Sources, Spectrum, Genetics, and Inheritance of *Phaseolus vulgaris* Resistance Against *Xanthomonas citri* pv. *fuscans*

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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 *Xanthomonas citri* pv. *fuscans* and *X. phaseoli* pv. *phaseoli*. In this work, we investigated the spectrum, genetics, and inheritance of common bean resistance to *X. citri* pv. *fuscans*. Inoculation of nine selected cultivars with an *X. citri* pv. *fuscans* strain showed that BRS Radiante and IAPAR 16 were resistant. These two cultivars were also resistant to six *X. phaseoli* pv. *phaseoli* strains of different geographic origins, demonstrating their broad-spectrum resistances. BRS Radiante sustained smaller *X. citri* pv. *fuscans* populations than two susceptible cultivars. Stomatal densities of IAPAR 16 and BRS Radiante were significantly higher than or not different from susceptible cultivars. BRS Radiante showed the lowest general combining ability values and the combination BRS Radiante × Carioca MG the lowest specific combining ability (SCA) values, revealing the capacity of BRS

Radiante to increase resistance to *X. citri* pv. *fuscans*. Positive and negative parental SCA values indicated dominant and recessive genes involved in *X. citri* pv. *fuscans* resistance. Resistance of the BRS Radiante × Carioca MG cross segregated in a 9:7 ratio in the F₂ population, indicating that it is governed by two complementary dominant genes. Maximum likelihood analysis showed that the resistance of BRS Radiante to *X. citri* pv. *fuscans* is conferred by a gene of major effect with contribution of additional polygenes. This study contributes with important knowledge on the resistance against CBB in Brazilian common bean cultivars as well as with molecular tools for confirmation of common bean hybrids.

Keywords: analytical and theoretical plant pathology, bacterial multiplication, common bacterial blight, common bean, disease control and pest management, genetics and resistance, stomatal density, *Xanthomonas phaseoli* pv. *phaseoli*

Common bean (*Phaseolus vulgaris* L.) is one of the most important food crops worldwide because of its high nutritional content and commercial value (Yu et al. 2000). It is an important source of proteins and energy for about 500 million people in African and Latin American countries (Broughton et al. 2003). India, Myanmar, and Brazil were the three major producers, contributing 19.5, 16.7, and 9.3% to the total production, respectively (FAOSTAT 2019). However, common bean crops in several areas around the world are threatened by common bacterial blight (CBB), a disease that can cause reductions in field production of up to 45% depending on the environmental conditions and the level of resistance of the host genotype (Fininsa 2003).

Bacteria that cause CBB penetrate primarily through stomata and wounds, colonize the mesophyll cells, and spread systemically throughout the plant, affecting different types of tissue, including stem causing cankers, pods causing water-soaked lesions that may become brown, and seeds that can develop extensive discoloration (Akhavan et al. 2013). Chemical control of CBB is difficult due to

the limited number of available products and their low efficacy as curative methods (Coyne and Schuster 1983). The use of plant genetic resistance is the preferred management 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 (Miklas et al. 2003; Singh and Muñoz 1999).

Sources of genetic resistance to CBB have been found in *P. vulgaris* as well as in other related species (Alladassi et al. 2018; Costa et al. 2008; Coyne and Schuster 1983; Kobayashi et al. 1999; Maringoni 1998; Silva et al. 2000; Singh and Miklas 2015). In studies carried out in Brazil, resistance to CBB was identified in the cultivar BRS Radiante in the field (Costa et al. 2008; Maringoni 1998) as well as in cultivars IAPAR 16 and Diamante Negro under greenhouse conditions (Silva et al. 2000). On the other hand, cultivars Ouro Negro, BRS Campeiro, IAPAR 81, Manteigão Fosco 11, and Carioca MG were classified as susceptible (Costa et al. 2008; Kobayashi et al. 1999; Maringoni 1998; Silva et al. 2000) and IPR Chopim as moderately resistant (da Silva et al. 2009). Most studies on the genetic resistance of common bean to CBB indicate that the inheritance is quantitative and polygenic in nature (Coyne and Schuster 1983; Singh and Miklas 2015; Singh and Muñoz 1999; Tryphone et al. 2012).

Traditionally, CBB has been attributed to the gram-negative bacteria *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (Young et al. 1996) and its variant *X. axonopodis* pv. *phaseoli* var. *fuscans* (Gilbertson and Maxwell 1992). Blight symptoms caused by these two pathogens are indistinguishable and both can cause significant negative impact on yield and seed quality, but the two bacteria can be easily differentiated by production of melanin in vitro by the *fuscans* variant, although this characteristic seems not to be involved in pathogenicity (Chan and Goodwin 1999). Several

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studies with strains from different geographic origins, based on repetitive genomic sequences (Bett and Banniza 2014; De Paiva et al. 2018; López et al. 2006; Mutlu et al. 2008), have provided strong evidence indicating that these two bacterial pathogens are genetically very different, justifying their separation into two different species. As a consequence, the *fuscans* variant was initially reclassified as *X. fuscans* subsp. *fuscans* (Schaad et al. 2005). Further support to the separation into two distinct species was provided by results of genome comparisons (Aritua et al. 2015). More recently, Constantin et al. (2016), based on polyphasic taxonomy, including average nucleotide identity (ANI) values proposed the reclassification of *X. fuscans* subsp. *fuscans* as *X. citri* pv. *fuscans* (Schaad et al. 2007) and *X. axonopodis* pv. *phaseoli* as *X. phaseoli* pv. *phaseoli* (Smith 1897).

Although *X. citri* pv. *fuscans* and *X. phaseoli* pv. *phaseoli* cause similar symptoms in common bean plants, several important differences between them should be considered when attempting to find sources of resistance and to understand the genetics and molecular mechanisms behind it. Firstly, diversity studies have consistently shown that *X. citri* pv. *fuscans* is genetically more homogeneous and appears to exhibit less aggressiveness variation than *X. phaseoli* pv. *phaseoli* (De Paiva et al. 2018; López et al. 2006; Mutlu et al. 2008), suggesting a shorter coevolution time of *X. citri* pv. *fuscans* with the host plant. Secondly, most studies have found that *X. citri* pv. *fuscans* strains cause more severe symptoms in the same common bean genotypes than *X. phaseoli* pv. *phaseoli* (Andrade et al. 2017; Mutlu et al. 2008; Opio et al. 1996), although some others indicate that there is no difference between the two bacterial species (Bett and Banniza 2014; De Paiva et al. 2018). Lastly, a single study suggested that *X. citri* pv. *fuscans* caused a more negative impact on seedling emergence, incidence of CBB, frequency of seed-to-seedling transmission, and seedling infection than *X. phaseoli* pv. *phaseoli* (He and Munkvold 2013). These results reinforce the need for a better understanding of the genetics and mechanisms involved in the resistance effective against each particular bacterial species, even more considering that these two bacterial pathogens have been shown to be sympatric in several geographic areas (Bett and Banniza 2014; De Paiva et al. 2018). Whether the same plant resistance genes are effective against both pathogenic species is largely unknown. So far, the vast majority of studies have investigated plant genetic resistance against *X. phaseoli* pv. *phaseoli* and very little effort has been put on *X. citri* pv. *fuscans* (Andrade et al. 2017; Kobayasti et al. 1999).

Thus, the main objectives of this work were to (i) identify common bean cultivars expressing resistance to *X. citri* pv. *fuscans* and determine if their resistances are related to restriction of bacterial growth in plant tissue or to stomatal density; (ii) estimate the contribution to *X. citri* pv. *fuscans* resistance of the resistant cultivars identified and the potential of their combinations with susceptible cultivars to enhance *X. citri* pv. *fuscans* resistance; and (iii) determine the genetics of the resistance to *X. citri* pv. *fuscans* acting in the most promising cross through inheritance and maximum likelihood function tests.

MATERIALS AND METHODS

Bacterial strains. The *X. citri* pv. *fuscans* and *X. phaseoli* pv. *phaseoli* strains used in this study either belong to the culture collection of the Laboratory of Molecular Phytobacteriology at the Universidade Federal de Viçosa (CFBM-UFV) or were obtained from the Laboratory of Phytobacteriology at the Universidade Federal de Lavras (Supplementary Table S1). Production of brown pigmentation was observed only for CFBM-UFV-0001 upon growth on YDC medium. Resistance to rifampicin in this strain was induced by growing it at 28°C for 24 h on solid 523 medium (Kado and Heskett 1970) amended with successive increasing concentrations of the antibiotic (from 1 to 100 µg ml⁻¹). Bacterial strains were routinely retrieved from 30% glycerol stocks at -80°C

and grown by streaking on solid 523 medium (Kado and Heskett 1970) and incubating at 28°C for 24 to 48 h.

Plant material and maintenance. Pure seeds of the nine common bean cultivars used in this study were obtained from the germplasm bank of the Universidade Federal de Viçosa (UFV), Viçosa, MG, Brazil (Supplementary Table S2). Accessions classified as resistant or susceptible in previous studies and that have phenotypic and agronomic characteristics similar to those of cultivars planted in Brazil were selected (Costa et al. 2008; Kobayasti et al. 1999; Maringoni 1998; Silva et al. 2000). The seeds were scarified by scrapping the tegument with the aid of sandpaper and then pregerminated on germination paper sterilized in an oven at 100°C for 5 h. After the emission of the radicle, the seeds were transplanted to 2-liter-capacity pots containing a 1:1 mixture of substrate Tropstrato HT Hortalças (Vida Verde, Mogi Mirim, SP, Brazil) and soil. Every 15 days the plants were fertilized with Niphokam (Fênix-Agro-Pecus Industrial Ltd., Tietê, SP, Brazil). The plants were maintained in a greenhouse at the Department of Plant Pathology of the UFV.

Plant inoculation. Bacterial cells from 24 to 48 h cultures were resuspended in 10 mM MgCl₂ and the optical density at 600 nm (OD₆₀₀) was adjusted to 0.1. Plants at the V3 stage (first fully expanded trifoliate) were inoculated by spraying the bacterial suspension onto both leaf surfaces with an atomizer Jet Master (Schulz S.A., Joinville, SC, Brazil) until run-off point. The plants were maintained in a mist chamber at 25°C for 24 h before and 24 h after inoculation and then kept in the greenhouse until the end of the experiments.

Evaluation of plant resistance. The response of nine common bean cultivars to inoculation with strain CFBM-UFV-0001 was evaluated in September 2016, and the whole experiment was repeated in March 2017. Seven plants of each common bean cultivar were treated with 10 mM MgCl₂ to serve as controls. Inoculated plants were kept in the greenhouse in a completely randomized design, in which the experimental unit consisted of two plants per pot (both of which were evaluated), with seven repetitions per cultivar. At 15 days after inoculation (DAI), the two most affected trifoliate of each plant were selected and scanned at 600 dpi resolution with an HP scanner model PSC 1500 series (Hewlett-Packard, Palo Alto, CA, U.S.A.), and 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 (ANOVA) and the means were grouped by the Scott-Knott test ($P \leq 0.05$).

To determine whether the resistance to *X. citri* pv. *fuscans* of cultivars IAPAR 16, BRS, Radiante was also effective against *X. phaseoli* pv. *phaseoli*, they were spray inoculated as described above with six strains of the latter pathogen from diverse origins. Carioca MG and strain CFBM-UFV-0001 were also included to serve as comparators. Each common bean cultivar was independently inoculated with each strain. The experiments were set up in completely randomized block designs, in which each block comprised one full repetition and each experimental unit consisted of two plants per pot (both of which were evaluated), with five repetitions per treatment. Disease symptoms were evaluated using the QUANT software (Vale et al. 2003) as indicated above. The data were subjected to ANOVA and the means were grouped by the Scott-Knott test ($P \leq 0.05$).

Quantification of in planta bacterial populations. With the help of a cork-borer, three 0.7-cm diameter discs were taken from the same leaves that were used for disease severity assessment, such that the location of the sampled area was standardized for all treatments. Four trifoliate leaves per treatment, each from a different plant, were evaluated. The discs were macerated in 1 ml of 10 mM MgCl₂ solution inside 2-ml microfuge tubes and serial dilutions of the macerates were prepared. A 20-µl aliquot of each dilution was dropped onto solid 523 medium amended with rifampicin (100 µg ml⁻¹) and cycloheximide (50 µg ml⁻¹), and the

plates were incubated at 28°C. After 24 h, the number of individual colonies on the medium was counted and the number of colony forming units per square centimeter of leaf tissue (CFU cm⁻²) was calculated. Each dilution was evaluated in triplicate. The data were subjected to ANOVA and the means were compared by the Tukey test ($P \leq 0.05$).

Determination of stomatal density. Plants were grown in the greenhouse as indicated above and stomata counted when they were at the V3 phenological stage. Six plants of each cultivar were used. Two fully expanded leaves from the trifoliolate of each plant were collected. A colorless cellulose acetate enamel film was carefully applied on the leaf surface and left to 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 40× objective. Images were captured with the camera of a cell phone J7 Prime (Samsung, Seoul, South Korea) and the stomata counted. The following formula was used to calculate the stomatal density: $D = (\text{number of stomata}) / 0.272902 \text{ mm}^2$. The data were log₁₀ transformed and subjected to ANOVA, and the transformed means were grouped with the Scott-Knott test ($P \leq 0.05$). The experiment was conducted twice with similar results.

Obtention of F₁ and F₂ plants. 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 (Paternelli and Borém 1999) with the aid of a stainless-steel forceps disinfested with 70% ethanol; the flower that received the pollen was carefully labeled with a tag. Because some of the parents utilized in the crosses were not easily distinguishable using morphological characters, a series of sequence characterized amplified region (SCAR) markers previously described in the literature were tested with all nine cultivars used in this study and evaluated to confirm hybridization in different crosses. The F₂ population was obtained by autogametic reproduction of F₁ plants.

DNA extraction and quantification. DNA was extracted with the Wizard Genomic DNA Purification System kit (Promega, Madison, WI, U.S.A.) according to the manufacturer's instructions or using the CTAB method (Doyle and 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. Primers (Supplementary Table S3) previously reported in the literature (Blair et al. 2007; Corrêa et al. 2000; Hegay et al. 2013; Larsen et al. 2010; Mahuku et al. 2004; Méndez de Vigo et al. 2000; Miklas et al. 2000a, b; Mutlu et al. 2005; O'Boyle et al. 2007) were used for SCAR amplification. PCR reactions were performed with the GoTaq Master Mix Green 2× (Promega) in a 25 µl of 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, U.S.A.), programmed for preheating at 95°C for 5 min, followed by 30 cycles at 94°C for 1 min, 50°C 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°C for 10 min. An electrophoresis in 1.5% agarose gel was carried out to resolve the amplification products, which were visualized and photographed using an L-PIX EX gel documentation system (Loccus, São Paulo, SP, Brazil). Polymorphic bands between the parents were used to confirm hybridization in the progenies.

Diallel analysis. Based on their reactions to strain CFBM-UFV-0001, cultivars BRS Radiante and IAPAR 16 (resistant) were chosen to compose group 1 of a partial diallel, whereas cultivars Diamante Negro, Ouro Negro, IAPAR 81, Carioca MG, and BRS

Campeiro, classified as susceptible, were chosen to compose group 2 (Supplementary Table S4). Plants of groups 1 and 2 were crossed in the form of partial diallels to obtain 10 combinations of plant candidates to hybrids.

Three plants of each parental and F₁ (in all possible combinations of resistance × susceptibility) were inoculated with a suspension of strain CFBM-UFV-0001 and maintained in the greenhouse as described above. The development of disease symptoms was monitored daily and their severity was evaluated at 15 DAI using the 1 to 9 rating scale of Schoonhoven and Pastor-Corrales (1987). Plants showing disease notes of 3 or lower were considered resistant. Disease severity data were subjected to ANOVA to estimate the means and variances of the populations by the weighted least squares method (Ramalho et al. 1993). The partial diallel analysis for parental and F₁ plants was performed according to the Geraldi and de Miranda-Filho (1988) model adapted from the Griffing (1956) method using the following equation:

$$Y_{ij} = \mu + \frac{1}{2}(d_1 + d_2) + g_i + g'_j + s_{ij} + e_{ij}$$

where Y_{ij} 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; g_i is the effect of the general combining ability of the i th parent of group 1; g'_j is the effect of the general combining ability of the j th parent of group 2; s_{ij} is the effect of specific combining ability (SCA); and e_{ij} is the mean experimental error. The standard deviation of the estimates of general (g_i and g'_j) and combination (s_{ij}) capacity was obtained by the square root of the variances, as in Cruz et al. (2012). Statistical analyses were performed with the Genes program (Cruz 2013).

Tests for inheritance hypotheses. Based on the lowest general combining ability (GCA) values for BRS Radiante and SCA values for BRS Radiante × Carioca MG in the partial diallel analysis, this cross was chosen to test inheritance hypotheses. One hundred and sixty F₂ plants were inoculated with strain CFBM-UFV-0001 and maintained in the greenhouse, and disease severity was rated using the scale proposed by Schoonhoven and Pastor-Corrales (1987), 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 F₂ generation, hypotheses of Mendelian inheritance of resistance by the χ^2 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 (1977) based on means and variances, with the following structure was used:

$$P1 = (\mu - [a] - A, \sigma^2)$$

$$P2 = (\mu + [a] + A, \sigma^2)$$

$$F1 = (\mu + [d] + D, \sigma^2)$$

$$F2 = \frac{1}{4} \left(\mu + \frac{[d]}{2} - A, \sigma^2 + V_A + V_D \right) + \frac{1}{2} \left(\mu + \frac{[d]}{2} + D, \sigma^2 + V_A + V_D \right) + \frac{1}{4} \left(\mu + \frac{[d]}{2} + A, \sigma^2 + V_A + V_D \right)$$

where μ is a reference constant; A is the additive effect of the major effect gene; D is the effect of dominance of the major effect gene;

[a] is the additive effect of polygenic; [d] is the effect polygenic dominance; V_A is the additive variance of the polygenics; V_D is the variance attributed to polygenic dominance deviations; and σ^2 is the environmental variance. The likelihood functions allow to compose models with tests of interest with the hypotheses described in Supplementary Table S5. 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 performed using the likelihood ratio proposed by Mood et al. (1974).

Statistical analysis. All inoculation experiments conducted in the present study were repeated at least once with similar results. Because interaction cultivar $\times$ experiment was found in the initial cultivar screening, results are shown separately for each experiment. Statistical analyses of experiments conducted in this study were applied using the following modules of the R package (R Development Core Team 2018): agricolae (Mendiburu 2017), easyanova (Arnhold 2013), ExpDes for ANOVA (Ferreira et al. 2018), lattice for data visualization (Sarkar 2008), and Scott-Knott, for mean comparisons (Jelihovschi et al. 2014).

RESULTS

Common bean cultivars express different levels of resistance to *X. citri* pv. *fuscans*. Differences in disease severity among cultivars 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, cultivars IAPAR 81, Carioca MG, BRS Campeiro, Diamante Negro, IPR Chopim, and Manteigão Fosco 11 were classified as susceptible, whereas IAPAR 16 and BRS Radiante were classified as resistant (Table 1). The disease severity exhibited by these two cultivars was consistently lower than those of the other cultivars tested in both experiments. Cultivar Ouro Negro grouped along with the resistant cultivars in one experiment, but in the other, it exhibited a response similar to those of susceptible cultivars, which explained the cultivar $\times$ experiment interaction (Table 1).

BRS Radiante sustains smaller populations of strain CFBM-UFV-0001. In order to determine if the reduction in disease symptoms could be associated with restriction of bacterial multiplication in planta, bacteria were recovered from inoculated plants by tissue maceration and dilution plating (Fig. 1). For this, the two resistant cultivars IAPAR 16 and BRS Radiante were contrasted with the two susceptible cultivars with the highest disease severity scores (Table 1). No significant differences were found in the bacterial population sizes recovered from Carioca MG, IAPAR 81 (classified as susceptible to strain CFBM-UFV-0001), and IAPAR

16 (considered resistant). However, the population size recovered from the resistant cultivar BRS Radiante was significantly smaller than those of the two susceptible cultivars (Fig. 1).

Stomatal density is not necessarily associated with resistance to CBB. Considering that *X. citri* pv. *fuscans* penetrates the host tissue mainly through stomata, a possible correlation between stomatal density and resistance was investigated. The stomatal density of resistant cultivars BRS Radiante and IAPAR 16 was not significantly different from that of susceptible cultivar Carioca MG. Interestingly, the susceptible cultivar IAPAR 81 had a significantly lower stomatal density than the other three cultivars (Table 2).

Cultivars resistant to strain CFBM-UFV-0001 are also resistant against *X. phaseoli* pv. *phaseoli* strains. Inoculation of six *X. phaseoli* pv. *phaseoli* strains of different geographic origins on the three common bean cultivars BRS Radiante, IAPAR 16, and Carioca MG revealed significant differences in aggressiveness, as demonstrated by the severity of disease symptoms caused on susceptible cultivar Carioca MG (Table 3). Strains CFBM-UFV-0003 and CFBM-UFV-0006 of *X. phaseoli* pv. *phaseoli* failed to cause disease symptoms to the same extent as the other strains in such a cultivar. Importantly, cultivars IAPAR 16 and BRS Radiante, classified as resistant to strain CFBM-UFV-0001, consistently showed resistance to all *X. phaseoli* pv. *phaseoli* strains tested (Table 3).

A set of SCAR markers identify common bean hybrids. Eight of the cultivars used in this study were tested and clearly distinguished by the banding patterns of SCAR markers (Table 4; Supplementary Figure S1). In all, 12 of 16 SCAR markers tested showed 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 with SCAR primers BC420, SB12, and SU91. Out of 346 hybrid candidate plants derived from artificial pollinations, 177 were confirmed as hybrids using the SCAR markers. It was possible to use at least one SCAR marker to confirm hybrids for each of the 10 cross combinations used in our genetic analyses (Supplementary Table S6).

Diallel analysis for resistance to strain CFBM-UFV-0001 reveals BRS Radiante as a highly promising donor. The GCA and SCA mean square values of group 2, but not of group 1, were significant for resistance to strain CFBM-UFV-0001 (Supplementary Table S7). Within group 1, cultivar 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, IAPAR 81, and Carioca MG showed the lowest GCA values (Table 5). In addition, the SCA values of the group 1 parents were negative in BRS Radiante and positive in IAPAR 16. With respect to

TABLE 1. Disease severity in common bean cultivars inoculated with *Xanthomonas citri* pv. *fuscans* strain CFBM-UFV-0001 in two independent experiments

Cultivar	Severity (% affected area) ^y		Classification ^z
	March 2017	September 2016	
BRS Campeiro	13.1 a	7.7 a	S
BRS Radiante	2.6 b	1.3 b	R
Carioca MG	14.1 a	12.1 a	S
Diamante Negro	12.6 a	7.6 a	S
IAPAR 16	7.8 b	3.4 b	R
IAPAR 81	19.8 a	10.7 a	S
IPR Chopim	11.5 a	6.6 a	S
Manteigão Fosco 11	12.2 a	8.3 a	S
Ouro Negro	14.1 a	3.9 b	S

^y Determined using QUANT version 1.0.1 (Vale et al. 2003). Within each column, means followed by the same letter are not significantly different according to the Scott-Knott test ($P \leq 0.05$).

^z R, resistant; S, susceptible.

Fig. 1. Bacterial populations recovered from bean cultivars resistant and susceptible to bacterial blight at 15 days after inoculation with *Xanthomonas citri* pv. *fuscans* strain CFBM-UFV-0001. Bars indicate the means and vertical lines indicate standard errors of four repetitions. Means of columns labeled with the same letter are not significantly different according to the Tukey test ($P \leq 0.05$).

the SCA effect, the lowest value observed was for BRS Radiante × Carioca MG (Table 6). This combination also showed the lowest mean disease severity values.

Resistance to *X. citri* pv. *fuscans* expressed in BRS Radiante is conditioned by at least two dominant genes. To study the inheritance to *X. citri* pv. *fuscans* contained in BRS Radiante, F₁ plants derived from its cross with Carioca MG and confirmed as hybrids by SCAR markers were advanced to the F₂ population. Of 160 F₂ plants evaluated, 94 were resistant and 66 were susceptible to strain CFBM-UFV-0001. The Mendelian segregation hypotheses tested were ratios corresponding to 3:1, 9:7, 13:3, 15:1, and 63:1 (Table 7). The hypothesis that best explained the R:S segregation observed in the F₂ population was a 9:7 ratio with a probability of 52%.

Tests of genetic models using the maximum likelihood function shows involvement of polygenes in the resistance to strain CFBM-UFV-0001. The significance of the contrast between models 2 and 6 indicated the presence of a major gene with additive effect and dominance. In addition, the significance of the contrast between models 7 and 8 showed the presence of additive and dominance effects of major resistance genes. Significance was also observed for the contrast between models 6 and 9 (Table 8), which test for the presence of polygenes only with additive effect on common bean resistance to strain CFBM-UFV-0001.

DISCUSSION

In this study, we identified common bean cultivars resistant and susceptible to *X. citri* pv. *fuscans* and suitable to undertake studies aimed at understanding the genetics underlying host resistance. In both experiments, the first CBB symptoms were readily noticeable on the common bean cultivars at 11 DAI, indicating that climatic conditions did not strongly influence the development of disease in most cultivars. Two cultivars (BRS Radiante and IAPAR 16), out of nine evaluated, were consistently resistant whereas six (IPR Chopim, IAPAR 81, Carioca MG, BRS Campeiro, Diamante Negro, and Manteigão Fosco 11) were consistently susceptible to *X. citri* pv. *fuscans* in both experiments. In contrast, cultivar Ouro Negro showed a resistance response in the experiment conducted

in September 2016, but in the March 2017 experiment, it was classified as susceptible. Nevertheless, Ouro Negro was classified as susceptible to strain CFBM-UFV-0001 since in most experiments conducted in this study it exhibited high disease notes concordant with susceptible responses (including the initial cultivar screening and the partial diallel experiments), which could not be considered as false positives. It is likely that the environmental conditions in March 2017 were not favorable for the development of disease symptoms in Ouro Negro. This interpretation is consistent with the notion that the development of CBB is influenced by climatic conditions (Gilbertson and Maxwell 1992).

Consistent with previous reports, cultivars IAPAR 16 and BRS Radiante were also resistant to *X. phaseoli* pv. *phaseoli* using different sources of inoculum (da Silva et al. 2009; Maringoni 1998). Similarly, cultivars IAPAR 81, Carioca MG, Manteigão Fosco 11, and Ouro Negro were also found susceptible to CBB (either *X. citri* pv. *fuscans* or *X. phaseoli* pv. *phaseoli*) in previous studies (Costa et al. 2008; Kobayashi et al. 1999; Rava and Romeiro 1990). On the contrary, cultivars Diamante Negro, IPR Chopim, and BRS Campeiro were classified as resistant to *X. phaseoli* pv. *phaseoli* in previous studies (da Silva et al. 2009; Silva et al. 2000), but here they exhibited a susceptible response to strain CFBM-UFV-0001. Nonetheless, direct comparisons are difficult to establish since in those studies the authors inoculated the strains using scissors, which is a more drastic method (since it directly delivers the pathogen inside the plant tissue through wounds, surpassing stomata structural and induced resistances) than by spraying. Whether the differences in responses to CBB of these common bean cultivars are related to the bacterial species inoculated remains to be determined. This information is important for the selection of common bean cultivars to be used in breeding programs for CBB resistance and for molecular studies aimed at understanding broad-spectrum plant resistance mechanisms.

Importantly, no common bean cultivar was found completely resistant (immune) to *X. citri* pv. *fuscans*. Instead, it was noticed that the cultivars presented quantitative resistance since different levels of disease severity were observed. These results agree with those of several previous studies indicating that the resistance of common bean to CBB is of quantitative type (Miklas et al. 2003; Singh and Muñoz 1999; Tryphone et al. 2012). Cultivar BRS Radiante is of Andean origin and IAPAR 16 is of Mesoamerican origin, which supports the notion that sources of quantitative resistance to CBB can be found in both gene pools (Barili et al. 2015; de Souza 2009). Cultivars BRS Radiante and IAPAR 16 showed resistance to *X. phaseoli* pv. *phaseoli* strains from a different state in Brazil (CFBM-UFV-0003 to CFBM-UFV-0007) as well as to a strain from the United States of America (CFBM-UFV-0002), suggesting that their resistances are of broad spectrum and effective against both CBB bacterial pathogens. Considering that the resistances observed in cultivar BRS Radiante and IAPAR 16 seem to be of broad spectrum, they represent excellent candidates to undertake studies aimed at understanding quantitative plant resistance.

TABLE 2. Stomatal density of common bean cultivars resistant and susceptible to *Xanthomonas citri* pv. *fuscans* strain CFBM-UFV-0001

Cultivar	Response to <i>X. citri</i> pv. <i>fuscans</i> ^y	Stomatal density (number per mm ²) ^z
BRS Radiante	R	208 b
Carioca MG	S	187 b
IAPAR 16	R	215 b
IAPAR 81	S	110 a

^y R, resistant; and S, susceptible.

^z Means followed by the same letter are not significantly different according to the Scott-Knott test ($P \leq 0.05$).

TABLE 3. Response to inoculation with *Xanthomonas phaseoli* pv. *phaseoli* strains of common bean cultivars resistant and susceptible to *Xanthomonas citri* pv. *fuscans* strain CFBM-UFV-0001

Strain	Geographic origin	Species/pathovar	Severity (% affected area)/cultivars ^z		
			BRS Radiante (R)	Carioca MG (S)	IAPAR 16 (R)
CFBM-UFV-0001	Brasília, Brazil	<i>X. citri</i> pv. <i>fuscans</i>	2.2 a B	12.7 a A	1.2 a B
CFBM-UFV-0002	United States	<i>X. phaseoli</i> pv. <i>phaseoli</i>	2.2 a B	14.1 a A	1.0 a B
CFBM-UFV-0003	Lavras, MG, Brazil	<i>X. phaseoli</i> pv. <i>phaseoli</i>	0.4 a A	3.1 b A	0.5 a A
CFBM-UFV-0004	Lavras, MG, Brazil	<i>X. phaseoli</i> pv. <i>phaseoli</i>	1.4 a B	11.7 a A	1.9 a B
CFBM-UFV-0005	Lavras, MG, Brazil	<i>X. phaseoli</i> pv. <i>phaseoli</i>	6.6 a B	11.6 a A	1.7 a B
CFBM-UFV-0006	Lavras, MG, Brazil	<i>X. phaseoli</i> pv. <i>phaseoli</i>	2.9 a A	2.0 b A	0.3 a A
CFBM-UFV-0007	Lavras, MG, Brazil	<i>X. phaseoli</i> pv. <i>phaseoli</i>	2.1 a B	11.0 a A	2.1 a B

^z Determined using QUANT version 1.0.1 (Vale et al. 2003). R, resistant; and S, susceptible to strain CFBM-UFV-0001. Within each column, means followed by the same lowercase letter are not significantly different according to the Scott-Knott test ($P \leq 0.05$). Within each row, means followed by the same uppercase letter are not significantly different according to the Scott-Knott test ($P \leq 0.05$).

Phytopathogenic bacteria that cause foliar spots penetrate their host plants mainly through stomata (Zeng et al. 2010). 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 bacterial growth and virulence is observed (Wang et al. 2017; Wilson et al. 1999). Consistently, it was found here that the population size recovered from resistant cultivar BRS Radiante was significantly lower than those of susceptible cultivars IAPAR 81 and Carioca MG. Nonetheless, significant differences between resistant cultivar IAPAR 16 and susceptible cultivars were not detected. It is likely that IAPAR 16 lacks some components of quantitative resistance involved in restriction of pathogen growth that are present in BRS Radiante. 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 with 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). Restriction of bacterial multiplication in plant tissue could be important to reduce the level of inoculum and bacterial survival in crop debris.

Stomata play important roles in structural and induced defense mechanisms utilized by plants to fight bacterial infection (Zeng et al. 2010). For instance, it has long been proven that stomatal density (number of stomata per leaf area) may be related to plant resistance against bacterial pathogens (Nicolas et al. 2018; Ramos and Volin 1987). Inducible plant defense responses also occur in stomata. In the first tier of the plant immune response, pathogen-associated molecular patterns (PAMPs) recognition by membrane-associated pattern recognition receptors (PRRs) activates signal cascades leading to the so-called PAMP-triggered immunity (Zeng et al. 2010). For example, recognition of flagellin (a PAMP) by its receptor FLS2 (Flagellin Sensing 2; a PRR) triggers a signaling that ends in stomatal closure to prevent bacterial entry into the plant tissue (Zeng et al. 2010). In response, gram-negative bacteria deliver effectors that promote stomata reopening (Zeng et al. 2010). Perception of PAMPs is thought to be related to plant basal defense (also called partial or quantitative) (Kushalappa et al. 2016). Here, we demonstrate that the resistance to strain CFBM-UFV-0001 exhibited by cultivars BRS Radiante and IAPAR 16 is not related to stomatal density, suggesting that the resistance is not based on structural barriers at the port of entry.

However, the possibility that the stomatal pore size is smaller or that stomatal reopening after PAMP perception occurs more slowly in BRS Radiante and IAPAR 16 compared with susceptible cultivars cannot be ruled out and remains to be investigated. For some plant–bacteria interactions, stomatal opening is more important. For instance, tomato cultivars susceptible and resistant to *X. perforans* showed differences in the timing of stomatal reopening but not in stomatal density (Wang et al. 2017). Another factor that can contribute to resistance of plants to bacteria is stomatal structure, whose importance was demonstrated for citrus resistance to *X. citri* subsp. *citri* in early studies (McLean 1921).

TABLE 5. Effect of the general combining ability (GCA) and specific combining ability (SCA) of common bean parents used in the partial diallel analysis for resistance to *Xanthomonas citri* pv. *fuscans* strain CFBM-UFV-0001

Parent	GCA	SCA	Severity
Group 1			
BRS Radiante	−0.25	−0.21	1.3
IAPAR 16	0.25	0.12	2.6
Group 2			
BRS Campeiro	0.80	−1.42	5.6
Carioca MG	−0.33	1.18	6.0
Diamante Negro	0.25	0.01	6.0
IAPAR 81	−0.30	0.45	5.3
Ouro Negro	−0.41	−0.31	4.3

TABLE 6. Specific combining ability (SCA) of crosses between common bean parents used in the partial diallel analysis for resistance to *Xanthomonas citri* pv. *fuscans* strain CFBM-UFV-0001

Number	Hybrid	CBB ^z	Severity
1	BRS Radiante × BRS Campeiro	0.679	5.0
2	BRS Radiante × Carioca MG	−1.848	1.3
3	BRS Radiante × Diamante Negro	0.568	4.3
4	BRS Radiante × IAPAR 81	−0.209	3.0
5	BRS Radiante × Ouro Negro	1.234	4.3
6	IAPAR 16 × BRS Campeiro	2.179	7.0
7	IAPAR 16 × Carioca MG	−0.515	3.1
8	IAPAR 16 × Diamante Negro	−0.598	3.6
9	IAPAR 16 × IAPAR 81	−0.709	3.0
10	IAPAR 16 × Ouro Negro	−0.598	3.0

^z Effect of the specific combining ability to characterize resistance against common bacterial blight (CBB) caused by strain CFBM-UFV-0001.

TABLE 4. Discrimination of common bean cultivars by sequence characterized amplified region (SCAR) markers^w

Marker	Resistant		Susceptible						Informative patterns ^x
	BRS Radiante	IAPAR 16	BRS Campeiro	Carioca MG	Diamante Negro	IAPAR 81	IPR Chopim	Ouro Negro	
A14	A1	A2	A2	A3	A3	A2	–	A3	Y
AS8	A1	A1	–	A1	A1	–	A1	A1	Y
AU05	–	A1	–	A1	–	A1	A1	A1	Y
BC420	–	–	–	–	–	–	–	–	N ^y
E-ACA	A2	A1	A1	A1	A1	A2	A1	A1	Y
I19	A1	A1	–	–	–	A1	A1	–	Y
Q14	A2	A1	–	A1	A1	A1	–	A1	Y ^z
ROC11	A1	–	–	–	A1	–	–	–	Y
SAP6	–	–	–	A1	–	–	–	A1	Y
SB12	–	–	–	–	–	–	–	–	N
SBD5	A2	A2	–	–	A1	–	A1	A1	Y ^z
SCARF10	A1	–	A2	–	–	A1	A1	A1	Y
SG6	A1	A1	A2	A1	–	A1	A1	A1	Y
SR2	A1	A1	A1	A1	A1	A1	A1	A1	N
SU91	–	–	–	–	–	–	–	–	N
SW13	–	A1	A2	A1	A1	A1	A1	–	Y

^w A1, A2, and A3, allele type according to the size of the amplified DNA fragment; and –, lack of DNA amplification.

^x Y, the SCAR marker discriminates between cultivars; and N, lack of discrimination between cultivars.

^y Nonspecific band amplification.

^z SCAR marker presents a codominant pattern for at least one cross between resistant and susceptible cultivars.

In the present study, the GCA of groups 1 and 2, which were composed of resistant and susceptible cultivars, respectively, indicated that BRS Radiante contributed genetically to common bean resistance against strain CFBM-UFV-0001. Cultivar BRS Radiante showed the lowest GCA mean indicating that it is a promising parental for resistance to *X. citri* pv. *fuscans*. The significance of the GCA effect for group 2 indicates that these cultivars contribute differently to susceptibility against the disease. Notably, a low SCA mean for BRS Radiante × Carioca MG indicates that BRS Radiante is a promising source for resistance against *X. citri* pv. *fuscans*. Nonetheless, although cultivar BRS Radiante from group 1 showed resistance to *X. citri* pv. *fuscans*, not all hybrids involving this parent were resistant to *X. citri* pv. *fuscans*, indicating that resistance is not determined by a few fully dominant genes. These results are consistent with results of hypotheses tests for resistance inheritance of the F₂ population derived from BRS Radiante × Carioca MG obtained in this study. In addition, group 1 parents had negative and positive values of SCA. Positive SCA values indicate that recessive genes increase trait value, while negative values indicate that dominant genes increase trait value (Cruz and Vencovsky 1989). The cultivar BRS Radiante exhibited a negative SCA value, while the cultivar IAPAR 16 had a positive value, indicating that there are both dominant and recessive genes involved in resistance to CBB.

Silva et al. (2000), in a complete diallel analysis without reciprocals on common bean resistance to *X. phaseoli* pv. *phaseoli*, observed that Diamante Negro contributed genetically to enhance resistance to CBB. Conversely, in the present study, cultivar Diamante Negro was allocated in group 2 (of susceptible cultivars), showed high disease severity (note 6.0), and contributed to the susceptibility of the hybrids. Differences in inoculum source (*X. citri* pv. *fuscans* versus *X. phaseoli* pv. *phaseoli*) may be responsible for the discrepancy between the two studies. Nonetheless, additional experiments using simultaneously strains from both bacterial species should be conducted in order to reach clearer conclusions.

TABLE 7. Inheritance hypotheses for the resistance of BRS Radiante to *Xanthomonas citri* pv. *fuscans* strain CFBM-UFV-0001 in the F₂ generation derived from a cross with susceptible cultivar Carioca MG

Hypothesis	Observed R:S ^z ratio	Expected R:S ratio	χ^2	Probability (%)
3:1	94 R:66 S	120 R:40 S	22.5	0
9:7		90 R:70 S	0.4	52
13:3		130 R:30 S	53.1	0
15:1		150 R:10 S	334.5	0
63:1		157 R:3 S	1,638.5	0

^z R, resistant, disease severity scores of 1 to 3; and S, susceptible, disease severity scores >3 at 15 days after inoculation with strain CFBM-UFV-0001.

TABLE 8. Hypotheses of genetic inheritance models of common bean resistance to *Xanthomonas citri* pv. *fuscans* strain CFBM-UFV-0001 by means of the maximum likelihood function^z

Models	Degree of freedom	χ^2	Probability
2 versus 4	1	19.93*	0.000008
2 versus 6	2	19.93*	0.000047
2 versus 7	2	4.27 ^{NS}	0.118043
2 versus 8	3	26.56*	0.000007
2 versus 9	4	39.54*	0.000000
4 versus 6	1	0.00 ^{NS}	1.000000
4 versus 8	2	6.62*	0.036388
4 versus 9	3	19.61*	0.000204
6 versus 9	2	19.61*	0.000055
7 versus 8	1	22.28*	0.000002
7 versus 9	2	35.27*	0.0000002
8 versus 9	1	—	—

^z —, negative value, perhaps due to convergence problems; *, significant at 5% probability by χ^2 test; and ^{NS}, not significant.

The hypotheses tests for inheritance of the BRS Radiante resistance against *X. citri* pv. *fuscans* 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. This result demonstrates that the resistance to *X. citri* pv. *fuscans* expressed in BRS Radiante is not monogenic and agrees with the oligogenic nature of the previously reported common bean resistance to *X. phaseoli* pv. *phaseoli* (Coyne and Schuster 1983; Singh and Muñoz 1999; Tryphone et al. 2012; Zhu et al. 2016). However, the results of this work contrast with those obtained in some previous inheritance studies indicating that resistance to CBB is conditioned by a major gene with partial effects (Tryphone et al. 2012) or explained by a single dominant resistance gene (Zapata et al. 2011). Taken together, these observations support the notion that the inheritance of common bean resistance to CBB is complex (Jung et al. 1996).

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 contrast between models 2 and 6 indicated the presence of a major gene with additive effect. In addition, the significance of the contrast between models 7 and 8 showed the presence of additive and dominance effects of major resistance genes. The dominance effect was also detected in this study by χ^2 inheritance hypotheses tests. Moreover, the significance of the contrast between models 6 and 9 indicated the presence of polygenes in addition to a dominant gene of major effect.

During the execution of this work, the necessity to distinguish between parents in genetic crosses emerged. Thus, a toolkit of SCAR markers that allowed discrimination of hybrids from their parents was assembled. The 16 SCAR markers used in this study have been previously used in common bean studies (Corrêa et al. 2000; Blair et al. 2007; Haley et al. 1993; Hegay et al. 2013; Larsen et al. 2010; Mahuku et al. 2004; Méndez de Vigo et al. 2000; Miklas et al. 2000a, b; Mutlu et al. 2005; O'Boyle et al. 2007) and some of them have been linked to resistance against fungal pathogens (Corrêa et al. 2000; Hegay et al. 2013; Miklas and Delorme 2003; Miklas et al. 1993, 2000a; Mutlu et al. 2005; Yu et al. 2000). With the exception of SCARF10, the SCAR markers here described had not previously been used in the cultivars 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 cross combination.

In conclusion, in this work BRS Radiante and IAPAR 16 were identified as cultivars exhibiting resistance against *X. citri* pv. *fuscans*, which in the case of BRS Radiante, was associated with restriction of bacterial multiplication in host tissue and not necessarily related to stomatal density. Partial diallel analysis indicated that BRS Radiante is a promising source of resistance and the BRS Radiante × Carioca MG combination has high potential for common bean breeding for resistance against *X. citri* pv. *fuscans*. Important insights into the genetic basis of resistance to *X. citri* pv. *fuscans* expressed in BRS Radiante have been obtained through inheritance hypotheses tests, which indicated that it is conditioned by two complementary dominant genes. Maximum likelihood analysis indicated that resistance of common bean to *X. citri* pv. *fuscans* 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 *X. citri* pv. *fuscans* 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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LITERATURE CITED

- Akhavan, A., Bahar, M., Askarian, H., Lak, M. R., Nazemi, A., and Zamani, Z. 2013. Bean common bacterial blight: Pathogen epiphytic life and effect of irrigation practices. *Springerplus* 2:41.
- Alladassi, B. M. E., Nkalubo, S. T., Mukankusi, C., Kayaga, H. N., Gibson, P., Edema, R., Urrea, C. A., Kelly, J. D., and Rubaihayo, P. R. 2018. Identification of common bean genotypes with dual leaf and pod resistance to common bacterial blight disease in Uganda. *Afr. Crop Sci. J.* 26:63-77.
- Andrade, F. A., Gonçalves, L. S. A., Fukui, A., Miglioranza, E., Takahashi, L. S. A., Balbi-Peña, M. I., and Rodrigues, R. 2017. Snap beans for organic farming and evaluation of resistance to the common bacterial blight. *Hortic. Bras.* 35:385-389.
- Aritua, V., Harrison, J., Sapp, M., Buruchara, R., Smith, J., and Studholme, D. J. 2015. Genome sequencing reveals a new lineage associated with lablab bean and genetic exchange between *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas fuscans* subsp. *fuscans*. *Front. Microbiol.* 6: 1080.
- Arnhold, E. 2013. Package in the R environment for analysis of variance and complementary analyses. *Braz. J. Vet. Res. Anim. Sci.* 50:488-492.
- Badel, J. L., Nomura, K., Bandyopadhyay, S., Shimizu, R., Collmer, A., and He, S. Y. 2003. *Pseudomonas syringae* pv. *tomato* DC3000 HopPtoM (CEL ORF3) is important for lesion formation but not growth in tomato and is secreted and translocated by the Hrp type III secretion system in a chaperone-dependent manner. *Mol. Microbiol.* 49:1239-1251.
- Barili, L. D., Vale, N. M., Moura, L. M., Paula, R. G., Silva, F. F., and Carneiro, J. E. S. 2015. Choice of parents for earliness and yield of carioca-type bean. *Pesqui. Agropecu. Bras.* 50:141-148.
- Blair, M. W., Rodriguez, L. M., Pedraza, F., Morales, F., and Beebe, S. 2007. Genetic mapping of the bean golden yellow mosaic geminivirus resistance gene *bgm-1* and linkage with potyvirus resistance in common bean (*Phaseolus vulgaris* L.). *Theor. Appl. Genet.* 114:261-271.
- Bett, K. E., and Banniza, S. 2014. Population study of *Xanthomonas* spp. from bean growing regions of Canada and response of bean cultivars to pathogen inoculation. *Can. J. Plant Pathol.* 36:341-353.
- Broughton, W. J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., and Vanderleyden, J. 2003. Beans (*Phaseolus* spp.) model food legumes. *Plant Soil* 252:55-128.
- Chan, J. W. Y. F., and Goodwin, P. H. 1999. Differentiation of *Xanthomonas campestris* pv. *phaseoli* from *Xanthomonas campestris* pv. *phaseoli* var. *fuscans* by PFGE and RFLP. *Eur. J. Plant Pathol.* 105:867-878.
- Constantin, E. C., Cleenwerck, I., Maes, M., Baeyen, S., van Malderghem, C., de Vos, P., and Cottyn, B. 2016. Genetic characterization of strains named as *Xanthomonas axonopodis* pv. *dieffenbachiae* leads to a taxonomic revision of the *X. axonopodis* species complex. *Plant Pathol.* 65:792-806.
- Corrêa, R. X., Costa, M. R., Good-God, P. I., Ragagnin, V. A., Faleiro, F. G., Moreira, M. A., and de Barros, E. G. 2000. Sequence characterized amplified regions linked to rust resistance genes in the common bean. *Crop Sci.* 40:804-807.
- Costa, J. G. C., Rava, C. A., Puríssimo, J. D., Del Peloso, M. J., Melo, L. C., and Faria, L. C. 2008. Reaction of common bean genotypes to common bacterial blight and bacterium wilt bacterial. *Rev. Ceres* 55:393-395.
- Coyne, D. P., and Schuster, M. L. 1983. Genetics of and breeding for resistance to bacterial pathogens in vegetable crops. *HortScience* 18:30-36.
- Cruz, C. D. 2013. GENES: A software package for analysis in experimental statistics and quantitative genetics. *Acta Sci. Agron.* 35:271-276.
- Cruz, C. D., Regazzi, A. J., and Carneiro, P. C. S. 2012. Componentes de variância genotípica. Pages 13-51 in: *Modelos Biométricos Aplicados ao Melhoramento Genético*. C. D. Cruz, A. J. Regazzi, and P. C. S. Carneiro, eds. UFV, Viçosa, MG.
- Cruz, C. D., and Vencovsky, R. 1989. Comparação de alguns métodos de análise dialélica. *Rev. Bras. Genet.* 12:425-438.
- da Silva, A., dos Santos, I., Balbinot, A. L., Matei, G., and de Oliveira, P. H. 2009. Reaction of bean genotypes to the common bacterial blight, evaluated by two inoculation methods. *Cienc. Agrotec.* 33:2019-2024.
- De Paiva, B. A. R., Wendland, A., Borba, T. C., and Araújo, L. G. 2018. Molecular characterization and differential interaction of *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas fuscans* subsp. *fuscans* with common beans (*Phaseolus vulgaris*). *Genet. Mol. Res.* 17:1-13.
- de Souza, T. L. P. O. 2009. SNP discovery, reaction of the common bean to soybean rust, and pyramiding of resistance genes to *Uromyces appendiculatus*. D.Sc. Thesis. Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.
- Doyle, J. J., and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19:11-15.
- FAOSTAT. 2019. Crops. Food and Agriculture Organization of the United Nations, Rome, Italy.
- Ferreira, E. B., Cavalcanti, P. P., and Nogueira, D. A. 2018. ExpDes: Experimental designs. *Appl. Math.* 5:2952-2958.
- Fininsa, C. 2003. Relationship between common bacterial blight severity and bean yield loss in pure stand and bean-maize intercropping systems. *Int. J. Pest Manage.* 49:177-185.
- Geraldi, I., and de Miranda-Filho, J. 1988. Adapted models for the analysis of combining ability of varieties in partial diallel crosses. *Rev. Bras. Genet.* 11: 419-430.
- Gilbertson, R. L., and Maxwell, D. P. 1992. Common blight of bean. Pages 18-39 in: *Diseases of International Importance*. H. S. Chaube, U. S. Singh, and A. N. Mukhopadhyay, eds. Prentice Hall, Englewood Cliffs, NJ.
- Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.* 9:463-493.
- Haley, S. D., Miklas, N., Stavely, J. R., and Kelly, D. 1993. Identification of RAPD markers linked to a major rust resistance gene block in common bean. *Theor. Appl. Genet.* 86:505-512.
- He, Y., and Munkvold, G. P. 2013. Comparison of seed transmission and survival of *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas fuscans* subsp. *fuscans* in common bean seeds. *Plant Health Prog.* 14:41.
- Hegay, S., Ortiz, R., Garkava-Gustavsson, L., Hövmalm, H. P., and Geleta, M. 2013. Marker-aided breeding for resistance to *Bean common mosaic virus* in Kyrgyz bean cultivars. *Euphytica* 193:67-78.
- Jelilovsch, E. G., Faria, J. C., and Allaman, I. B. 2014. ScottKnott: A Package for performing the Scott-Knott clustering algorithm in R. *Trends Appl. Comp. Math.* 15:3-17.
- Jung, G., Coyne, D. P., Skroch, P. W., James, N., Arnaud-Santana, E., Bokosi, J., Ariyaratne, H. M., Steadman, J. R., Beaver, J. S., and Kaeppler, S. M. 1996. Molecular markers associated with plant architecture and resistance to common blight, web blight, and rust in common beans. *J. Am. Soc. Hortic. Sci.* 121:794-803.
- Kado, C. I., and Heskett, M. G. 1970. Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas* and *Xanthomonas*. *Phytopathology* 60:969-976.
- Kobayashi, L., de Souza, R. M., and Santos, J. B. 1999. Evaluation of common bean cultivars and lines as to foliar and pod reaction to *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans*. *Cienc. Agrotec.* 23:40-47.
- Kushalappa, A. C., Yogendra, K. N., and Karre, S. 2016. Plant innate immune response: Qualitative and quantitative resistance. *Crit. Rev. Plant Sci.* 35: 38-55.
- Larsen, R. C., Kurowski, C. J., and Miklas, P. N. 2010. Two independent quantitative trait loci are responsible for novel resistance to *Beet curly top virus* in common bean landrace G122. *Phytopathology* 100:972-978.
- López, R., Asensio, C., and Gilbertson, R. L. 2006. Phenotypic and genetic diversity in strains of common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) in a secondary center of diversity of the common bean host suggests multiple introduction events. *Phytopathology* 96:1204-1213.
- Mahuku, G., Montoya, C., Henríquez, M. A., Jara, C., Teran, H., and Beebe, S. 2004. Inheritance and characterization of angular leaf spot resistance gene present in common bean accession G 10474 and identification of an AFLP marker linked to the resistance gene. *Crop Sci.* 44:1817-1824.
- Maringoni, A. C. 1998. Virulence of *Xanthomonas campestris* pv. *phaseoli* (Smith) dye strains on bean plants. *Pesqui. Agropecu. Bras.* 33:861-867.
- Mather, K., and Jinks, J. L. 1977. Additive and dominance effects. Pages 32-59 in: *Introduction to Biometrical Genetics*. K. Mather and J. L. Jinks, eds. Springer, Boston, MA.
- McLean, F. T. 1921. A study of the structure of the stomata of two species of citrus in relation to citrus canker. *J. Torrey Bot. Soc.* 48:101-106.
- Méndez de Vigo, B., Rodríguez, C., Pañeda, A., and Giraldez, R. 2000. Development of a SCAR marker linked to *Co-9* in common bean. *Annu. Rep. Bean Improv. Coop.* 45:1998-1999.
- Mendiburu, F. 2017. Package "agricolae" statistical procedures for agricultural research. R Package Version 1.3-1. <https://cran.r-project.org/web/packages/agricolae/index.html>
- Miklas, P. N., Coyne, D. P., Grafton, K. F., Mutlu, N., Reiser, J., Lindgren, D. T., and Singh, S. P. 2003. A major QTL for common bacterial blight resistance derives from the common bean great northern landrace cultivar Montana No. 5. *Euphytica* 131:137-146.
- Miklas, P. N., and Delorme, R. 2003. Identification of QTL conditioning resistance to white mold in snap bean. *J. Am. Soc. Hortic. Sci.* 128: 564-570.
- Miklas, P. N., Delorme, R., Stone, V., Daly, M. J., Stavely, J. R., Steadman, J. R., Basset, M. J., and Beaver, J. S. 2000a. Bacterial, fungal, and viral disease resistance loci mapped in a recombinant inbred common bean population ('Dorado'/XAN 176). *J. Am. Soc. Hortic. Sci.* 125:476-481.

- Miklas, P. N., Smith, J. R., Riley, R., and Grafton, K. F. 2000b. Marker-assisted breeding for pyramided resistance to common bacterial blight in common bean. *Annu. Rep.* 43:39-40.
- Miklas, P. N., Staveland, J. R., and Kelly, J. D. 1993. Identification and potential use of a molecular marker for rust resistance in common bean. *Econ. Bot.* 85:745-749.
- Mood, A. M., Graybill, F. A., and Boes, D. C. 1974. *Introduction to the Theory of Statistics*. McGraw-Hill, Tokyo.
- Mutlu, N., Miklas, P., Reiser, J., and Coyne, D. 2005. Backcross breeding for improved resistance to common bacterial blight in pinto bean (*Phaseolus vulgaris* L.). *Plant Breed.* 124:282-287.
- Mutlu, N., Vidaver, A. K., Coyne, D. P., Steadman, J. R., Lambrecht, P. A., and Reiser, J. 2008. Differential pathogenicity of *Xanthomonas campestris* pv. *phaseoli* and *X. fuscans* pv. *fuscans* strains for bean genotypes with common blight resistance. *Plant Dis.* 92:546-554.
- Nicolas, O., Charles, M. T., Jenni, S., Toussaint, V., and Beaulieu, C. 2018. Relationships between *Xanthomonas campestris* pv. *vitians* population sizes, stomatal density and lettuce resistance to bacterial leaf spot. *Can. J. Plant Pathol.* 40:399-407.
- O'Boyle, P. D., Kelly, J. D., and Kirk, W. W. 2007. Use of marker-assisted selection to breed for resistance to common bacterial blight in common bean. *J. Am. Soc. Hort. Sci.* 132:381-386.
- Opio, A. F., Allen, D. J., and Teri, J. M. 1996. Pathogenic variation in *Xanthomonas campestris* pv. *phaseoli*, the causal agent of common bacterial blight in *Phaseolus* beans. *Plant Pathol.* 45:1126-1133.
- Peternelli, L. A., and Borém, A. 1999. Hibridação artificial em feijão. Pages 269-294 in: *Hibridação Artificial de Plantas*. A. Borém, ed. UFV, Viçosa, MG.
- R Development Core Team. 2018. R: A Language and Environment for Statistical Computing. Vienna, Austria. <http://www.R-project.org>
- Ramalho, M. A. P., dos Santos, J. B., and Zimmermann, M. J. de O. 1993. *Genética Quantitativa em Plantas Autógamas: Aplicações ao Melhoramento do Feijoeiro*. Eds. UFG, Goiânia GO, Brazil.
- Ramos, L. J., and Volin, R. B. 1987. Role of stomatal opening and frequency on infection of *Lycopersicon* spp. by *Xanthomonas campestris* pv. *vesicatoria*. *Phytopathology* 77:1311-1317.
- Rava, C. A., and Romeiro, R. da S. 1990. Variability of isolates of *Xanthomonas campestris* pv. *phaseoli* on pathogenicity in *Phaseolus vulgaris* cultivars. *Summa Phytopathol.* 16:225-232.
- Rigault, M., Buellet, A., Masclaux-Daubresse, C., Fagard, M., Chardon, F., and Dellagi, A. 2017. Quantitative methods to assess differential susceptibility of *Arabidopsis thaliana* natural accessions to *Dickeya dadantii*. *Front. Plant Sci.* 8:394.
- Sarkar, D. 2008. *Lattice: Multivariate Data Visualization with R*. Springer, New York. <http://lmdvr.r-forge.r-project.org>
- Schaad, N. W., Postnikova, E., Lacey, G., Lacy, G. H., Agarkova, I., Stromberg, P. E., Stromberg, V. K., and Vidaver, A. K. 2005. Reclassification of *Xanthomonas campestris* pv. *citri* (ex Hasse 1915) Dye 1978 forms A, B/C/D, and E as *X. smithii* subsp. *citri* (ex Hasse) sp. nov. nom. rev. comb. nov., *X. fuscans* subsp. *aurantifolii* (ex Gabriel 1989) sp. nov. nom. rev. comb. nov., and *X. alfalfae* subsp. *citrumelo* (ex Riker and Jones) Gabriel et al. 1989 sp. nov. nom. rev. comb. nov.; *X. campestris* pv. *malvacearum* (ex Smith 1901) Dye 1978 as *X. smithii* subsp. *smithii* nov. comb. nov. nom. nov.; *X. campestris* pv. *alfalfae* (ex Riker and Jones, 1935) dye 1978 as *X. alfalfae* subsp. *alfalfae* (ex Riker et al. 1935) sp. nov. nom. rev.; and "var. *fuscans*" of *X. campestris* pv. *phaseoli* (ex Smith, 1987) Dye 1978 as *X. fuscans* subsp. *fuscans* sp. nov. Syst. Appl. Microbiol. 89:494-518.
- Schaad, N. W., Postnikova, E., Lacey, G., Sechler, A., Agarkova, I., Stromberg, P. E., Stromberg, V. K., and Vidaver, A. K. 2007. List of new names and new combinations previously effectively, but not validly, published. *Int. J. Syst. Evol. Microbiol.* 57:893-897.
- Schoonhoven, V., and Pastor-Corrales, M. A. 1987. Measurement of damage caused by diseases. Pages 40-41 in: *Sistema Estándar para la Evaluación de Germoplasma de Fríjol*. V. Schoonhoven and M. A. Pastor-Corrales, eds. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.
- Silva, S. A. G., de Moraes, O. P., Rava, C. A., and Costa, J. G. C. 2000. Generalized method for analysis of unbalanced diallels. *Pesqui. Agropecu. Bras.* 35:1999-2005.
- Silva, W. 2003. *Estimadores de Máxima Verossimilhança em Mistura de Densidades Normais: Uma Aplicação em Genética*. MSc. Thesis. Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil.
- Singh, S. P., and Miklas, P. N. 2015. Breeding common bean for resistance to common blight: A review. *Crop Sci.* 55:971-984.
- Singh, S. P., and Muñoz, C. G. 1999. Resistance to common bacterial blight among *Phaseolus* species and common bean improvement. *Crop Sci.* 39:80-89.
- Smith, E. F. 1897. Description of *Bacillus phaseoli* n. sp. *Bot. Gaz.* 24:192.
- Tryphone, G. M., Chilagane, L. A., Protas, D., Mbogo, P., and Nchimbi-Msolla, S. 2012. Introgression of common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) resistance to common bean (*Phaseolus vulgaris* L.) adapted to Tanzania facilitated by marker assisted selection. *Int. J. Agric. Sci.* 2:285-290.
- Vale, F., Fernandes Filho, E., and Liberato, J. 2003. Quant-A Software for Plant Disease Severity Assessment. Page 105 in: *International Congress of Plant Pathology Christchurch Proceedings*. Plant Pathology Society, Christchurch, New Zealand.
- Wang, Y. Q., Zhang, X. F., Li, N., and Liu, X. 2017. Comparison of cellular responses to *Xanthomonas perforans* infection between resistant and susceptible tomato accessions. *J. Plant Physiol.* 209:105-114.
- Wilson, M., Hirano, S. S., and Lindow, S. E. 1999. Location and survival of leaf-associated bacteria in relation to pathogenicity and potential for growth within the leaf. *Appl. Environ. Microbiol.* 65:1435-1443.
- Young, J., Saddler, G., Takikawa, Y., De Boer, S., Vauterin, L., Gardan, L., Gvozdyak, R. I., and Stead, D. E. 1996. Names of plant pathogenic bacteria 1864-1995. *Rev. Plant Pathol.* 75:721-763.
- Yu, K., Park, S. J., and Poysa, V. 2000. Marker-assisted selection of common beans for resistance to common bacterial blight: Efficacy and economics. *Plant Breed.* 119:411-415.
- Zapata, M., Beaver, J. S., and Porch, T. G. 2011. Dominant gene for common bean resistance to common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli*. *Euphytica* 179:373-382.
- Zeng, W., Melotto, M., and He, S. Y. 2010. Plant stomata: A checkpoint of host immunity and pathogen virulence. *Curr. Opin. Biotechnol.* 21:599-603.
- Zhu, J., Wu, J., Wang, L., Blair, M. W., Zhu, Z., and Wang, S. 2016. QTL and candidate genes associated with common bacterial blight resistance in the common bean cultivar Longyundou 5 from China. *Crop J.* 4:344-352.