\mu, A, D, [a], [d], Va, Vd, S_{AD}, \sigma^2$
2 = larger gene with additive effect and dominance polygenes only with additive effect	$\mu, A, D, [a], Va, \sigma^2$
3 = larger gene only with additive effect; polygenes with additive and dominance effect	$\mu, A, [a], [d], Va, Vd, S_{AD}, \sigma^2$
4 = larger gene only with additive effect; polygenes only with additive effects	$\mu, A, [a], [d], Va, \sigma^2$
5 = polygenes with additive and dominance effect	$\mu, [a], [d], Va, Vd, S_{AD}, \sigma^2$
6 = polygenes only with additive effect	$\mu, [a], Va, \sigma^2$
7 = major gene with additive and dominance effects	μ, A, D, σ^2
8 = larger gene only with additive effect	μ, A, σ^2
9 = only effect of the environment	μ, σ^2

S_{AD}, component of product-related variation of the additive polygenic effects for the polygenic effects of dominance. Adapted from Silva (2003).

Table 4. Discrimination of common bean varieties by SCAR markers.

	Group 1			Group 2					
Marker	BRS Radiante	IAPAR 16	IPR Chopim	BRS Campeiro	Carioca MG	Diamante Negro	IAPAR 81	Ouro Negro	Informative Patterns[2]
A14	A1[1]	A2	-	A2	A3	A3	A2	A3	Y
AS8	A1	A1	A1	A1	A1	A1	-	A1	N[4]
AU05	-	A1	A1	-	A1	A1	A1	A1	Y
BC420	-	-	-	-	-	-	-	-	N
E-ACA	A2	A1	A1	A1	A1	A1	A2	A1	Y
I19	A1	A1	A1	-	-	-	A1	-	Y
Q14	A2	A1	-	-	A1	A1	A1	A1	Y[3]
ROC11	A1	-	-	-	-	A1	-	-	Y
SAP6	-	-	-	-	A1	-	-	A1	Y
SB12	-	-	-	-	-	-	-	-	N
SBD5	A2	A2	A1	A1	A1	A1	-	A1	Y[3]
SCARF10	A1	-	A1	A2	-	-	A1	A1	Y
SG6	A1	A1	A1	A2	A1	-	A1	A1	Y
SR2	A1	A1	A1	A1	A1	A1	A1	A1	N
SU91	-	-	-	-	-	-	-	-	N
SW13	-	A1	A1	A2	A1	A1	A1	-	Y

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[1] A1, A2, A3, allele type according to the size of the amplified DNA fragment; -, lack of DNA amplification.

[2] Y, the SCAR marker discriminates between varieties; N, lack of discrimination between varieties.

[3] SCAR marker presents a co-dominant pattern for at least one cross between group 1 and group 2 varieties.

[4] Nonspecific band amplification.

Table 5. SCAR markers useful in discriminating F_1 hybrids derived from 15 combinations of common bean varieties.

Cross	SCAR marker confirmed in hybrids	Possible alternatives
Ouro Negro × IPR Chopim	SW13, SAP6, I19	A14, Q14
IPR Chopim × Diamante Negro	ROC11, I19	A14, Q14, SCARF10, SG6
BRS Campeiro × IPR Chopim	A14	AU05, E-ACA, Q14, ROC11, SBD5, SW13
IPR Chopim × IAPAR 81	E-ACA	A14, AS8, Q14, SBD5
IPR Chopim × Carioca MG	SCARF10	A14, I19, Q14, SAP6
Ouro Negro × IAPAR 16	I19	A14, SAP6, SBD5, SCARF10, SW13
Diamante Negro × IAPAR 16	I19	A14, ROC11, SBD5, SG6
BRS Campeiro × IAPAR 16	SG6, Q14	AU05, I19, SBD5, SCARF10, SW13
IAPAR 16 × IAPAR 81	SCARF10	AS8, E-ACA, SBD5
Carioca MG × IAPAR 16	I19	A14, SAP6, SBD5
BRS Radiante × Ouro Negro	Q14	A14, AU05, E-ACA, I19, ROC11, SAP6, SBD5
BRS Radiante × Diamante Negro	A14, AU05	E-ACA, I19, Q14, ROC11, SBD5, SW13
BRS Radiante × BRS Campeiro	A14	E-ACA, I19, Q14, ROC11
BRS Radiante × IAPAR 81	SW13	A14, AS8, AU05, Q14, ROC11, SAP6, SBD5
BRS Radiante × Carioca MG	SBD5	A14, AU05, E-ACA, I19, Q14, ROC11, SAP6, SCARF10, SW13

Table 6. Analysis of variance of the general combining ability (GCA) and specific combining ability (SCA) of common bean parents of groups 1 and 2 and their hybrid combinations for their reactions to Common Bacterial Blight.

Source[1]	DF[2]	SS[2]	MS[2]
Treatments	22	142	6*[3]
Group	1	52	52*
GCA Group 1 (R)	2	13	6*
GCA Group 2 (S)	4	19	4*
SCA 1 × 2	14	56	3*
Residue	46	63	1

[1] R, resistant; S, susceptible.

[2] DF, degree of freedom; SS, sum of squares; MS, mean square.

[3] An asterisk (*) at the end of the row indicates statistical significance.

Table 7. General combining ability (GCA) of common bean parents used in the partial diallel analysis for Common Bacterial Blight (CBB) resistance.

Parent	CBB	Severity
Group 1		
BRS Radiante	-0.48	1.3
IAPAR 16	0.00	2.6
IPR Chopim	0.48	3.3
Group 2		
Diamante Negro	0.13	6.0
Ouro Negro	-0.53	4.3
Carioca MG	-0.15	6.0
IAPAR 81	-0.20	5.3
BRS Campeiro	0.75	5.6

Table 8. Specific combining ability (SCA) of crosses between common bean parents used in the partial diallel analysis for Common Bacterial Blight (CBB) resistance.

Number	Hybrid	CBB[1]	Severity
1	IPR Chopim × Ouro Negro	-0.636	3.3
2	IPR Chopim × Diamante Negro	-0.636	4.0
3	IPR Chopim × BRS Campeiro	-0.255	5.0
4	IPR Chopim × IAPAR 81	0.698	5.0
5	IPR Chopim × Carioca MG	1.150	5.5
6	IAPAR 16 × Ouro Negro	-0.488	3.0
7	IAPAR 16 × Diamante Negro	-0.488	3.6
8	IAPAR 16 × BRS Campeiro	2.227	7.0
9	IAPAR 16 × IAPAR 81	-0.820	3.0
10	IAPAR 16 × Carioca MG	-0.702	3.1
11	BRS Radiante × Ouro Negro	1.346	4.3
12	BRS Radiante × Diamante Negro	0.679	4.3
13	BRS Radiante × BRS Campeiro	0.727	5.0
14	BRS Radiante × IAPAR 81	-0.321	3.0
15	BRS Radiante × Carioca MG	-2.035	1.3

[1] Effect of the specific combining ability to characterize resistance against Common Bacterial Blight.

Table 9. Inheritance hypotheses for the resistance of BRS Radiante to Common Bacterial Blight in the F_2 generation derived from a cross with susceptible variety Carioca MG.

Hypothesis	Observed R:S[1] ratio	Expected R:S ratio	x^2	Probability (%)
3:1	94 R : 66 S	120 R : 40 S	22.50	0
9:7		90 R : 70 S	0.40	52
13:3		130 R : 30 S	53.10	0
15:1		150 R : 10 S	334.50	0
63:1		157 R : 3 S	1638.50	0

[1] R, resistant, disease severity scores of 1–3; S, susceptible, disease severity scores > 3 at 15 days after inoculation with *Xap*

Table 10. Hypotheses of genetic inheritance models of common bean resistance to Common Bacterial Blight by means of the maximum likelihood function.

Models	Degree of freedom	X^2_c	Probability
2 *vs* 4	1	19.93 *	0.000008
2 *vs* 6	2	19.93 *	0.000047
2 *vs* 7	2	4.27 ^{NS}	0.118043
2 *vs* 8	3	26.56 *	0.000007
2 *vs* 9	4	39.54 *	0.000000
4 *vs* 6	1	0 ^{NS}	1.000000
4 *vs* 8	2	6.62 *	0.036388
4 *vs* 9	3	19.61 *	0.000204
6. *vs* 9	2	19.61 *	0.000055
7 *vs* 8	1	22.28 *	0.000002
7 *vs* 9	2	35.27 *	0.0000002
8 *vs* 9	1	- [1]	-

[1] Negative value, perhaps due to convergence problems.

* Significant at 5% probability by chi-square test.

^{NS} Not significant.

FIGURES

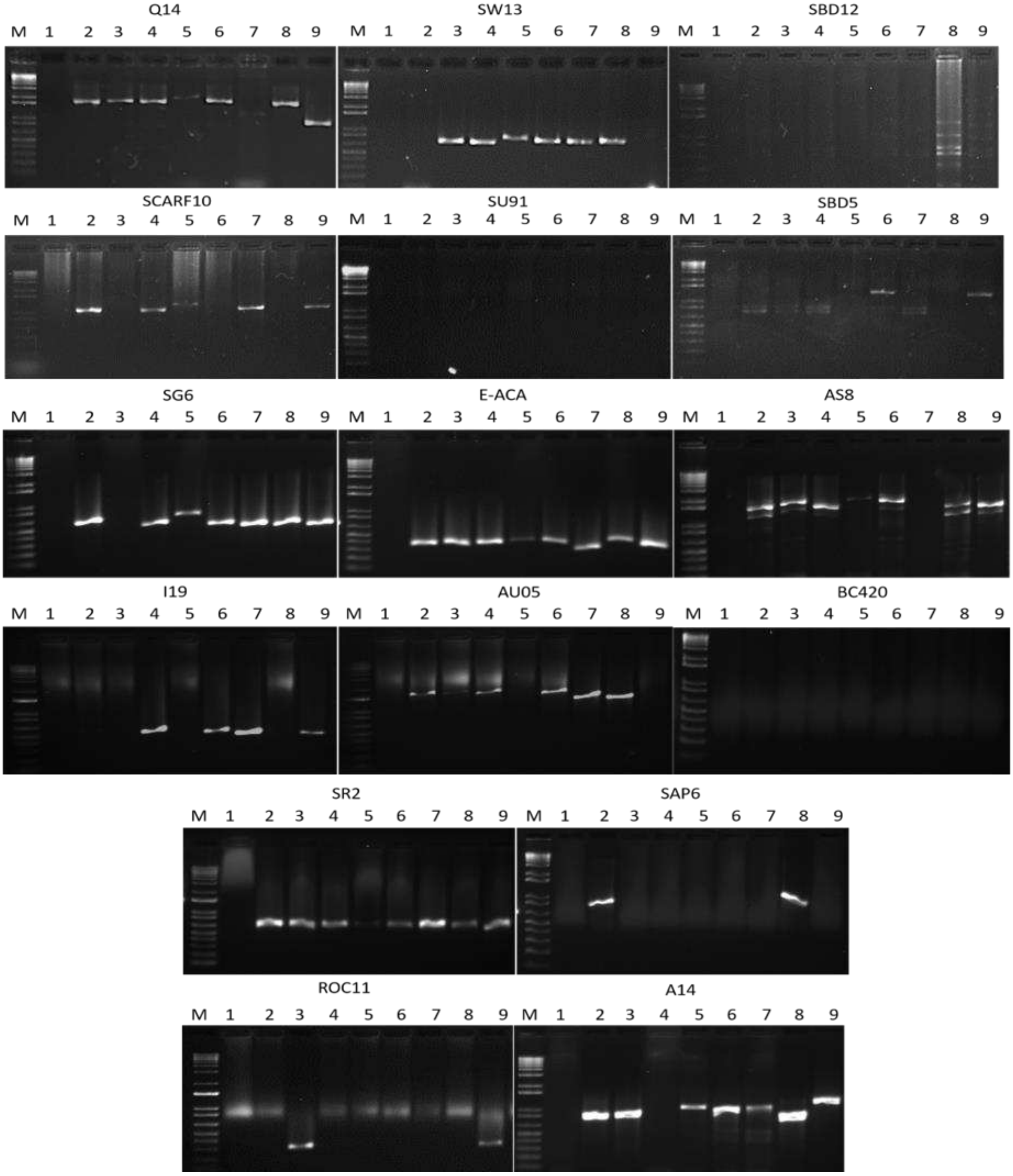

Figure S1. SCAR banding patterns in eight common bean varieties used in this study. M, molecular marker 1 kb (Invitrogen, Carlsbad, CA, USA), 1, control without template DNA, 2, Ouro Negro, 3, Diamante Negro, 4, IPR Chopim, 5, BRS Campeiro, 6, IAPAR 16, 7, IAPAR 81, 8, Carioca MG and 9, BRS Radiante.

Chapter 3 - Short Communication

Development of backcross *Phaseolus vulgaris* plants resistant to Common Bacterial Blight

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ABSTRACT

Common bean is one of the main food crops in Brazil, with significant economic expression and social importance. Common Bacterial Blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) is the main disease of bacterial etiology that causes significant losses in common bean production in several growing regions in Brazil. Plant genetic resistance is the most desirable method for disease management due to its reduced cost to the farmer and its low environmental impact. This study aimed to obtain

BC$_1$F$_1$ and BC$_2$F$_1$ plants resistant to CBB derived from crosses between BRS Radiante (highly resistant) and Carioca MG (highly susceptible). F$_1$ plants were successively backcrossed with Carioca MG to obtain BC$_1$F$_1$ and BC$_2$F$_1$ seeds. In all crosses, plant hybridization was confirmed by genotyping with SCAR markers Q14 and SBD5 and phenotyping for resistance to CBB by inoculating with a *Xap* isolate. Six BC$_1$F$_1$ and seven BC$_2$F$_1$ plants were used to confirm hybridization. Genotyping with the SCAR markers indicated that the backcross plants presented the expected banding pattern when compared to the donor and recurrent parents. Although in some cases such a genotyping was not sufficient to correlate the SCAR markers with hybridization (since not all backcross plants presented a heterozygous pattern) the expression of enhanced CBB resistance confirmed a gain in this phenotypic trait, indicating that SCAR Q14 and SBD5 markers are not linked to resistance against *Xap*. The backcross plants developed in this study exhibited a very variable response to CBB; two BC$_1$F$_1$ and BC$_2$F$_1$ plants each were highly resistant, indicating that they are excellent candidates to be advanced to the next generations. The backcross plants developed in this study represent important biological tools in order to undertake studies aimed at elucidating the molecular mechanisms involved in quantitative resistance of common bean to *Xap*.

KEYWORDS: Common bean, genetic resistance, SCAR, *Xanthomonas axonopodis* pv. *phaseoli*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most traditional foods of the Brazilian table, consequently Brazil is amongst the three largest producers in the world (FAO, 2018). However, the production of this crop is threatened by diseases, among which Common Bacterial Blight (CBB), caused by the Gram-negative bacterium *Xanthomonas axonopodis* pv. *phaseoli* (Smith 1897) Vauterin *et al.* 1995 (*Xap*), affects leaves, pods and, is considered one of the most important in producing areas around the world (Belete & Bastas, 2017). The disease caused by *Xap* can be highly destructive in conditions of high humidity and temperature causing losses in productivity and seed quality (Fininsa, 2003).

Initially, the bacterium causes water-soaked spots that increase in size and coalesce, forming larger necrotic lesions surrounded by chlorotic haloes. The lesions progress causing the premature fall of the leaves. Water-soaked spots with longitudinal cracks can be observed on the pods, which progress to reach the seeds. The bacteria can also colonize the stem of plants causing canker. The main source of long-distance dissemination of the pathogen are contaminated seeds (Karavina *et al.*, 2011). The recommended approach for disease control is integrated management involving pathogen-free seeds, elimination of residues from the affected crops, crop rotation and especially the use of resistant varieties, which is the most desirable method because it is economically favorable for the grower and, in general, highly effective (Maringoni & Lauretti, 1999; Viteri & Singh, 2014).

Bean varieties with different levels of CBB resistance have been reported in previous studies, including some performed by our research group (Monteiro *et al.*, unpublished). With respect to resistance inheritance, most studies indicate that resistance is quantitative or polygenic, of a complex nature and under strong environmental influence (Coyne & Schuster, 1983; Rodrigues *et al.*, 1999, Ferreira *et al.*, 2003; Monteiro *et al.*, unpublished)

Several studies have shown that variety BRS Radiante is highly resistant and Carioca MG highly susceptible to CBB (Silva *et al.*, 2000; Costa *et al.*, 2008; Monteiro *et al.*, unpublished). Hence, these two common bean genotypes constitute excellent candidates to undertake studies on the genetic and molecular mechanisms underlying resistance. However, in order to achieve a clear identification of genes involved in the resistant response, the difference in the genetic background that is not associated with this phenotypic trait between any two varieties being compared must be minimized *i.e.* to obtain genotypes with the smallest possible difference in genes not involved in resistance.

Transfer of desirable alleles can be performed through backcrossing, where small genomic portions carrying the gene(s) of interest present in nonadapted genotypes or whose agronomic characteristics are not desirable are introgressed into genotypes deficient in such alleles, but that have desirable agronomic traits (Lorencetti *et al.*, 2006). In order to confirm the effectiveness of the introgression, either phenotypic or molecular markers must be used. When individuals have contrasting phenotypes, morphological markers could be used, and it is the generalized basis of conventional genetic breeding, in which desirable parental traits are selected prior to making crosses. The use of molecular markers can aid when differences in the phenotypic characteristics between parents (and between parents and offspring) are not easily scorable. Besides helping confirm hybridization and select promising plant genotypes, molecular markers could also help estimate the degree of kinship with the recurrent parent (Benchimol *et al.*, 2005; Turchetto-Zolet *et al.*, 2017).

Several molecular markers have been developed and used for common bean genotyping (Gill-langarica *et al.*, 2011; Yu *et al* 2012, Gyang *et al.*, 2017). Among them, Sequence Characterized Amplified Region (SCAR) markers have proved very useful because of their low-cost, simplicity, and easy implementation (Yu *et al.*, 2000). The molecular markers Q14 and SBD5 are already used in studies to genotype common bean and identify quantitative trait loci (QTL) linked to resistance against the viruses *Beet curly top virus* (BCTV), *Bean common mosaic virus* (BCMV) and *Bean golden yellow mosaic virus* (BGYMV) (Blair *et al.*, 2007; Larsen *et al.*, 2010; Hegay *et al.*, 2013).

The present work aimed to obtain BC_1F_1 and BC_2F_1 plants resistant to CBB derived from a cross between Carioca MG and BRS Radiante. Since backcrossing decreases differences in the genetic background with the recurrent parent, the populations generated can be used to identify genes potentially associated with the resistance of *P. vulgaris* to CBB.

MATERIALS AND METHODS

Bacterial strain

The rifampicin-resistant *Xap-7* strain (Lilian Cação Costa, unpublished results) used in this study belongs to the culture collection of the Laboratory of Molecular Phytobacteriology at the Universidade Federal de Viçosa (LBM-UFV). It was routinely grown on solid 523 medium (Kado & Heskett, 1970) at 28 °C for 48 h.

Common bean accessions and plant growth

The accessions of *P. vulgaris* BRS Radiante and Carioca MG were classified as highly resistant and highly susceptible to *Xap*, respectively, in previous studies (Silva *et* *al.*, 2000; Costa *et al.*, 2008; Monteiro *et al.*, unpublished). These accessions were kindly provided by Prof. José Eustáquio de Souza Carneiro from the Department of Plant Science at UFV. Plants were individually grown in 2 l plastic pots containing substrate Tropstrato HT Hortaliças® (Vida Verde, Mogi Mirim, SP, Brazil) and soil in a 1:1 ratio. Every 15 days, the plants were fertilized with Niphokam® (Fênix-Agro-Pecus Industrial Ltda, Tietê, SP, Brazil) and watered according to their daily requirement. The plants were maintained in a greenhouse of the Department of Plant Pathology at the UFV.

Development of backcross populations

The F_1 plants derived from reciprocal crosses between Carioca MG and BRS Radiante were obtained in our previous studies (Monteiro *et al.*, unpublished). The BC_1F_1 and BC_2F_1 plants were obtained by backcrossing F_1 plants of each generation with Carioca MG. These crosses were made either between 07:00 and 08:30 or between 17:00 and 18:00 (solar time) during the days when the temperature was milder in order to minimize the number of abortive pollinations. Plants selected as female parents presented flowers in the balloon stage and plants with open flowers on the same day were selected as male parents. With the aid of a stainless-steel forceps number 5, previously disinfested with 70% ethanol, the flower flag of the female parent was opened to display the keel, without emasculation. After exposing the keel, the flower of the parental donor was detached from the original plant, the wings propelled downward such that the stamen was exposed, detached and inserted into the keel of the recipient flower. The flower that received the pollen was closed and carefully labeled with a tag.

For each generation, hybridization was confirmed and highly resistant plants were selected to advance to the next populations. In addition, morphological traits that differed between individual plants in the population in comparison with the parents were noticed. Some BC_1F_1 seeds originated from these backcrosses were preserved while others were planted to perform the next round of backcrosses with Carioca MG to obtain the BC_2F_1 population.

Selection of highly resistant plants

Strain *Xap-7* was recovered from a 30% glycerol stock at -80 °C by streaking on solid 523 medium (Kado & Heskett, 1970). A suspension in 10 mM $MgCl_2$ solution was prepared using bacterial cells from cultures grown on solid medium at 28 °C for 24 h and the OD_{600} adjusted to 0.1 (to contain approx. 10^8 colony forming units per milliliter (CFU ml^{-1})). The plants were inoculated when they had the first fully expanded trifoliate (V3 stage) by spraying the bacterial suspension onto both leaf surfaces with an atomizer Jet Master (Schulz, Joinville, SC, Brazil) until run-off point. The plants were kept for 24 h before and after inoculation in a mist chamber, and then kept in the greenhouse until the end of the experiment. Disease symptoms were evaluated daily for up to 15 days using a rating scale adopted from Schoonhoven & Pastor-Corrales (1987), ranging from 1 to 9. Plants showing disease note ≤ 3 were considered highly resistant, >3-5 moderately resistant, and >5 susceptible.

DNA extraction and quantification

DNA was extracted from the BC_1F_1 and BC_2F_1 and parental plants with the Wizard Genomic DNA Purification System kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. For that, cotyledonary leaf tissue was collected and immediately frozen in liquid nitrogen. A total of 50 mg of leaf tissue was macerated in liquid nitrogen and used for DNA extraction. The DNA was quantified using Nanodrop™ 2000 (Thermo Fisher Scientific; Wilmington, MA, USA) and stored at -20 °C until used. Dilutions of the DNA solution were prepared to obtain a final concentration of 10 ng μl^{-1} for PCR.

SCAR markers amplification

Specific primers to amplify the SCAR markers Q14 and SBD5, previously described in the literature were used (Larsen *et al.*, 2010; Hegay *et al.*, 2013). The Q14 SCAR is a 1000 and 2000 bp fragment amplified by the following primer pair: forward – GGA CGC TTC ATG ACA TTG GAT GAA CAG / reverse – GGA CGC TTC ACC CTT TGT GGT ATT G. The SBD5 SCAR is a 1600 and 1000 bp fragment amplified by the following primer pair: forward – GTG CGG AGA GGC CAT CCA TTG GTG / reverse – GTG CGG AGA GTT TCA GTG TTG ACA.

A 25 µl reaction containing 12.5 µl of GoTaq Green Master Mix 2X (Promega, Madison, WI, USA), 1 µl of each primer (10 µM), 1.5 µl of DNA (10 ng μl^{-1}) and 10 µl of nuclease-free water was prepared. PCR was performed on a Peltier Thermal Cycler PTC-100 (Bio-Rad, Hercules, CA, USA), programmed for preheating at 95 °C for 5 min, followed by 30 cycles at 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1.5 min and a final extension step at 72 °C for 10 min. The PCR products were electrophoresed on 1.5% agarose and the gels visualized and photographed using a L-PIX EX (Loccus, São Paulo, SP, Brazil) gel documentation system. Presence and size of the bands were used to determine hybridization in the backcross plants.

RESULTS

Identification of backcross plants using SCAR markers

A total of 13 BC_1F_1 (six) and BC_2F_1 (seven) potential hybrid plants obtained after pollination of Carioca MG flowers with pollen from appropriate F1 plants were tested with the SCAR markers. The SCAR Q14 primers amplified a 1000 bp fragment in BRS Radiante and a 2000 bp fragment in Carioca MG whereas the SCAR SBD5 primers amplified single 1650 bp and 1000 bp fragments in BRS Radiante and Carioca MG, respectively. Bands of the same size were also amplified in hybrid plants (Figure S1).

Genotyping with these two markers confirmed hybridization in most of the backcross plants. Amplification with SCAR Q14 indicated that five (numbers 1, 2, 4, 7 and 9) out of the six evaluated BC_1F_1 plants exhibited a heterozygote genotype whereas one (number 6) showed a homozygote genotype similar to that of Carioca MG. Genotyping of the BC_1F_1 plants with SCAR SBD5 indicated heterozygocity in only one plant (number 9) whereas the others (numbers 1, 2, 4, 6 and 7) exhibited the Carioca MG genotype (Table 1).

With regard to the BC_2F_1 plants, the SCAR Q14 marker indicated that only two (numbers 1 and 2) of the seven evaluated were heterozygous whereas five (numbers 3, 5, 6, 7 and 8) showed the Carioca MG genotype. On the other hand, amplification with the SCAR SBD5 primers identified five heterozygous plants (numbers 1, 5, 6, 7 and 8) and two plants (numbers 2 and 3) that exhibited the Carioca MG genotype (Table 1).

Resistance to CBB and morphological characters of the backcross plants

The BC_1F_1 and BC_2F_1 plants were evaluated for CBB resistance under greenhouse conditions using Carioca MG and BRS Radiante as controls. As expected, variety Carioca MG was highly susceptible (mean scale note = 8) whereas BRS Radiante was highly resistant (mean scale note = 2) to CBB (Figures 1 and 2; Table 1). The resistance to CBB of the BC_1F_1 plants was variable and exhibited a continuous and normal distribution, in which the disease severity scores ranged from 2 (highly resistant) to 9 (highly susceptible) (Figure 1; Table 1). BC_1F_1 plants with the lowest disease severity scores were selected to advance the backcrossing. For BC_2F_1 plants, the disease severity scores ranged from 2 to 7 (Figure 2, Table 1).

BRS Radiante has a determinate type I growth habit (Figure 3A) and produces pink flowers (Figure 3B) whereas Carioca MG has an indeterminate type II growth habit (Figure 3C) and produces white flowers (Figure 3D). It was possible to observe a characteristic admixture in the BC_1F_1 population, as indicated by these morphological characters. For instance, the growth habit of BC_1F_1 plant number 3 was of type II (Figure 3E) and produced flowers of a color typical of BRS Radiante (pink wings and light violet banners) (Figure 3F). Furthermore, the opposite happened in other plants (*e.g.* number 1) whose growth habit was determinate (Figure 3G) but produced white flowers (Figure 3H).

DISCUSSION

In this study, we generated BC_2F_1 plants derived from a cross between Carioca MG (recurrent parent) and BRS Radiante (parental donor) useful to undertake studies on the molecular mechanisms underlying resistance of common bean to CBB. The plants were genotyped with SCAR markers Q14 and SDB15. The results indicate that these two molecular markers are not tightly linked to resistance against CBB, but were both very useful in the identification of recombinant plants (Monteiro *et al.*, unpublished). Previous studies showed that the SCAR Q14 marker is linked to a Quantitative Trait Loci (QTL) associated with resistance to *Beet curly top virus* in common bean Landrace G122 whereas SCAR SBD5 is linked to a QTL associated with resistance to *Bean golden yellow mosaic virus* and *Bean common mosaic virus* and are both used in marker-assisted selection (MAS) (Blair *et al.*, 2007; Larsen *et al.*, 2010; Hegay *et al.*, 2013).

Taken together, the genotypic and phenotypic information obtained from the BC_1F_1 and BC_2F_1 plants indicated that all of them were true hybrids resulting from the cross between the F_1 plants and Carioca MG. Two particular cases worth mentioning are those of BC_1F_1 and BC_2F_1 plants numbers 6 and 3, respectively, which showed a highly resistance response even though their genotypes corresponded to that of the recurrent parent (based on both molecular markers). These results along with the enhanced resistant response exhibited by BC_1F_1 plant number 7 (whose SCAR SBD5 genotype was similar to that of Carioca MG) and by BC_2F_1 plant number 6 (whose SCAR Q14 genotype was the same as that of Carioca MG) demonstrated that neither SCAR marker is linked to resistance of common bean to CBB. These genotypic patterns are in agreement with the notion that in backcross generations, the banding pattern is expected to be either homozygous similar to the recurrent parent or heterozygous, one band derived from the recurrent and the other from the parental donor (Carneiro *et al.*, 2010).

In this study, it was possible to observe the admixture of morphological characters in BC_1F_1 plants which possessed the color of the flowers of one parent and the growth habit of the other parent. Variety Carioca MG presents indeterminate growth habit and white-colored flowers, whereas variety BRS Radiante exhibits determinate growth habit and flowers with pink wings and light-violet banners (Machado *et al.*, 2000; Teixeira *et al.*, 2015). Some backcross plants showed a determinate growth habit and produced white flowers while others showed indeterminate growth habit and produced flowers with pink wings and light violet banners. Amongst the morphological markers of common bean, the color of the flower, which is a so-called fixed descriptor because it is influenced by one or a few genes and is easily differentiated, is considered a good genetic marker (Ramalho *et al.*, 1993; Rodrigues *et al.*, 2002).

The results of this study demonstrated that the incorporation of genes conferring horizontal resistance to CBB from BRS Radiante to Carioca MG was possible through backcrossing, but that additional molecular markers, tightly associated to resistance, are required for marker-assisted selection (Silva *et al.*, 2008). In the long run, the purpose of developing backcross plants is to narrow down the gap between the genetic backgrounds of highly resistant and highly susceptible genotypes by assorting out genes that are not associated with *Xap* resistance. These plants, together with Carioca MG (the highly susceptible recurrent parent), will be used in the identification of *P. vulgaris* genes associated with resistance to CBB through comparative transcriptomics.

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TABLES

Table 1. SCAR banding patterns and reactions to Common Bacterial Blight of BC_1F_1 and BC_2F_1 plants.

	Marker pattern type			
Plants	Q14*	SBD5*	CBB score	Phenotype **
BC_1F_1				
1	H	C	6	HS
2	H	C	9	HS
4	H	C	5	MR
6	C	C	2	HR
7	H	C	4	MR
9	H	H	6	HS
BC_2F_1				
1	H	H	7	HS
2	H	C	7	HS
3	C	C	2	HR
5	C	H	5	MR
6	C	H	3	HR
7	C	H	5	MR
8	C	H	5	MR
BRS Radiante	R	R	2	HR
Carioca MG	C	C	8	HS

* H, heterozygote; R, homozygous - parental donor; C, homozygous - recurrent parent.

** HS, highly susceptible; HR, highly resistant; MR, moderately resistant.

FIGURES

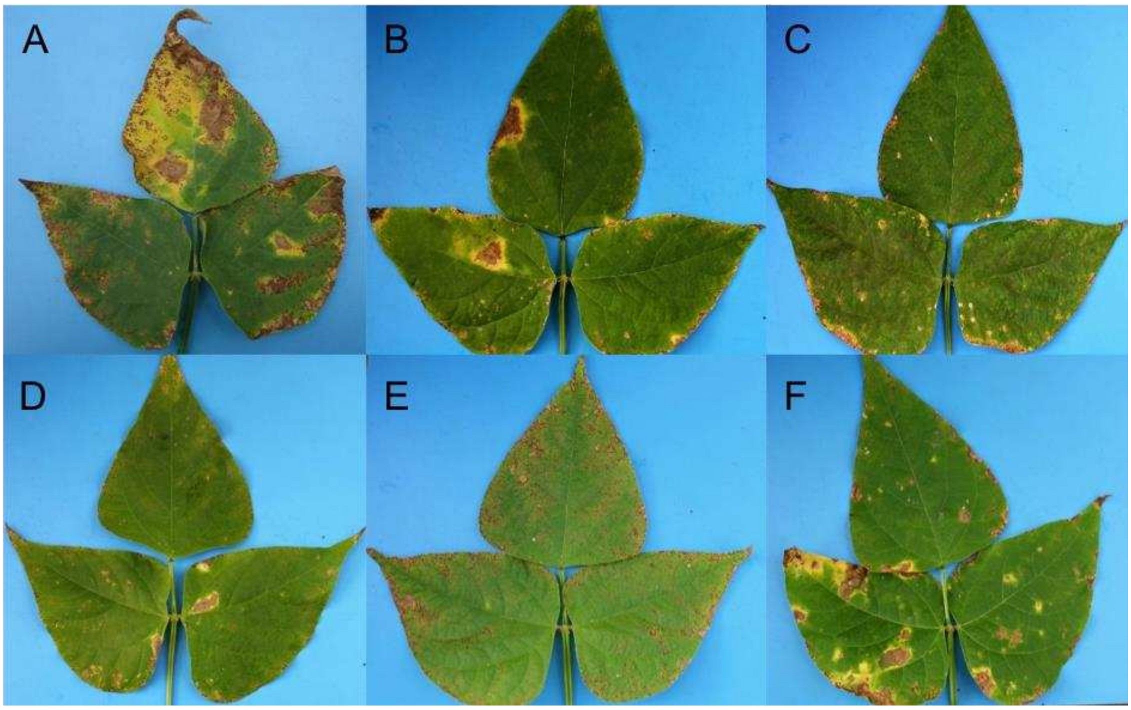

Figure 1. Common Bacterial Blight symptoms of representative BC_1F_1 plants. (A) Carioca MG – recurrent parent, (B) BC_1F_1-5, (C) BC_1F_1-6, (D) BC_1F_1-7, (E) BC_1F_1-8 and (F) BC_1F_1-9 inoculated with *Xanthomonas axonopodis* pv. *phaseoli Xap*-7 at 15 days after inoculation.

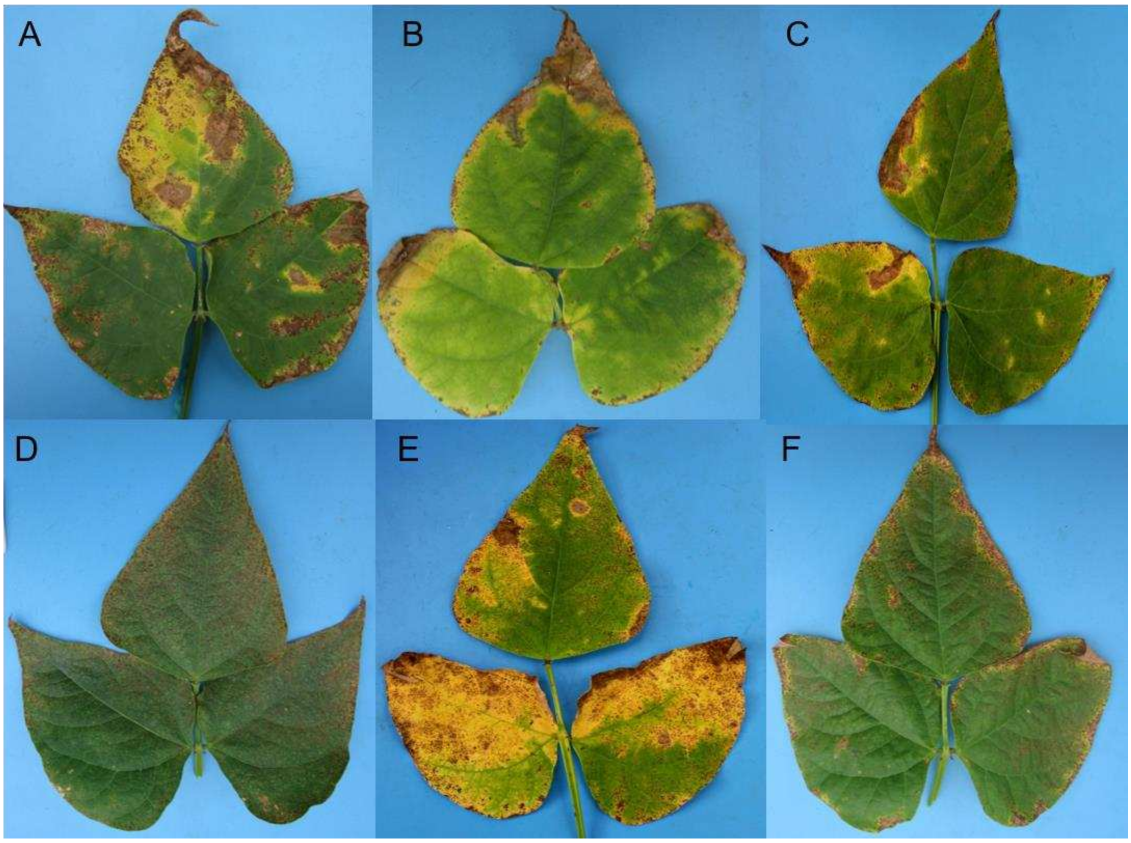

Figure 2. Common Bacterial Blight symptoms of representative BC_2F_1 plants. (A) Carioca MG – recurrent parent, (B) BC_2F_1-1, (C) BC_2F_1-2, (D) BC_2F_1-3, (E) BC_2F_1-4, (F) BC_2F_1-6 inoculated with *Xanthomonas axonopodis* pv. *phaseoli Xap*-7 at 15 days after inoculation.

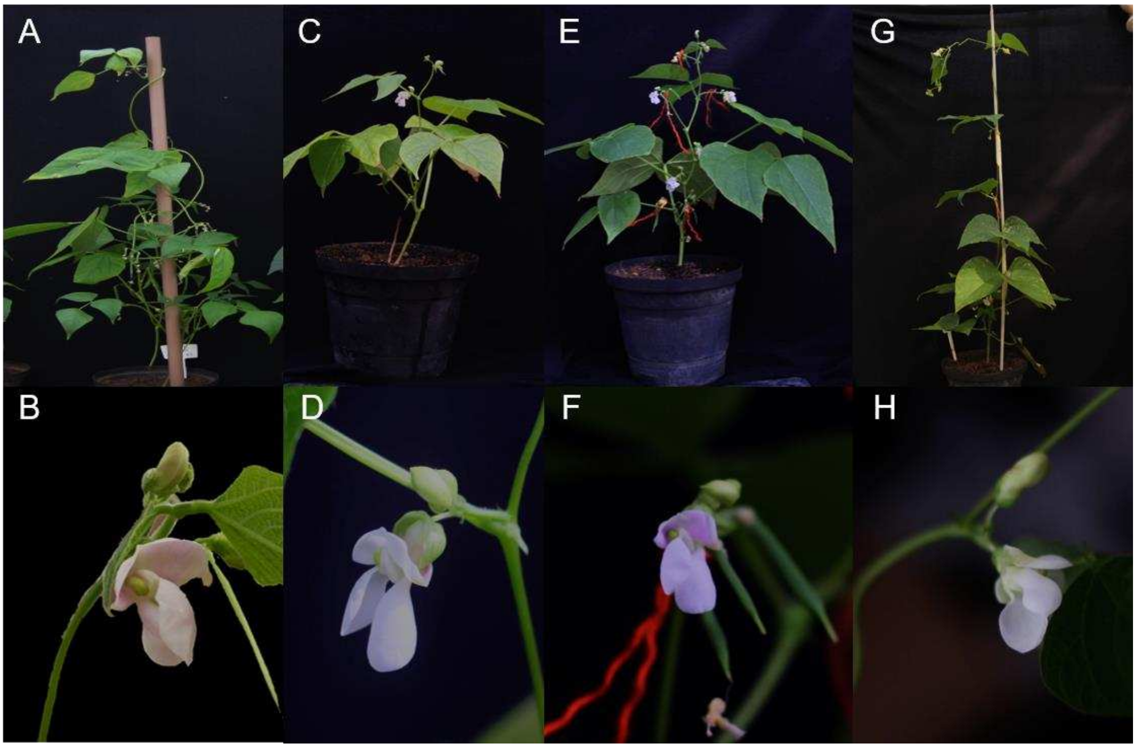

Figure 3. Admixture of morphological characters in BC_1F_1 plants. (A) and (B) BRS Radiante - determinate type I growth habit and pink flower, (C) and (D) Carioca MG - indeterminate type II growth habit and white flower, (E) and (F) BC_1F_1 plant 3- indeterminate type II growth habit and pink flowers, (G) and (H) BC_1F_1 plant 1 - determinate type I growth habit and white flower.

SUPPLEMENTARY MATERIAL

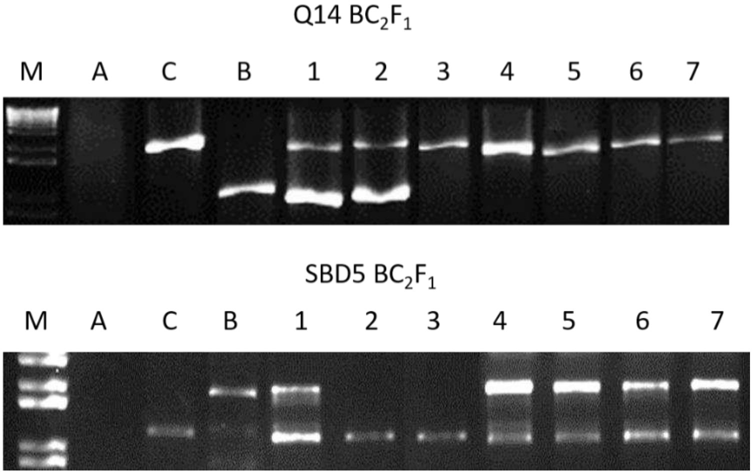

Figure S1. Representative SCAR banding patterns of BC$_2$F$_1$ plants. Top panel: SCAR marker Q14. Bottom panel: SCAR marker SBD5. M, molecular marker, 1 kb ladder (Invitrogen, Carlsband, CA, USA); A, control without template DNA; C, Carioca MG; B, BRS Radiante. Lane numbers indicate backcross BC$_2$F$_1$ plants.

Chapter 4 - Research Article

Sources of resistance and genome-wide association study (GWAS) of *Phaseolus vulgaris* to Common Bacterial Blight

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ABSTRACT:

Common bean is one of the main legumes used for food around the world. Amongst the main problems for crop production is Common Bacterial Blight (CBB) caused by the Gram-negative bacterium *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*). The most efficient and desirable alternative of disease control is plant genetic resistance, which is generally highly effective, especially if applied in integrated management programs. In the present study, we aimed to classify 103 common bean varieties for resistance to CBB and to evaluate disease progression by determining the area under disease progress curve (AUDPC). In addition, a broad genome-wide association study (GWAS) of single nucleotide polymorphism (SNP) markers with resistance to CBB in eighty common bean varieties was investigated. The varieties evaluated for CBB resistance were classified into four groups according to the severity of disease symptoms at 15 days after inoculation, 29 of which were classified as highly resistant. The highly resistant response to CBB of 15 of these common bean varieties is shown for the first time in this work. A positive correlation between AUDPC and disease severity was observed. Although statistical significance for association of the SNPs investigated with CBB resistance was not found, annotation of the genes linked to the ten SNP markers with the highest -log(P) values revealed a striking overrepresentation of genes involved in plant defense against pathogens. These SNP markers are distributed over eight common bean chromosomes and are linked to genes that code for diverse functions, including some that belong to serine-threonine protein kinase, glutamine synthase, lectin domain protein, and pentatricopeptide repeat protein families. These results contribute to the identification of common bean germplasm resistant to CBB and genes putatively involved in the resistance response.

KEYWORDS: Common bean, quantitative resistance, SNP, *Xanthomonas axonopodis* pv. *phaseoli.*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the main leguminous foods around the world because of its high nutritional value (Asgharipour *et al.*, 2019). Amongst the factors that cause significant loss in the world production of common bean is the Gram-negative bacterium *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), etiologic agent of the disease called Common Bacterial Blight (CBB). The disease causes crop losses in tropical, subtropical and temperate climates (Coyne & Schuster, 1974). Initially, small water-soaked lesions appear on the leaves, which in time grow to become necrotic lesions that subsequently develop yellow haloes around them and increase in size. In the pods, the lesions also begin as water-soaked lesions that later become dark. On seeds, the bacterium can cause severe discoloration (Singh & Muñoz, 1999). Common Bacterial Blight is difficult to control in the field since the use of curative chemical compounds is unfeasible. The best form of bacterial disease control is the use of resistant varieties, especially if this practice is incorporated in integrated management programs (Mutlu *et al.*, 2005).

Several sources of resistance to CBB have been found in *P. vulgaris* as well as in other closely-related species such as *P. acutifolius* and *P. coccineus* (Singh & Miklas, 2015). Most studies on the resistance of common bean to CBB state that it is of quantitative, partial or horizontal type (Coyne & Schuster, 1983; Silva *et al.*, 1989; Singh & Muñoz, 1999; Tryphone *et al.*, 2012, 2013). Quantitative resistance is generally conferred by several genes that jointly contribute to fight pathogen attack and consequently, different levels of disease severity are observed among different plant genotypes. These differences in levels of resistance are generally not easily distinguishable, which makes it important to measure disease progression through estimation of the area under the disease progress curve (AUDPC) (Simko & Piepho, 2012). Some studies have used AUDPC to access the resistance of common bean to CBB (Hailu *et al.*, 2015; Trindade *et al.*, 2015; Andrade *et al.*, 2017).

The development of disease resistant varieties is one of the main objectives of bean breeding programs worldwide. After identification of sources of resistance, it is useful to identify markers that assist the selection of desirable genotypes and/or that pinpoint specific genomic loci linked to resistance in order to reduce the phenotyping time (Miklas *et al.*, 2006). Molecular markers of several different types have been used in the construction of genetic linkage maps and localization of quantitative trait loci (QTL) (Grattapaglia & Ferreira, 2016). Amongst the most commonly used molecular markers are single nucleotide polymorphisms (SNPs), which consist of variations in a single nucleotide position in homologous sequences of the DNA and have been considered as new generation markers, especially after the advent of new generation sequencing technologies (Ganal *et al.*, 2009; Wang *et al.*, 2012).

Sequencing genotyping technologies have allowed discovery variant and genotyping in a single step, thus facilitating research in the area of plant breeding. They allow obtaining a large number of SNP markers covering a high percentage of the genome in a rapid, effective and relatively inexpensive manner. From this high genome coverage, the SNPs can be used in genomic selection, genome-wide association studies (GWAS) as well as in genetic diversity and evolution studies (Elshire *et al.*, 2011; Mourad *et al.*, 2018). In the case of GWAS, one of its major advantages is that the long process of crossing between phenotypically contrasting parents and obtaining segregating progenies is avoided by accessing the natural variation within populations (Silva, 2017). This genotyping approach has been utilized to find association of specific genomic loci with several phenotypic traits of interest, such as plant architecture, productivity, biomass production, and yield components (Kamfwa *et al.*, 2015; Resende *et al.*, 2018). Once candidate SNPs are identified, annotated genes physically linked to them and putatively involved in the phenotypic trait can be retrieved from reference genome sequences (González *et al.*, 2017). With respect to resistance of common bean to diseases, reports on the use of GWAS are scarce; the very few studies reported are restricted to resistance against Anthracnose and Angular Leaf Spot (Perseguini *et al.*, 2016). No GWAS has been reported for resistance of common bean to bacterial diseases.

In this study, we set out to first determine the resistance of 103 common bean varieties to CBB by assessing severity of disease symptoms and estimating the AUDPC after inoculation with a *Xap* isolate. In order to identify SNPs potentially associated with resistance to CBB, a broad GWAS using 384 SNPs on 80 of the phenotyped common bean varieties was conducted. Genes putatively associated with common bean resistance to CBB were physically mapped and annotated based on the sequence of a common bean reference genome.

MATERIALS AND METHODS

Bacterial strain

The *Xap-7* strain used in this study belongs to the culture collection of the Laboratory of Molecular Phytobacteriology at the Universidade Federal de Viçosa (LBM-UFV). It was routinely grown on solid 523 medium (Kado & Heskett, 1970) at 28 °C for 48 h.

Plant material and maintenance

One-hundred and three *varieties* (Table S1) were used in this study. Pure seeds of these varieties were obtained from the Common Bean Bank of Germplasm of the Universidade Federal de Viçosa (UFV), Viçosa, Brazil. These accessions were kindly provided by Prof. José Eustáquio de Souza Carneiro from the Department of Plant Sciences at UFV. The seeds were germinated in 2 l pots containing 1:1 mixture of substrate Tropstrato HT Hortaliças® (Vida Verde, Mogi Mirim, SP, Brazil) and soil. The plants were fertilized with Niphokam® (Fênix-Agro-Pecus Industrial Ltda, Tietê, SP, Brazil) every 15 days and watered according to daily requirement. During warmer climate, they were watered twice a day. The plants were kept in a greenhouse at the Department of Plant Pathology (DPP) of the UFV.

Plant phenotyping

The one-hundred and three common bean varieties were planted from May to July, 2018. For inoculation, cultures of the *Xap-7* strain were grown on solid 523 medium (Kado & Heskett, 1970) at 28 ºC for 24-48 h. Bacterial cells were resuspended in 10 mM $MgCl_2$ solution and the OD_{600} adjusted to 0.1. At the V3 phenological stage, they were inoculated with the *Xap*-7 strain, by spraying the bacterial suspension onto both leaf surfaces with an atomizer Jet Master (Schulz, Joinville, SC, Brazil) until run-off point. At least five plants of each genotype were treated with 10 mM $MgCl_2$ solution to serve as controls. The plants were kept for 24 h before and after inoculation in a mist chamber and then kept in the greenhouse until the end of the experiment. Disease symptoms were evaluated daily for up to 15 days using a rating scale ranging from 1 to 9 adopted from Schoonhoven & Pastor-Corrales (1987). Varieties showing mean disease notes of 2.6 or lower (with no significant difference with the lowest mean value) were considered highly resistant. Inoculated plants were kept in the greenhouse using a completely randomized design, in which the experimental unit consisted of one plant per pot, with five repetitions per genotype. At 15 days after inoculation (dai), the most affected trifoliate of each plant were selected for evaluation. The severity data were used to calculate the AUDPC according to Campbell & Madden (1990). To estimate the AUDPC, the disease notes at 9, 11, 13, 15, 17 and 19 dai were considered. The whole experiment was replicated once.

Plant genotyping

Genotyping was performed with 80 of the common bean varieties used in this study (Table S1). Genomic DNA samples of the varieties were genotyped in the Biotechnology Laboratory of Embrapa (Santo Antônio de Goiás, GO, Brazil) with the Veracode 1 BeadXpress platform (Illumina, San Diego, CA, USA) using 384 SNPs according to the methodology used by Nascimento *et al.* (2018). These SNP set was previously identified based on polymorphic sites between 88 common Andean and Mesoamerican bean varieties/lines with the aim of developing an SNP-based operational panel to discover diversity, study genetic structure and to be applied in common bean breeding (Müller *et al.*, 2015). The genotyping of the 80 common bean accessions used in this study was performed in a previous study (Nascimento *et al.*, 2018). Genotype call was performed using the Genome Studio software version 1.8.4 (Illumina, San Diego, CA, USA). Identification of the valid SNPs was done using Call Rate (CR) values ranging from 0.8 to 0.9 and a Minimum Allele Frequency (MAF) of 5%, according to criteria adapted from Anderson *et al.*, (2010).

Genome-wide association study

The analysis of genome-wide association of SNPs to CBB resistance was performed applying the polygenic randomized regression method of Yu *et al.* (2006) using the GWAS function of the rrBLUP package (Endelman, 2011) for R (R Development Core Team, 2018). The -log10(P) threshold was calculated based on the Bonferroni method for multiple testing to identify SNPs significant at 5% probability.

Functional annotation of candidate genes

The SNPs with -log(P) values equal or greater than 0.9 were selected to conduct gene annotation. The identification of genes physically linked to these SNPs was performed by searching for their positions in the whole genome sequence of common bean (PhaVulg1_0; variety G19833) deposited at National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/assembly/GCF_000499845.1). Taken the position of the SNPs in the chromosomes as references, a list of closely linked genes, mRNA and protein sequences was retrieved. The position of the SNPs in introns or exons and the annotation of the gene models of the *Phaseolus vulgaris* v2.1 genome (Schmutz *et al.*, 2014) was obtained using Phytozome v11.0 (Goodstein *et al.*, 2012; http://phytozome.jgi.doe.gov).

Statistical analysis

Statistical analyses of data from phenotyping experiments were conducted using either the agricolae (Mendiburu, 2017), easyanova (Arnhold, 2013) and ExpDes (Ferreira *et al.*, 2018) packages for analysis of variance; lattice (Sarkar, 2008) for graphical visualization of the data; and ScottKnott (Jelihovschi *et al.*, 2014) for mean comparisons. The audpc function of the agricolae package was used to calculate the AUDPC. The function cor.test of R basis was used to correlate the results of disease severity and AUDPC. The above-mentioned packages were applied with the software R version 1.1.453 (R Development Core Team, 2018). A factorial analysis was performed to determine if there was interaction between the genotype response to inoculation and the environment using the Genes program (Cruz, 2013. Since no interaction was found, the data from the two experiments were combined, transformed to square root, subjected to analysis of variance, and the means compared by the Scott-Knott test ($P \leq 0.05$).

RESULTS

Resistance of common bean varieties to Common Bacterial Blight

Inoculation of the bean varieties with *Xap-7* was conducted twice, in May and July of year 2018 and similar results were obtained. Early symptoms of CBB were observed as water-soaked spots at 9 dai, at which time differences in the foliar area affected by the disease among genotypes were readily observed by naked eye. Significant differences in disease severity among common bean genotypes were found at 15 dai. The disease severity means were separated into four groups according to the Scott-Knott test (Table 1); 16 varieties were classified as highly susceptible, 20 as moderately susceptible, 38 as moderately resistant and 29 as highly resistant. The mean severity of the highly resistant varieties ranged between 0.5 and 2.5 while that of the highly susceptible varieties were between 5.8 and 7.5.

There were significant differences in AUDPC among the genotypes tested. The AUDPC means ranged from 18 to 55 (Table 2) and were separated into four groups according to the Scott-Knott test. Seventeen varieties composed the group with the highest and 24 varieties with the lowest AUDPC means. The disease severity and AUDPC data had a significant correlation with an $R^2 = 0.76$ (Figure 1).

Genome-wide association of common bean SNPs to resistance against CBB

Amongst the 384 SNP markers used in this study, 103 remained after applying the quality control criteria and were used to determine their association with resistance to CBB. The GWAS did not reveal statistically significant association of any SNP marker with resistance to CBB. Nonetheless, an examination of the ten SNP markers with the highest -log(P) values, located on chromosomes 1, 3, 6, 7, 8, 9, 10 and 11 (Figure 2; Table 3), indicated that they were linked to a set of genes, all of which, except gene *Phvul.003G182700* (which codes for a SRF-type transcription factor) code for proteins that belong to families that have been previously associated with plant resistance to pathogens (Table 3). Notably, SNP marker 142 located on chromosome 7, whose -log(P) value was the greatest and clearly separated from those for other SNPs in the Manhattan plot (Figure 2), lays within a gene coding for a serine-threonine kinase and is a *Solanum lycopersicum* CTR1 homolog. Other SNPs with -log(P) equal or greater than 0.9 were linked or laid within genes that code for diverse biochemical functions, including serine-threonine kinase, glutamine synthetase, pentatricopeptide repeat protein, plastid-lipid associated protein, lectin domain-containing kinase and transcription factors.

DISCUSSION

In this study, amongst 103 common bean varieties evaluated, 29 highly resistant to CBB were identified based on disease severity, which positively correlated with AUDPC. In addition, ten SNP markers with the highest -log(P) values for association with resistance to CBB within or closely linked to genes coding for proteins of diverse biochemical functions were found in a GWAS using 80 of the phenotyped common bean varieties.

The inoculation of the common bean varieties was conducted twice and the data were subjected to statistical analysis to determine whether interaction between plant response and environment occurred, and no significant interaction was found. Amongst the 29 varieties with the lowest mean disease notes, the highly resistant response to *Xap* of varieties IPR Bem-te-vi, IAC-Ybaté, RP 1, IAPAR 44, Rico 1735, IPR Inhambu, IPR Tangará, IPR Colibri, IPR Saracura, IPR Quero-Quero, Milionário 1732, Rudá, Varre-Sai, IPR Graúna, FT Bonito, is reported for the first time.

Consistent with previous reports, varieties IAPAR 80, BR 6-Barriga Verde and Diamante Negro were found to be resistant to *Xap-7* (Silva *et al.*, 2000; Vavassori *et al.*, 2006; da Silva *et al.*, 2009). Conversely, varieties BRS Majestoso, BRSMG Madrepérola, BRS Cometa that were previously classified as susceptible (Wendland *et al.*, 2006; Rezende *et al.*, 2011; Azevedo *et al.*, 2015) were found to be resistant to *Xap-7* in the present study. These observations suggest that the resistance components of common bean genotypes may be effective against specific groups of *Xap* strains. Nonetheless, additional studies comparing the response of common bean genotypes to different *Xap* strains in the same experiment must be conducted to prove or rule out this interpretation.

It is worth mentioning that the common bean varieties presented different levels of resistance to CBB, indicating that it is of horizontal type, which is consistent with results of previous reports (Miklas *et al.*, 2003; Chataika *et al.*, 2011; Tryphone *et al.*, 2012). Moreover, the AUDPC estimates varied from 19 to 55, a range that is similar to that reported by Durham, (2011), who found a maximum value of 59.9 in a similar study. The AUDPC is widely used to assess quantitative resistance to disease in plants, particularly to identify genotypes that express different patterns of disease progression (Jeger & Viljanen-Rollinson, 2001), which could be associated with some components of quantitative resistance. Nonetheless, to date, no genes involved quantitative resistance of common bean to CBB have been identified.

As for disease severity, the common bean genotypes were separated into four AUDPC groups. However, although a positive correlation of severity with AUDPC was observed, not all of the varieties that were in the lowest disease severity group were also in the lowest AUDPC group. The highly resistant varieties BRS Majestoso, BR 6-Barriga Verde, and IAPAR 80, that had mean disease severity of one or lower at 15 dai, were also grouped in the lowest AUDPC means group. Most of the other varieties classified as highly resistant were also classified in the lowest or intermediate AUDPC mean groups, except varieties IPR Bem-te-vi, IAC-Ybaté, RP 1, BRSMG Madrepérola and Rudá R that were classified in the group with the second largest AUDPC means. These results suggest that some plant resistance components may restrain disease symptoms progression while others may limit the extent of tissue damage caused by the pathogen.

Even though the GWAS did not reveal significant association of any of the 103 SNPs analyzed with CBB resistance, it is very striking that all of the genes linked to SNPs whose -log(P) value was equal or greater than 0.9 (with the exception of gene *Phvul.003G182700* coding for an SRF-type transcription factor) code for proteins that belong to families that have previously been associated with plant response to pathogens (Frye and Innes, 1998; Daniel *et al.*, 1999; Swiderski & Innes, 2001; Durian *et al.*, 2016; Nirmala *et al.*, 2006; Geddy & Brown, 2007; Lin *et al.*, 2008; Silva *et al.*, 2009b; Zhong & Chang, 2012; González *et al.*, (2017); Huang *et al.*, 2017; Wang & Bouwmeester, 2017). These genes are distributed over eight of the common bean chromosomes.

The SNP with the largest -log(P) value found in this study was marker 142, which lays within the sequence of the gene *Phvul.007G184600* that codes for a protein with functional annotation as a serine-threonine protein kinase, protein tyrosine kinase, and ethylene-responsive protein kinase Le-CTR1. Serine-threonine protein kinases participate in the control of a large number of signal transduction pathways in cells (Silva *et al.*, 2009). With regard to their involvement in plant disease resistance, four out of the approx. 50 cloned plant resistance genes encode serine-threonine kinases (Nirmala *et al.*, 2006). Proteins involved in recognition of bacterial phytopathogens with serine-threonine kinases include the rice Xa21 kinase that confers resistance to species of *Xanthomonas*, Pto and Prf that confer resistance in tomato to *Pseudomonas syringae* pv. *tomato* and the *Arabidopsis* Pbs1 which is required by the product of the *Rps5* gene to mediate resistance against *P. syringae* (Swiderski & Innes, 2001; Liu *et al.*, 2002; Martin *et al.*, 2003). The Triple Constitutive Response 1 (CTR1) protein also belongs to the family of serine-threonine kinases and is an important negative regulator of ethylene signaling (Zhong & Chang, 2012). A *Lycopersicum esculentum* CTR1 homolog (LeCTR2) has been shown to exhibit serine-threonine kinase activity and to be implicated in plant defense against *Botrytis cinerea* (Lin *et al.*, 2008). Also, mutation in another *CTR1*-like gene, Enhanced Disease Resistance 1 (*EDR1*), in *Arabidopsis* confers enhanced disease resistance against *P. syringae* and the ascomycete fungus *Erysiphe cichoracearum* (Frye and Innes, 1998).

Single nucleotide polymorphism marker 299 was found to be within gene *Phvul.009G155900* coding for a member of the pentatricopeptide repeat (PPR) protein family, which are modular RNA-binding proteins that mediate various aspects of gene expression (Manna, 2015). It has been shown that some members of this protein family share features with plant resistance proteins (Geddy & Brown, 2007). In a search for QTLs associated with common bean resistance to *P. syringae* pv. *phaseolicola*, González *et al.*, (2017) identified a marker on chromosome 10 linked to a gene coding for a putative PPR protein.

Two of the SNPs (155 and 240) are located inside genes (*Phvul.006G155800* and *Phvul.006G155800*) coding for glutamine synthetases. It is well known that the enzyme glutamine synthetase plays a key role in the glutamate route. Glutamate metabolism is involved in the defense response of at least ten host plant species against biotrophic, necrotrophic and hemibiotrophic pathogens. Amongst the hemibiotrophic pathogens in which the glutamate metabolism plays a defense role are the Gram-negative bacteria *P. syringae* pv. *syringae, P. syringae* pv. *tabaci*, *P. syringae* pv. *tomato* and *X. axonopodis* pv. *vesicatoria* (Seifi *et al.*, 2013; Huang *et al.*, 2017).

Common bean gene *Phvul.008G188700* linked to SNP marker 255 codes for a lectin domain kinase, which are enzymes that, in general, play a key role in the response of plants to pathogens. For instance, it has been demonstrated in *Arabidopsis* that lectin kinases are involved in defense responses against *P. syringae* and the oomycetes *Phytophthora brassicae* and *Phytophthora capsici* (Wang & Bouwmeester, 2017). A similar involvement in plant defense against pathogens has been demonstrated for phosphoserine phosphatases, the biochemical function coded by gene *Phvul.010G007300* linked to SNP marker 93. For example, silencing of protein phosphatase PP4 in *Nicotiana benthamiana* showed it to exert a role as a negative regulator of the defense responses against *P. syringae* and *Cladosporium fulvum* since the silenced plants exhibited enhanced resistance to these two pathogens (Durian *et al.*, 2016). On the other hand, MYB transcription factors, the function encoded in gene *Phvul.011G034900* linked to SNP marker 211, play an important role in the control of diverse cellular processes, including the first steps of defense responses associated with an active and quick induction of the hypersensitivity (HR) response in *Arabidopsis* against *Xanthomonas campestris* and *P. syringae* pv. *tomato* (Daniel *et al.*, 1999).

In summary, this study identified 29 varieties of common bean with high levels of horizontal resistance to CBB, mostly related to lower AUDPC values. The resistance response of 15 common bean varieties to CBB is reported for the first time. An exploratory GWAS to identify SNPs associated with CBB resistance using 80 phenotyped common bean varieties pointed to genes with putative functions related to plant resistance to disease, such as serine-threonine kinases, glutamine synthetases, lectin domain proteins, transcription factors, plastid-lipid associated proteins, phosphoserine phosphatase and pentatricopeptide repeat proteins. The results of this work not only provide valuable information on the resistance of common bean varieties to CBB, but also important insights into genes potentially involved in the resistance response.

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TABLES

Table 1. Disease severity and classification (susceptibility/resistance) of common bean varieties to *Xanthomonas axonopodis* pv. *phaseoli.*

Variety	Severity*		Classification**	Variety	Severity*		Classification**
Rico 23	7.5	a	HS	Ouro Negro	4.8	b	MS
IPR 139	7.3	a	HS	BRS Grafite	4.8	b	MS
VP 22	7	a	HS	BRSMG Talismã	4.8	b	MS
BR-2 Grande Rio	7	a	HS	Rio Tibagi	4.8	b	MS
BRS Requinte	6.5	a	HS	IAC Tunã	4.5	b	MS
BRS Supremo	6.3	a	HS	IRAÍ	4.5	b	MS
IAC Votuporanga	6.3	a	HS	Rio doce	4.5	b	MS
IAC Carioca	6	a	HS	IAC Formoso	4.5	b	MS
Macotaço	6	a	HS	BRS Valente	4.5	b	MS
IAC-Maravilha	5.8	a	HS	Aporé	4.5	b	MS
BRS Agreste	5.8	a	HS	BR- IPA 11-Brígida	4.3	b	MS
Princesa	5.8	a	HS	BR 1- Xodó	4	c	MR
Pampa	5.8	a	HS	IAPAR 31	4	c	MR
BRS Notável	5.8	a	HS	Carioca MG	4	c	MR
IAC-Una	5.8	a	HS	Preto Uberabinha	4	c	MR
IAC Imperador	5.8	a	HS	BRSMG Pioneiro	4	c	MR
IPR Siriri	5.3	b	MS	BR-IPAGRO 1- Macanudo	4	c	MR
FT 120	5.3	b	MS	VC15	3.8	c	MR
IAPAR 81	5.3	b	MS	VP 33	3.8	c	MR
Carioca 80	5.3	b	MS	BRS Expedito	3.8	c	MR
IPR Andorinha	5.3	b	MS	BRS Esteio	3.8	c	MR
IAC-Apuã	5	b	MS	IPR Chopin	3.8	c	MR
IAC Ayso	5	b	MS	IPR Tiziu	3.5	c	MR
BR- IPA 10	5	b	MS	Onix	3.5	c	MR
IAPAR 8-Rio Negro	4.8	b	MS	BRS Campeiro	3.5	c	MR

Table 1. Continued.

Variety	Severity*		Classification**	Variety	Severity*		Classification**
Pérola	3.5	c	MR	BRS Estilo	2.5	d	MR
Porto Real	3.5	c	MR	Rudá R	2.5	d	HR
IAC Alvorada	3.5	c	MR	BRS Pontal	2.5	d	HR
BRS Esplendor	3.3	c	MR	IAC - Carioca Pyatã	2.5	d	HR
IAPAR 16	3.3	c	MR	FT bonito	2.2	d	HR
Minuano	3.3	c	MR	IPR Graúna	2.2	d	HR
SCS Guará	3.3	c	MR	Varre-Sai	2.2	d	HR
Moruna	3.3	c	MR	BRS Cometa	2.2	d	HR
Capixaba Precoce	3.3	c	MR	BRS Ametista	2.2	d	HR
Carioca 1030	3.3	c	MR	Rudá	2.2	d	HR
Carioca 1070	3	c	MR	Milionário 1732	2.2	d	HR
Meia Noite	3	c	MR	IPR Quero-Quero	2.2	d	HR
VC 17	3	c	MR	IPR Saracura	2	d	HR
IPR Gralha	3	c	MR	IPR Colibri	2	d	HR
BRS Horizonte	3	c	MR	IPR Tangará	2	d	HR
IPR Uirapurú	3	c	MR	IPR Inhambu	2	d	HR
IAPAR 20	3	c	MR	Rico 1735	1.7	d	HR
IAC- Diplomata	2.8	c	MR	BRSMG Madrepérola	1.6	d	HR
IAC - Carioca Akytá	2.8	c	MR	IAPAR 44	1.5	d	HR
IAPAR 57	2.8	c	MR	RP 1	1.5	d	HR
BR-3 Ipanema	2.8	c	MR	IAC-Ybaté	1.5	d	HR
IPR Campos Gerais	2.8	c	MR	IPR Bem-te-vi	1.5	d	HR
IPR Curió	2.8	c	MR	Diamante Negro	1.2	d	HR
IAC Milênio	2.8	c	MR	BRS Majestoso	1	d	HR
IPR Tuiuiú	2.5	d	HR	BR 6-Barriga verde	0.8	d	HR
IAPAR 65	2.5	d	MR	IAPAR 80	0.5	d	HR
IPR Eldourado	2.5	d	MR				

* Within this column, means followed by the same letter are not significantly different according to the Scott-Knott test ($p \leq 0.05$) on square root transformed data.

** HS, highly susceptible; HR, highly resistant; MR, moderately resistant; MS, moderately susceptible.

Table 2. Area under the disease progress curve (AUDPC) in common bean varieties in response to Common Bacterial Blight.

Variety	AUDPC*		Variety	AUDPC*	
IPR 139	55	a	IAC Formoso	35	c
Rico 23	55	a	Milionário 1732	35	c
VP 22	53	a	BRS Esteio	35	c
IAC Votuporanga	51	a	IPR Tuiuiú	34	c
BRS Requinte	51	a	IAC-Apuã	33	c
Pampa	48	a	IPR Campos Gerais	33	c
IAC-Maravilha	48	a	IRAÍ	33	c
BRS Notável	47	a	IAC - Carioca Akytá	33	c
BR-2 Grande Rio	47	a	BRS Pontal	33	c
Princesa	46	a	SCS Guará	32	c
IAPAR 16	46	a	IAC Milênio	32	c
BRS Grafite	45	a	BRS Cometa	32	c
IAC Carioca	45	a	BRS Esplendor	32	c
BRS Agreste	45	a	FT bonito	31	c
IAPAR 8-Rio Negro	45	a	Diamante Negro	30	c
Carioca MG	45	a	VC 17	30	c
VC15	44	a	VP 33	30	c
BRS Supremo	44	b	IAC Alvorada	30	c
IAPAR 81	44	b	IPR Eldourado	30	c
IAC Imperador	44	b	IAPAR 65	30	c
Aporé	43	b	Pérola	30	c
BRS Valente	43	b	BR- IPA 10	29	c
IPR Andorinha	43	b	IAPAR 31	29	c
BR 1- Xodó	42	b	Meia Noite	29	c
IPR Siriri	42	b	Rico 1735	28	c
IAC Ayso	42	b	IAPAR 57	28	c
Onix	42	b	IPR Saracura	28	c
Rio Tibagi	41	b	IAC - Carioca Pyatã	28	d
IAC-Una	41	b	BRS Campeiro	27	d
FT 120	41	b	Carioca 1070	27	d
RP 1	41	b	IPR Graúna	27	d
BR-IPAGRO 1- Macanudo	41	b	IPR Uirapurú	27	d
Rio doce	41	b	Minuano	27	d
BRSMG Talismã	41	b	Rudá	26	d
Preto Uberabinha	40	b	BRS Estilo	26	d
Capixaba Precoce	40	b	Carioca 1030	26	d
IAC-Ybaté	40	b	Varre-Sai	26	d
BRS Horizonte	40	b	Porto Real	25	d
BR-3 Ipanema	39	b	BRS Ametista	25	d
Macotaço	39	b	IPR Inhambu	25	d
IAPAR 20	39	b	IAC- Diplomata	25	d
Carioca 80	39	b	IPR Tangará	25	d
BRS Expedito	39	b	IPR Curió	24	d
IPR Bem-te-vi	38	b	Moruna	24	d
Ouro Negro	38	b	IAPAR 80	23	d
BRSMG Madrepérola	38	b	IAPAR 44	23	d
BRSMG Pioneiro	37	b	BRS Majestoso	23	d
IAC Tunã	37	b	IPR Colibri	22	d
BR- IPA 11-Brígida	36	b	IPR Tiziu	22	d
Rudá R	36	b	BR 6-Barriga verde	19	d
IPR Gralha	36	b	IPR Quero-Quero	18	d
IPR Chopin	36	b			

* Within this column, means followed by the same letter are not significantly different according to the Scott-Knott test ($p \leq 0.05$).

Table 3. Common bean genes linked to Single Nucleotide Polymorphism (SNP) markers with the highest -log(P) values and putatively associated with Common Bacterial Blight resistance.

SNP	Chr.*	Genome position (pb)	Candidate gene	Distance (bp)**	Position in the gene***	Putative function
142	7	42072686	Phvul.007G184600	Within	E14/14	Serine-threonine protein kinase, protein tyrosine kinase, ethylene-responsive protein kinase Le-CTR1
240	6	26864717	Phvul.006G155800	Within	E12/12	Glutamine synthetase
155	6	23676256	Phvul.006G155800	Within	I6/7	Glutamine synthetase
299	9	22662290	Phvul.009G155900	Within	E1/1	Pentatricopeptide repeat protein
197	1	6782067	Phvul.001G058000	Within	I2/3	Plastid-lipid associated protein (PAP)
211	11	3058811	Phvul.011G034900	+ 2576	-	MYB transcription factor homolog
340	11	3129740	Phvul.011G035600	Within	I7/11	Serine/threonine protein kinase
93	10	1131266	Phvul.010G007300	Within	I3/5	Phosphoserine phosphatase (serB, PSPH)
191	3	39427394	Phvul.003G182700	Within	I2/6	SRF-type transcription factor (DNA-binding and dimerization domain), K-box region
255	8	49262096	Phvul.008G188700	-832	-	Legume lectin domain, protein tyrosine kinase, serine/threonine protein kinase

* Chr, chromosome.

** Distance from the closest gene model; within, lays in the candidate gene sequence; +, upstream; -, downstream.

*** I, intron; E, exon; numbers indicate (position of the SNP in an intron or exon)/(total number of introns or exons in the gene model).

FIGURES

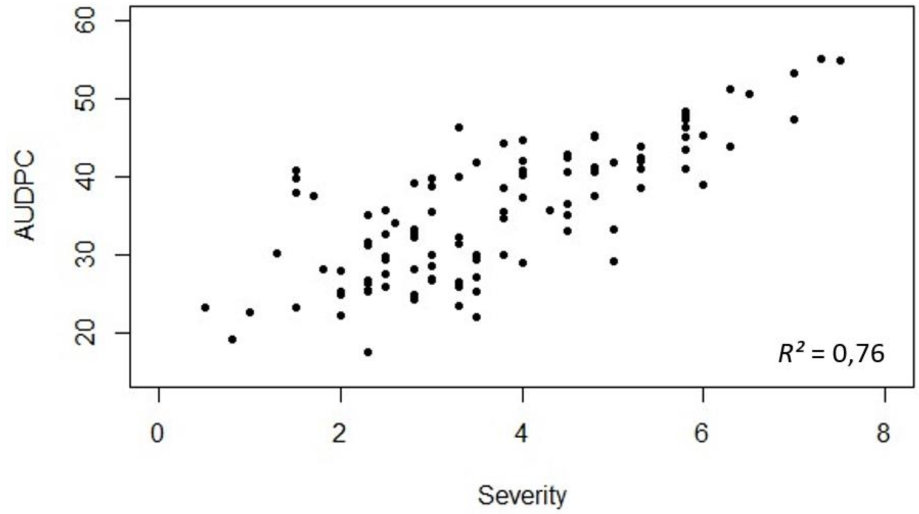

Figure 1. Correlation between the area under the disease progress curve (AUDPC) and disease severity of 103 common bean varieties at 15 days after inoculation with *Xanthomonas axonopodis* pv. *phaseoli*.

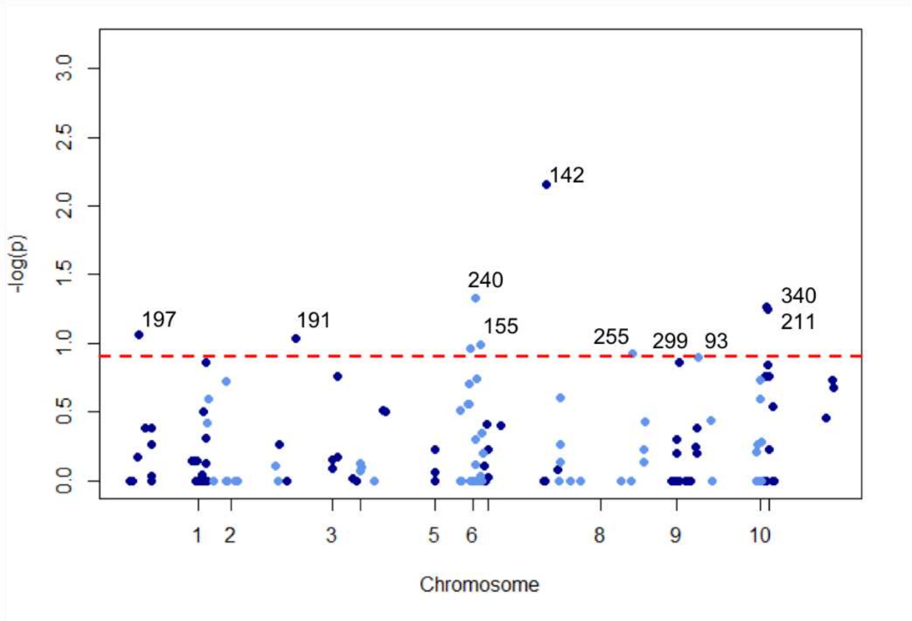

Figure 2. Manhattan plot of the genomic association of 80 common bean SNPs with resistance to Common Bacterial Blight. The red dashed line indicates the -log(P) threshold of 0.9 used to select SNPs for gene annotation. The numbers indicate the SNPs identification.

SUPPEMENTARY MATERIAL

Table S1. Common bean varieties used in this study.

Variety	Source	Variety	Source
Aporé*	EMGOPA	IAPAR 31*	IAPAR
BR 1- Xodó*	PESAGRO	IAPAR 44*	IAPAR
BR 6-Barriga verde	EMPASC	IAPAR 57*	IAPAR
BR- IPA 10*	IPA	IAPAR 65*	IAPAR
BR- IPA 11-Brígida*	IPA	IAPAR 80	IAPAR
BR-2 Grande Rio*	PESAGRO	IAPAR 81*	IAPAR
BR-3 Ipanema*	PESAGRO	IAPAR 8-Rio Negro*	IAPAR
BR-IPAGRO 1- Macanudo *	IPAGRO	IPR 139*	IAPAR
BRS Agreste	EMBRAPA	IPR Andorinha*	IAPAR
BRS Ametista	EMBRAPA	IPR Bem-te-vi	IAPAR
BRS Campeiro*	EMBRAPA	IPR Campos Gerais*	IAPAR
BRS Cometa*	EMBRAPA	IPR Chopin	IAPAR
BRS Esplendor*	EMBRAPA	IPR Colibri*	IAPAR
BRS Esteio	EMBRAPA	IPR Curió	IAPAR
BRS Estilo*	EMBRAPA	IPR Eldourado*	IAPAR
BRS Expedito*	EMBRAPA	IPR Gralha*	IAPAR
BRS Grafite*	EMBRAPA	IPR Graúna*	IAPAR
BRS Horizonte	EMBRAPA	IPR Inhambu	IAPAR
BRS Majestoso*	EMBRAPA	IPR Quero-Quero	IAPAR
BRS Notável*	EMBRAPA	IPR Saracura*	IAPAR
BRS Pontal*	EMBRAPA	IPR Siriri	IAPAR
BRS Requinte*	EMBRAPA	IPR Tangará*	IAPAR
BRS Supremo*	EMBRAPA	IPR Tiziu*	IAPAR
BRS Valente*	EMBRAPA	IPR Tuiuiú*	IAPAR
BRSMG Madrepérola*	UFV	IPR Uirapurú*	IAPAR
BRSMG Pioneiro*	EMBRAPA	IRAÍ*	IPAGRO
BRSMG Talismã*	UFV/UFLA	Macotaço	EMBRAPA
Capixaba Precoce*	EMCAPA	Meia Noite	EMBRAPA
Carioca 1030*	IAC	Milionário 1732*	EPAMIG
Carioca 1070*	IAC	Minuano	EMBRAPA
Carioca 80*	IAC	Moruna*	IAC
Carioca MG	EMBRAPA	Onix*	EMGOPA
Diamante Negro*	EMBRAPA	Ouro Negro*	EMBRAPA
FT 120*	FT- sementes	Pampa*	EMCAPA
FT bonito*	FT- sementes	Pérola*	EMBRAPA
IAC - Carioca Pyatã*	IAC	Porto Real	EMBRAPA
IAC - Carioca Akytá*	IAC	Preto Uberabinha*	IPEACO
IAC Alvorada*	IAC	Princesa	IPAGRO

Table S1. Continued.

Variety	Source**	Variety	Source**
IAC Ayso	IAC	Rico 1735*	EPAMIG
IAC Carioca*	IAC	Rico 23*	UFV
IAC- Diplomata	IAC	Rio doce*	EMCAPA
IAC Formoso*	IAC	Rio Tibagi*	UFV
IAC Imperador*	IAC	RP 1	UFV
IAC Milênio	IAC	Rudá*	EMCAPA
IAC Tunã*	IAC	Rudá R*	EMCAPA
IAC Votuporanga*	IAC	SCS Guará*	EPAGRI
IAC-Apuã*	IAC	Varre-Sai*	PESAGRO
IAC-Maravilha	IAC	VC 17	UFV
IAC-Una*	IAC	VC15*	UFV
IAC-Ybaté*	IAC	VP 22*	UFV
IAPAR 16*	IAPAR	VP 33*	UFV
IAPAR 20*	IAPAR		

* Varieties used in genome-wide association study.

**EMBRAPA, Empresa Brasileira de Pesquisa Agropecuária; EMCAPA, Empresa Capixaba de Pesquisa Agropecuária; EMGOPA, Empresa Goiana de Pesquisa Agropecuária; EMPASC, Empresa Catarinense de Pesquisa Agropecuária; EPAGRI, Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina; EPAMIG, Empresa de Pesquisa Agropecuária de Minas Gerais; IAC, Instituto Agronômico de Campinas; IAPAR, Instituto Agronômico de Paraná; IPA, Instituto Agronômico de Pernambuco; IPAGRO, Instituto de Pesquisas Agronômicas; IPEACO, Instituto de Pesquisa e Experimentação Agropecuária do Centro-Oeste; PESAGRO, Empresa de Pesquisa Agropecuária do Estado do Rio de Janeiro; UFV, Universidade Federal de Viçosa.

GENERAL CONCLUSIONS

The following conclusions can be drawn from the results obtained in this study:

1. Varieties BRS Radiante and IAPAR 16 exhibit high levels of broad-spectrum horizontal resistance, which may be involved in limitation of bacterial multiplication in leaf tissues but not necessarily associated with stomatal density.
2. A set of eleven SCAR markers was developed to identify hybrid plants derived from crosses using the varieties BRS Radiante, BRS Campeiro, Carioca MG, Diamante Negro, IAPAR 16, IAPAR 81, IPR Chopim and Ouro Negro.
3. Diallelic analysis indicated that BRS Radiante, IAPAR 16 and Chopim IPR contribute to genetic resistance against CBB according to GCA values.
4. Based on SCA values, the crosses BRS Radiante × Carioca MG, IAPAR 16 × IAPAR 81 and IPR Chopim × Ouro Preto were shown to be promising combinations to obtain enhanced resistance against *Xap*.
5. According to results of inheritance hypotheses tests, the resistance present in variety BRS Radiante is conditioned by at least two dominant genes.
6. Maximum likelihood analysis indicated that, in addition to the dominant genes involved in the resistance of the BRS Radiante variety to CBB, additive effects and polygenes are also involved.
7. BC_1F_1 and BC_2F_1 plants highly resistant to CBB derived from a cross between BRS Radiante × Carioca MG were obtained, which will be useful in further studies aimed at understanding the molecular basis of common bean horizontal resistance to CBB.
8. Twenty-nine varieties with high levels of horizontal resistance were identified, fifteen of which were classified as resistant for the first time.

9. An exploratory GWAS allowed the identification of SNP potentially associated with resistance to CBB. These SNPs lay on or are in close proximity to genes coding for proteins, such as serine-threonine kinases, glutamine synthetases, lectin-domain proteins, transcription factors, plastid-associated protein lipids, phosphoserine phosphatase and pentatricopeptide repeat proteins, some of which have previously been associated with plant resistance to disease.