LARISSA CARVALHO COSTA

MAPEAMENTO DE GENES DE RESISTÊNCIA A DIFERENTES ISOLADOS DE *Colletotrichum lindemuthianum* E SELEÇÃO RECORRENTE VISANDO À RESISTÊNCIA A ANTRACNOSE DO FEIJOEIRO

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento de Plantas, área de concentração em Genética Molecular e de Fitopatógenos, para a obtenção do título de Doutora.

Profa. Dra. Elaine Aparecida de Souza
Orentadora

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MAPPING OF RESISTENCE GENES TO DIFFERENT STRAINS OF *Colletotrichum lindemuthianum* AND RECURRENT SELECTION AIMING RESISTANCE TO ANTHRACNOSE IN COMMON BEAN

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento de Plantas, área de concentração em Genética Molecular e de Fitopatógenos, para a obtenção do título de Doutora.

APROVADA em 26 de março de 2019.

Dr. Alisson Fernando Chiorato	IAC
Dra. Ângela de Fatima Barbosa Abreu	Embrapa Arroz e Feijão
Dr. Magno Antônio Patto Ramalho	UFLA
Dr. Thiago Livio Pessoa Oliveira de Souza	Embrapa Arroz e Feijão

Profa. Dra. Elaine Aparecida de Souza
Orientadora

LAVRAS -MG
2019

A Deus, pela saúde, sabedoria e por todas as bençãos em minha vida

OFEREÇO

À minha mãe, Maria da Penha, por ser o meu maior exemplo de vida, de amor e de força para nunca desistir. Nosso amor vai além da vida.
Ao meu pai, Valdir e aos meus irmãos, Ivan e Karina pelo apoio, amor e carinho em todas as etapas da minha vida.

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“Lembre-se de olhar para as estrelas
e não para baixo, para os seus pés.
Tente achar sentido no que você vê
e pergunte o que faz o universo existir.
Seja curioso.”

Stephen Hawking

RESUMO

A grande variabilidade do fungo *Colletotrichum lindemuthianum*, agente causal da antracnose do feijoeiro, tem dificultado a obtencão de cultivares com resistência durável a esse patógeno. Além da variabilidade de raças fisiológicas que tem sido identificada, variabilidade dentro de raças também tem sido relatada. Nesse contexto, os objetivos desse estudo foram: i) Identificar regiões genômicas associadas à resistência a diferentes isolados da raça 65 de *C. lindemuthianum*, uma das mais importantes raças deste fungo no Brasil. ii) Desenvolver e avaliar a eficiência de um programa de seleção recorrente como alternativa para a obtenção de resistência durável à antracnose do feijoeiro. Para atingir o primeiro objetivo, foram utilizadas duas abordagens de mapeamento: O mapeamento tradicional por ligação, utilizando uma população F_2, derivada do cruzamento entre as cultivares de feijoeiro, BRS Estilo x Ouro Vermelho, as quais são contrastantes quanto a reação a dois isolados diferentes da raça 65 de *C. lindemuthianum* e, também, o mapeamento associativo, utilizando um painel diverso de 189 acessos do banco de germoplasma de feijoeiro da Universidade Federal de Lavras, com variação fenotípica para cinco isolados diferentes dessa mesma raça. As 189 linhagens do banco de germoplasma e 400 indivíduos da população F_2 do cruzamento entre BRS Estilo x Ouro Vermelho, bem como os parentais, foram genotipados, utilizando o *BARCBEAN6K_3 Illumina SNP chip* com 5398 SNPs. Para o desenvolvimento do programa de seleção recorrente, uma mistura de sementes de 45 populações F_2 (população S_0), derivadas do cruzamento dialélico entre dez linhagens de feijoeiro, foi utilizada para a formação da população base. Essas linhagens possuem variabilidade quanto a reação a diferentes isolados de *C. lindemuthianum.* Cinco ciclos de avaliação, seleção e recombinação entre as plantas S_0 mais resistentes a isolados das raças 65, 73, 81 e 89 foram realizados e cerca de 40 progênies $S_{0:2}$ foram obtidas em cada ciclo. O resultado do mapeamento, utilizando a população biparental derivada do cruzamento entre BRS Estilo x Ouro Vermelho e dois isolados diferentes da raça 65 de *C. Lindemuthianum*, revelou a presença de marcadores completamente ligados a regiões genômicas associadas a resistência cada um dos dois isolados utilizados, distantes 1.8cM um do outro, no cromossomo Pv04. Pelo mapeamento associativo foram identificados *Quantitative Trait Loci* (QTLs) de grande efeito associados à resistência a esses dois isolados, nessa mesma posição e, também, em outras posições do Pv04. Além disso, foram identificados QTLs associados a resistência a outros três isolados da raça 65 nos cromossomos Pv04, Pv05, Pv10 e Pv11. Este é o primeiro trabalho de mapeamento de genes de resistência em *Phaseolus vulgaris*, que utiliza isolados diferentes pertencentes a mesma raça de *C. lindemuthianum*. Os resultados indicam que as regiões genômicas que controlam a resistência à raça 65 de *C. lindemuthianum* variam de acordo com o isolado utilizado. Algumas cultivares com alelos de resistência à raça 65 de *C. lindemuthianum*, já identificados, também foram avaliadas quanto à reação aos cinco isolados da raça 65 utilizados no presente trabalho, e, estas, apresentaram reação diferente a cada um desses isolados. Em relação à seleção recorrente, por este método foi possível realizar três ciclos seletivos por ano. Após cinco ciclos seletivos, foi observado progresso genético de 7,4% e 10,7% para dois isolados diferentes da raça 65 e de 9,0%, 9,0% e 8,0% para os isolados das raças 73, 81 e 89, respectivamente. Ademais, houve um aumento no número de progênies resistentes a um maior número de isolados de *C. lindemuthianum* no decorrer dos ciclos seletivos, evidenciando a eficiência do método. Se as progênies oriundas desse programa forem conduzidas pelo método bulk dentro de progênies S_0, sabe-se que ao final do processo as progênies serão compostas por misturas de linhas puras. Se o melhorista optar por utilizar essa mistura de linhas puras como cultivar, esta apresentará alelos de resistência piramidados entre e dentro das linhas puras que a compõe, o que pode propiciar uma maior estabilidade e durabilidade da resistência frente a grande variabilidade entre e dentro de raças de *C. lindemuthianum*.

Palavras-chave: *Phaseolus vulgaris*. Resistência a doenças. Melhoramento genético.

ABSTRACT

The large variability of *Colletotrichum lindemuthianum* fungus, the causal agent of anthracnose in the common bean, has made it difficult to obtain cultivars with durable resistance to this pathogen. Variability within races has been reported in addition to the variability of identified physiological races. In this context, the objectives of this study were to i) identify genomic regions associated with the resistance to different *C. lindemuthianum* strains, one of the most significant races of this fungus in Brazil; and ii) develop and evaluate the efficiency of a recurrent selection program as an alternative to obtain durable resistance to anthracnose in common bean. To reach the first objective, we employed two mapping approaches: the traditional mapping by linkage analysis, using an F_2 population derived from the cross between BRS Estilo x Ouro Vermelho common bean cultivars, which are contrasting regarding the reaction to two different *C. lindemuthianum* strains of race 65; and the association mapping, using a panel of 189 accessions of the common bean germplasm bank of the Universidade Federal de Lavras, with phenotypic variation for five different strains of the same race. We genotyped the 189 common bean lines of the germplasm bank and 400 individuals from the F_2 population of the cross between BRS Estilo x Ouro Vermelho, as well as the parental, using the BARCBEAN6K_3 Illumina SNP chip with 5398 SNPs. For the development of the recurrent selection program, we used a mixture of seeds from 45 F_2 populations (S_0 population) derived from the diallelic cross among ten common bean lines to form the base population (Cycle 0).. These common bean lines present variability regarding different *C. lindemuthianum* strains. From cycle 0, we performed five evaluation, selection and recombination cycles among the S_0 plants most resistant to strains of races 65, 73, 81 and 89, obtaining approximately 40 $S_{0:2}$ progenies in each cycle. The result of the mapping using the biparental population derived from the cross between BRS Estilo x Ouro Vermelho and two different strains of *C. lindemuthianum*, race 65, revealed the presence of markers completely linked to genomic regions associated with resistance to each of the two strains used, on the Pv04 chromosome, distant 1.8cM from each other. Through the association mapping, we identified significant Quantitative Trait Loci (QTLs) associated with the resistance to both strains this same position as well as in other positions of the Pv04. Furthermore, we identified QTLs associated with resistance to three other strains of race 65 on chromosomes Pv04, Pv05, Pv10, and Pv11. This is the first study about mapping of resistance genes in *Phaseolus vulgaris* using different strains belonging to the same race of *C. lindemuthianum*. The results indicate that the genomic regions that control resistance to *C. lindemuthianum*, race 65, vary according to the strain used. Some common bean lines with, already identified resistance alleles to *C. lindemuthianum*, race 65, were also evaluated regarding the reaction to the five strains of race 65 used in the present study, presenting a different reaction to each of these strains. Concerning the recurrent selection, we were able to perform three selective cycles per year using this method. After five selective cycles, we observed genetic progress of 7.4% and 10.7% for two different strains of race 65 and 9.0%, 9.0%, and 8.0% for the strains of races 73, 81, and 89, respectively. Moreover, there was an increase in the number of progenies resistant to a higher number of *C. lindemuthianum* strains during the selective cycles, demonstrating the efficiency of the method. If the progenies derived from this program are conducted by the bulk method within S_0 progenies, the progenies will be composed of mixtures of pure common bean lines at the end of the process. If the breeder chooses to use this mixture of pure lines as a cultivar, it will present pyramided resistance alleles among and within the pure lines that compose the mixture, which can provide higher stability and durability of the resistance against the large variability among and within *C. lindemuthianum* races.

Keywords: *Phaseolus vulgaris*. Resistance to diseases. Genetic breeding.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

Um dos principais problemas que acomete a cultura do feijoeiro e causa grande instabilidade de produção é a ocorrência de doenças. A antracnose, causada pelo fungo *Colletotrichum lindemuthianum,* destaca-se com uma das doenças mais destrutivas da cultura, podendo reduzir drasticamente o rendimento dos grãos, quando são utilizadas sementes contaminadas e ou cultivares susceptíveis, sob condições climáticas favoráveis à epidemia. (PADDER et al., 2017; SINGH; SCHWARTZ, 2010). A utilização de cultivares resistentes tem sido a forma mais econômica e eficaz de controle da doença (FERREIRA; CAMPA; KELLY, 2013; MIKLAS et al., 2006). No entanto, a variabilidade genética e patogênica desse fungo, evidenciada pela grande quantidade de raças fisiológicas já identificadas, dificulta a obtenção de cultivares com resistência durável. Atualmente, quase 200 raças de *C. lindemuthianum* já foram identificadas em todo o mundo, utilizando o conjunto internacional padrão de cultivares diferenciadoras de antracnose do feijoeiro (PADDER et al., 2017). No Brasil, há um predomínio das raças 65, 73, 81 e 89 (CARBONELL et al., 2012; PINTO et al., 2012; RIBEIRO et al., 2016; SILVA; SOUZA; ISHIKAWA, 2007).

A interação altamente específica entre *P. Vulgaris* e *C. lindemuthianum* tem feito desse patossistema um modelo de referência em estudos de resistência-raça-específica em plantas. Mais de vinte alelos de resistência, provenientes de diferentes genes, já foram identificados em cultivares de feijoeiro, conferindo resistência qualitativa a diferentes raças de *C. lindemuthianum* (COELHO et al., 2013; FERREIRA; CAMPA; KELLY, 2013; GONZÁLEZ et al., 2015). Esses genes têm sido mapeados em vários grupos de ligação do feijoeiro e muitos deles são, na verdade, complexos blocos gênicos (*clusters*), dentro dos quais, genes individuais conferem resistência a uma raça específica (FERREIRA; CAMPA; KELLY, 2013; MEZIADI et al., 2016; MURUBE; CAMPA; FERREIRA, 2019). O advento das tecnologias de sequenciamento de nova geração e as recentes publicações do genoma do feijoeiro (SCHMUTZ et al., 2014; VLASOVA et al., 2016), têm oferecido a oportunidade para a realização de mapeamento fino de vários genes relacionados à resistência a *C. lindemuthianum*, o que permite o desenvolvimento de marcadores moleculares intimamente ligados ao gene de interesse e viabiliza a seleção assistida por marcadores moleculares (BURT et al., 2015; MURUBE; CAMPA; FERREIRA, 2019; RICHARD et al., 2014; VALENTINI et al., 2017).

Além do mapeamento tradicional, envolvendo populações segregantes oriundas de cruzamentos biparentais, estudos de associação genômica ampla (*Genome Wide Association Study* - GWAS), também têm sido realizados na cultura do feijoeiro para a identificação de regiões genômicas associadas a várias características de interesse, incluindo resistência a doenças (KAMFWA; CICHY; KELLY, 2015; MOGHADDAM et al., 2016; PERSEGUINI et al., 2016; TOCK et al., 2017; ZUIDERVEEN et al., 2016). Nesse caso, não há necessidade de sintetizar uma população para realizar o mapeamento, esta pode ser, por exemplo, um conjunto diverso de indivíduos, que represente algum germoplasma. Comparado ao mapeamento por ligação, utilizando populações biparentais, GWAS pode oferecer maior resolução de mapeamento para identificação de *Quantitative Trait Loci* (QTLs) (SCHMUTZ et al., 2014; ZHU et al., 2008). No entanto, enquanto que, em populações segregantes oriundas de cruzamentos biparentais, todo o desequilíbrio de ligação entre o marcador e o QTL acontece, exclusivamente, em razão da ligação física, em GWAS, outros fatores, tais como: seleção, deriva genética, estrutura populacional e parentesco, podem afetar o desequilíbrio de ligação e levar a associações espúrias entre o marcador e o QTL.

Além da variabilidade patogênica entre raças de *C. lindemuthianum*, variabilidade dentro de raça também tem sido observada. Sob o ponto de vista do melhoramento do feijoeiro, essa questão é muito importante, pois as cultivares podem ser resistentes a alguns isolados e suscetíveis a outros, de mesma raça (DAVIDE; SOUZA, 2009; ISHIKAWA; RAMALHO; SOUZA, 2011; RODRÍGUEZ-SUÁREZ et al., 2005). O controle genético da resistência de cultivares de feijoeiro a seis diferentes isolados pertencentes à raça 65 de *C. lindemuthianum* foi recentemente estudado por Costa et al. (2017). O diferente padrão de reação das cultivares de feijoeiro quando inoculadas com os diferentes isolados da raça 65 e as segregaçõesde 15R:1S, observadas na geração F_2 de vários cruzamentos, indicaram que genes duplicados poderiam estar conferindo resistência específica a cada um dos isolados utilizados. Esse resultado evidencia que a especificidade da interação patógeno-hospedeiro ocorre também em nível de isolado, dentro de raça. Portanto, uma grande quantidade de alelos, provenientes de diferentes genes, estão envolvidos na resistência à antracnose, dificultando a obtenção de cultivares com resistência durável. Ademais, genes de resistência a raças específicas, já identificados em algumas linhagens de feijoeiro (GONÇALVES-VIDIGAL et al., 2012; GONÇALVES-VIDIGAL; LACANALLO; VIDIGAL FILHO, 2008; LACANALLO; GONÇALVES-VIDIGAL, 2015; SOUSA et al., 2015), provavelmente não conferem resistência a todos os isolados pertencentes a essas raças. Consequentemente, os

marcadores moleculares ligados a esses genes podem não ser úteis em todas as regiões onde o feijão é cultivado.

Uma estratégia de melhoramento que tem sido utilizada em muitas culturas, visando a aumentar a frequência de alelos favoráveis de várias características, de herança quantitativa, de forma gradativa, por meio de sucessivos ciclos de avaliação, seleção e recombinação, é a seleção recorrente (NELSON et al., 2018; RAMALHO, 2012). Na cultura do feijoeiro, esse método tem sido utilizado com sucesso visando à resistência à mancha angular (*Pseudocercospora griseola*) e ao mofo branco (*White mold*). Linhagens provenientes dos vários ciclos seletivos, com boas características agronômicas e bons níveis de resistência à essas doenças, têm sido obtidas (LEITE et al., 2016; REZENDE et al., 2014). No entanto, não há informações, na literatura, sobre a eficiência da utilização dessa estratégia, para a obtenção de cultivares resistentes à antracnose do feijoeiro. Apesar do caráter reação do feijoeiro a *C. lindemuthinaum* tipicamente apresentar herança qualitativa, muitos genes estão envolvidos na resistência às diferentes raças do patógeno e, além disso, resistência parcial, ou quantitativa, também tem sido relatada em algumas ocasiões (GONZÁLEZ et al., 2015; OBLESSUC et al., 2014).

Diante do exposto, o presente trabalho foi realizado com os seguintes objetivos: i) Identificar regiões genômicas associadas a resistência a diferentes isolados da raça 65 de *C. lindemuthianum*, por meio do mapeamento tradicional por ligação e GWAS. ii) Desenvolver e avaliar a eficiência de um programa de seleção recorrente visando à resistência a diferentes isolados, pertencentes a várias raças de *C. lindemuthianum*, como alternativa para a obtenção de cultivares com resistência durável a antracnose do feijoeiro.

2 REFERENCIAL TEÓRICO

2.1 A Cultura do feijoeiro no Brasil

O feijoeiro comum (*Phaseolus vulgaris* L.), é uma cultura de grande importância no cenário agropecuário mundial, sobretudo nos países em desenvolvimento da América do Sul, América Central e sudoeste da África. Seus grãos possuem elevado teor de proteína, carboidratos e minerais, tornando o seu consumo altamente vantajoso, principalmente para as populações de baixa renda (BROUGHTON et al., 2003; RON et al., 2016). No Brasil, historicamente, o feijoeiro apresenta uma grande importância social, sendo cultivado por um grande contingente de pequenos produtores, voltados, sobretudo, para a agricultura de subsistência. No entanto, nas últimas décadas, o interesse pela cultura por parte de grandes produtores, com elevado nível tecnológico, tem aumentado, fazendo com que a cultura apresente, cada vez mais, grande importância econômica para o país (CARNEIRO; PAULA JÚNIOR; BORÉM, 2015).

O Brasil destaca-se por ser o maior produtor e consumidor mundial de feijão, com uma produção de cerca de 3,12 milhões de toneladas em uma área de 3,17 milhões de hectares no período de 2017/2018 (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2019). A maior produção no cenário nacional ocorre no estado do Paraná, seguido por Minas Gerais, Mato Grosso, Bahia e Goiás, os quais respondem por cerca de 64% do total produzido no país (CONAB, 2019). No entanto, mesmo produzindo mais de três milhões de toneladas, anualmente, a produção brasileira de feijão ainda não é suficiente para atender ao mercado interno em sua totalidade. O país importa cerca de 150 mil toneladas por ano, sendo a maioria de feijão tipo preto, cujo principal fornecedor é a Argentina (RUAS, 2017). Na maioria das regiões brasileiras, a preferência é pelo feijão de grãos tipo carioca, já nos estados do Rio de Janeiro e Rio Grande do Sul, o preferido é o grão tipo preto (COMISSÃO TÉCNICA SUL-BRASILEIRA DE FEIJÃO - CTSBF, 2012).

O cultivo dessa leguminosa no país é realizado durante todo o ano, de tal forma que há produção de feijão em praticamente todos os meses do ano, em alguma região do país. No entanto, de maneira geral, a produção concentra-se em três safras bem definidas: A primeira safra, ou “safra das águas”, semeada entre outubro e novembro, a “safra da seca” ou de segunda época, semeada entre fevereiro e março e a terceira safra, também chamada de ‘safra de “outono-inverno”, com semeadura entre os meses de junho e julho (BARBOSA; GONZAGA, 2012). Nessa última safra, via de regra, é necessário o uso da irrigação, que,

aliada a tecnologias avançadas, tem permitido a obtenção dos maiores índices de produtividade do país, principalmente nos estados localizados na região do planalto central brasileiro (SOUZA; WANDER, 2014) Os Estados de Goiás e Distrito Federal alcançaram produtividade de cerca de 3.000Kg.ha na terceira safra de 2017/2018, praticamente o triplo da produtividade média nacional que foi de 1.056Kg.ha, nesse mesmo período (CONAB, 2019).

Em decorrência do uso de novas tecnologias e melhorias desde o manejo adequado do solo ao uso de sementes melhoradas, a produtividade do feijoeiro tem aumentado nas últimas décadas. No entanto, a produtividade média nacional ainda está muito aquém do potencial produtivo da cultura. Isso ocorre, porque o feijão é cultivado ao longo de todo o ano em uma ampla diversidade de ecossistemas, sob diferentes sistemas de produção, estando exposto a diversos fatores que causam instabilidade à sua produção. Entre esses fatores, a ocorrência de doenças, especialmente as de etiologia fúngica, são responsáveis por perdas significativas de produção (CARNEIRO; PAULA JÚNIOR; BORÉM, 2015; PAULA JÚNIOR; WENDLAND, 2012). A alternativa mais econômica e ecologicamente correta para o controle dessas doenças é o emprego de cultivares resistentes (FERREIRA; CAMPA; KELLY, 2013; MIKLAS et al., 2006). Dessa forma, pesquisas relacionadas à resistência de cultivares de feijão aos principais fitopatógenos presentes no território nacional são fundamentais para dar sustentabilidade e suporte aos programas de melhoramento do feijoeiro no país.

2.2 Aspectos gerais da antracnose do feijoeiro

A antracnose do feijoeiro, causada pelo fungo *Colletotrichum lindemuthianum*, é uma das doenças mais agressivas e de maior importância para a cultura, podendo provocar perda total de produção quando são utilizadas sementes contaminadas e/ou cultivares suscetíveis, sob condições climáticas favoráveis para o desenvolvimento da doença durante o ciclo da cultura (BARBOSA; GONZAGA, 2012; KIMATI et al., 1997). Além disso, em ataques mais severos, a doença pode ocasionar manchas nos grãos, depreciando a qualidade do produto final e tornando-os indesejáveis ao consumo (PAULA JÚNIOR; ZAMBOLIM, 2006). Trata-se de uma doença cosmopolita, sendo encontrada em todas as regiões onde o feijoeiro é cultivado, principalmente em regiões tropicais e subtropicais da América Latina e África Central e Oriental, onde as condições climáticas favorecem o desenvolvimento da doença (MIKLAS et al., 2006).

No Brasil, há uma alta incidência dessa doença nas lavouras de feijão da maioria dos estados produtores, sendo mais frequente em Minas Gerais, São Paulo, Paraná, Santa Catarina

e Rio Grande do Sul. Na primeira safra, ou safra das águas, normalmente ocorre uma maior incidência do patógeno. Isso porque a doença é favorecida por temperaturas moderadas, entre 15 e 25°C, associadas à alta umidade relativa do ar, acima de 91% e precipitação frequente (CARBONELL et al., 2012; PINTO et al., 2001).

O agente etiológico da antracnose, *C. lindemuthianum*, é um fungo filamentoso, de comportamento hemibiotrófico, ou seja, apresenta uma fase inicial biotrófica, em que extrai nutrientes das células vivas do hospedeiro e uma segunda fase, necrotrófica, em que se nutre do tecido morto (PERFECT; GREEN; O'CONNELL, 2001). Em sua fase assexual ou anamórfica, o fungo produz micélio septado e ramificado, de coloração hialina a quase negra à medida que envelhece. Os conídios são hialinos, unicelulares, de formato cilíndrico, com as pontas arredondadas. Estes são produzidos em corpos de frutificação denominados acérvulos e germinam após um período de 6 a 9 horas sob condições favoráveis, formando um tubo germinativo curto, com um apressório em sua extremidade (PERFECT et al., 1999). A penetração ocorre mecanicamente pela cutícula e epiderme do hospedeiro, por meio da formação da hifa de infecção, que se desenvolve a partir do apressório (O'CONNELL; BAILEY; RICHMOND, 1985).

À medida que as hifas do patógeno se ramificam intra e extracelularmente nos tecidos do hospedeiro, ocorre o rompimento das células do vegetal e os sintomas macroscópicos da doença começam a ser visualizados (PERFECT; GREEN; O'CONNELL, 2001). Tais sintomas são de fácil reconhecimento e dependendo da intensidade da doença, podem se manifestar em toda parte aérea da planta, a partir do sétimo dia da infecção. Os sintomas típicos da doença são lesões necróticas de coloração marrom escura nas nervuras da parte abaxial das folhas. Em infestações mais severas, as lesões estendem-se ao limbo foliar, resultando em necrose de parte do tecido vegetal. As lesões produzidas no caule e nos pecíolos são alongadas, escuras e deprimidas, podendo apresentar cancros. Já nas vagens, as lesões são circulares, de coloração marrom, com os bordos escuros e salientes, circundados por um anel pardo avermelhado. Em condições favoráveis ao desenvolvimento do patógeno, uma massa rósea de esporos pode ser observada no centro das lesões. As sementes infectadas podem apresentar desde uma leve descoloração até lesões escuras e deprimidas, as quais são mais facilmente visualizadas em sementes de tegumentos claros (BARBOSA; GONZAGA, 2012; CARBONELL et al., 2012; PADDER et al., 2017).

A curtas distâncias, o patógeno dissemina-se por meio de respingos de chuva, por insetos, animais e pela ação do homem, que transporta sementes e restos culturais infectados entre as lavouras, principalmente quando opera máquinas agrícolas. No entanto, a fonte

primária de inóculo, do ponto de vista epidemiológico, são as sementes infectadas, as quais são responsáveis pela disseminação a longas distâncias, tanto dentro quanto entre países (CARBONELL et al., 2012; FERREIRA; CAMPA; KELLY, 2013). No Brasil, muitos produtores utilizam os grãos colhidos como sementes para a próxima semeadura, o que aumenta o risco de contaminação de uma safra para outra.

O uso de sementes de alta qualidade sanitária é de fundamental importância para o controle da antracnose, pois, quanto mais tardio for o aparecimento da doença na lavoura, menor será a perda de rendimento de grãos. A eliminação de restos de cultura e, quando possível, a rotação de culturas com plantas não hospedeiras, também são medidas preventivas recomendadas para evitar a transmissão de inóculo entre safras (CARBONELL et al., 2012; FERREIRA; CAMPA; KELLY, 2013; REIS; CASA; BIANCHIN, 2011). O sistema de plantio direto, comumente utilizado no Brasil, caso não seja utilizado em um esquema racional de rotação de culturas, pode favorecer o desenvolvimento de algumas doenças, entre elas, a antracnose. Embora vários fungicidas tenham sido recomendados para o controle da antracnose (LIMA et al., 2010; SARTORATO, 2006), este onera os custos de produção. Dessa forma, a resistência genética tem sido uma das estratégias mais eficientes, econômicas e ecologicamente viável para o controle da antracnose (MIKLAS et al., 2006; SINGH; SCHWARTZ, 2010).

O fungo possui ainda uma fase sexual ou telomórfica, denominada *Glomerella lindemuthiana*. Nessa fase, o fungo produz peritécios, dentro destes se encontram os ascos, os quais possuem oito ascósporos (conídios) (KIMATI; GALLI, 1970; SHEAR; WOOD, 1913). Recentemente, tem sido observado que um complexo de diferentes espécies pode estar co-habitando lesões de antracnose (BARCELOS et al., 2014; MOTA et al., 2016).

2.3 Variabilidade patogênica de Colletotrichum lindemuthianum

Apesar do uso de cultivares resistentes ser o método mais eficiente no controle da antracnose, a grande variabilidade genética e patogênica de *C. lindemuthianum*, evidenciada pela grande quantidade de raças fisiológicas que têm sido identificadas, dificulta a obtenção de cultivares com resistência durável (FERREIRA; CAMPA; KELLY, 2013; PADDER et al., 2017; PEREIRA et al., 2010; PINTO et al., 2012). A primeira evidência da variabilidade patogênica entre isolados de *C. lindemuthianum* foi relatada por Barrus (1911) que, utilizando algumas cultivares de feijão, observou que estas apresentavam reação diferente quando inoculadas com dois isolados diferentes de *C. lindemuthianum*, os quais foram denominados

como Alfa e Beta. A partir de então, várias raças patogênicas começaram a ser relatadas em todo mundo. No Brasil, o estudo pioneiro de identificação de raças de *C. lindemuthianum* foi realizado por Kimati em 1966. Neste trabalho, a partir de isolados coletados no estado de São Paulo, foram identificadas duas raças do patógeno, as quais pertenciam aos grupos, Alfa mexicano II e Delta.

Nos primeiros estudos de identificação de raças de *C. lindemuthianum,* cada pesquisador utilizava um conjunto particular de linhagens de feijoeiro, a fim de se averiguar a reação destas a diferentes isolados do patógeno. Nessa época, não havia consenso quanto à nomenclatura das raças identificadas e estas eram, normalmente, designadas por códigos específicos locais, tais como: Alfa, Beta, Gama, Lambda, Epsilon, Mexicano I e II, Brasileiro I e II, entre outras (CARBONELL et al., 1999). Com o objetivo de facilitar o intercâmbio de informações e permitir a comparação dos resultados dos estudos realizados por diferentes pesquisadores, na Primeira Reunião Latino Americana da Antracnose do Feijoeiro, realizada no CIAT (Cali, Colômbia), Pastor-Corrales (1991) sugeriu uma metodologia padronizada para classificar as raças do patógeno, a qual é utilizada até os dias atuais. Por essa metodologia, os isolados são inoculados em um conjunto internacional de doze cultivares diferenciadoras, sendo quatro de origem andina (Michigan Dark Red Kidney, Perry Marrow, Widusa e Kaboon) e oito de origem mesoamericana (Michelite, Cornell 49-242, México 222, PI 207.262, TO, TU, AB 136 e G2333). Um valor binário (HABGOOD, 1970) é atribuído a cada uma dessas cultivares e a raça é determinada numericamente, de acordo com a soma dos valores binários de todas as cultivares para as quais o isolado inoculado é virulento. Como exemplo, a raça 65 é virulenta às cultivares Michelite (2^0) e México 222 (2^6), $2^0 + 2^6 = 65$.

Vários estudos de levantamento de raças vêm sendo realizados desde então. Atualmente, mais de 180 raças já foram identificadas entre isolados de *C. lindemuthianum*, coletados em todo o mundo, utilizando o conjunto internacional de cultivares diferenciadoras e o método binário para nomear as raças, comprovando a eficiência do método (PADDER et al., 2017). Dessa forma, o monitoramento das raças patogênicas, prevalecentes em cada região produtora de feijão, deve ser um processo rotineiro, assim como a avaliação de germoplasma de feijoeiro com as raças de *C. lindemuthianum* mais frequentes (BARCELOS et al., 2013; SALA; ITO; CARBONELL, 2006).

Balardin, Jarosz e Kelly (1997) observaram que algumas raças do patógeno, a exemplo das raças 65 e 73, ocorrem em várias regiões geográficas, enquanto outras raças são encontradas apenas em países ou regiões específicas. Estudos de levantamento de raças em regiões produtoras de feijoeiro do Brasil têm sido realizados e as raças 65, 73, 81 e 89 tem

sido as mais frequentes (FERREIRA et al., 2008; ISHIKAWA et al., 2005; MAHUKU; RIASCOS, 2004; PINTO et al., 2012; RIBEIRO et al., 2016; SILVA; SOUZA; ISHIKAWA, 2007). Entre estas, a raça 65, tem sido relatada como uma raça estável e amplamente distribuída na maioria das regiões produtoras.

Ainda que o sistema atual de determinação de raças tenha facilitado a comparação e troca de informações entre diferentes pesquisadores, além de permitir um maior entendimento sobre a dinâmica populacional do patógeno, ele não discrimina toda a variabilidade do patógeno. Isso ocorre porque o conjunto de cultivares diferenciadoras não representa todos os alelos de resistência à antracnose do hospedeiro, o que impossibilita a identificação da real variabilidade patogênica entre isolados de *C. lindemuthianum* (DAVIDE; SOUZA, 2009; FERREIRA; CAMPA; KELLY, 2013; RODRÍGUEZ-SUÁREZ et al., 2005). Mahuku e Riascos (2004), utilizando 200 isolados coletados em vários países, verificaram que todas as cultivares diferenciadoras já tiveram sua resistência quebrada por isolados de *C. lindemuthianum*. Por outro lado, a raça 0 não é virulenta a nenhuma das doze cultivares diferenciadoras, no entanto, outras cultivares de feijoeiro já foram descritas como suscetíveis a essa raça (GONZÁLEZ-CHAVIRA et al., 2004; SHARMA et al., 2007).

Carbonell et al. (1999), ao inocularem dois isolados das raças 31 e 65 e três isolados da raça 81, em cultivares recomendadas para o plantio no estado de São Paulo, observaram diferenças dentro de raças para o caráter patogenicidade. Rodríguez-Suárez et al. (2005) verificaram que isolados da raça 65 coletados no Brasil eram mais virulentos quando inoculados nas linhagens AB321 e A493 quando comparados com isolados da mesma raça, pertencentes à micoteca da *Michigan State University*, EUA. Davide e Souza (2009) inoculando seis diferentes isolados da raça 65 em sete cultivares comerciais de feijão, observaram que houve diferença significativa na agressividade dos isolados utilizados. Recentemente, Costa et al. (2017), verificaram que a cultivar Ouro Negro, descrita em muitos trabalhos, como suscetível à raça 65 (ALZATE-MARIN et al., 2003; GONÇALVES-VIDIGAL et al., 2011), mostrou-se resistente a cinco de seis isolados da raça 65 de *C. lindemuthianum* utilizados. Neste mesmo trabalho, a cultivar Talismã, descrita como resistente à raça 65 (ABREU et al., 2004), foi completamente suscetível a todos os isolados utilizados.

Quando uma cultivar de feijão é descrita como resistente a uma determinada raça de *C. lindemuthianum*, pressupõe-se que ela seja resistente a todos os isolados pertencentes a essa raça. Caso contrário, isso poderá comprometer a credibilidade dos programas de melhoramento, além de gerar prejuízos econômicos (ISHIKAWA; RAMALHO; SOUZA,

2011). Em razão da alta frequência de ocorrência e a grande variabilidade encontrada dentro de isolados da raça 65 de *C. lindemuthianum,* Ishikawa, Ramalho e Souza (2011), sugeriram a utilização de um conjunto de cultivares diferenciadoras, adicional, para discriminar a variabilidade dentro de isolados pertencentes a essa raça. As cultivares recomendadas foram: BRS Estilo, BRSMG Majestoso, BRS Supremo, BRSMG União, BRS Valente, Ouro Vermelho, BRSMG Madrepérola e BRSMG Talismã, as quais são comercialmente disponíveis e adaptadas às condições brasileiras de cultivo, o que facilita a sua utilização. Posteriormente, Ishikawa et al. (2012) verificaram que esse conjunto de cultivares também apresentavam capacidade para discriminar a variabilidade dentro de isolados da raça 81 de *C. lindemuthianum*.

Variação dentro de raças também tem sido relatada nas raças 73, 81 e 89 (FERREIRA; CAMPA; KELLY, 2013; ISHIKAWA et al., 2012; SANTOS et al., 2008), sendo importante a identificação de fontes de resistência que discriminem melhor a variação existente dentro dessas raças, assim como tem sido realizado para as raças 65 e 81 de *C. lindemuthianum* (ISHIKAWA, et al., 2012; ISHIKAWA; RAMALHO; SOUZA, 2011).

A ampla variabilidade patogênica encontrada na população de *C. lindemuthianum* pode ser atribuída a vários mecanismos de ampliação de variabilidade, comumente encontrados em fungos. Além da recombinação sexual, por meio da meiose, o patógeno também apresenta outros mecanismos de recombinação assexual, como tubos anastomoses entre conídios (CATs) (ISHIKAWA et al., 2012) e anastomose de hifas e formação do heterocário, que leva a ocorrência ciclo parassexual (CASTRO-PRADO et al., 2007; ISHIKAWA et al., 2013) e, consequentemente, ao polimorfismo cromossômico, relatado em linhagens de *C. lindemuthianum* (GONÇALVES, 2016; O`SULIVAN et al., 1998).

2.4 Controle genético da resistência do feijoeiro à antracnose

Em razão da especificidade dos alelos de resistência, já idenficados em diferentes cultivares de feijoeiro, às diferentes raças de *C. lindemuthianum*, a resistência do feijoeiro a esse patógeno, geralmente segue um modelo de herança qualitativo, em que os fenótipos, resistente e suscetível, são claramente diferenciados (CAMPA et al., 2014; FERREIRA; CAMPA; KELLY, 2013). Esse tipo de resistência, também conhecida como resistência vertical é governada por poucos genes, de grande efeito (ROBINSON, 1971; VANDERPLANK, 1963) e está de acordo com a teoria gene-a-gene proposta por Flor (1956), pela especificidade dos genes de resistência do hospedeiro às raças do patógeno.

Bulkholder (1918), foi quem primeiro estudou a herança da resistência do feijoeiro à *C. lindemuthianum* por meio de análises genéticas clássicas, baseadas na interpretação de resultados obtidos a partir de uma população segregante F_2 derivada do cruzamento entre as cultivares White Marrow (resistente à raça Alpha) e Wells' Red Kidney (suscetível à raça Alpha). Neste trabalho foram observadas 362 plantas resistentes e 111 plantas suscetíveis na geração F_2, sugerindo que um gene com alelo dominante, mais tarde denominado *Co*-1, estava envolvido na resistência à essa raça. A partir de então, até os dias atuais, o controle genético da resistência de cultivares de feijoeiro às mais diversas raças de *C. lindemuthianum* tem sido estudado e vários alelos de resistência, provenientes de diferentes genes têm sido identificados. (COSTA et al., 2017; FERREIRA; CAMPA; KELLY, 2013; LACANALLO; GONÇALVES-VIDIGAL, 2015; SOUSA et al., 2015). Além de populações segregantes F_2, Famílias $F_{2:3}$ e linhagens endogâmicas recombinantes (*Recombinant Imbreed Lines - RILs*) também têm sido sugeridas para esse tipo de estudo, sobretudo por possibilitar a repetibilidade dos experimentos e confiabilidade dos resultados obtidos (CAMPA et al., 2014; FERREIRA; CAMPA; KELLY, 2013).

Em 1996, Kelly e Young propuseram o uso do símbolo "*Co*" seguido por um número ou letra para se referir aos alelos de resistência às raças de *C. lindemuthianum* identificados em cultivares de feijoeiro. Até o momento, já foram identificados 23 alelos *Co*'s (Tabela 1), provenientes de diferentes genes. Alguns desses alelos, como por exemplo o *Co*-4^2, têm sido utilizados com frequência em programas de melhoramento de feijoeiro visando à resistência à antracnose. Alelismo múltiplo também tem sido relatado nos locos *Co*-1, *Co*-3, *Co*-4 e *Co*-5 (Tabela 1). Com exceção do alelo *co*-8, identificado na cultivar AB136 (ALZATE-MARIN et al., 1997), no qual a resistência é relatada como sendo conferida por um alelo recessivo, nos demais casos, o alelo dominante é descrito como o responsável pela resistência. Diferentes tipos de marcadores ligados a esses genes de resistência à antracnose do feijoeiro, têm sido identificados, viabilizando a seleção assistida por marcadores (FERREIRA; CAMPA; KELLY, 2013; GONÇALVES-VIDIGAL et al., 2011, 2015; KELLY; VALLEJO, 2004; MEZIADI et al., 2016).

Tabela 1 - Alelos de resistência a *C. lindemuthianum*, cultivar em que o alelo foi identificado e seu respectivo cromossomo.

Loco	Cultivar	Cromossomo	Referência
Co-1	MDRK	Pv01	(MCROSTIE, 1919)
Co-1^2	Kaboon	Pv01	(MELOTTO; KELLY, 2000)
Co-1^3	Perry Marrow	Pv01	(MELOTTO; KELLY, 2000)
Co-1^4	Widusa	Pv01	(GONÇALVES-VIDIGAL et al., 2011)
Co-1^5	Widusa	Pv01	(GONÇALVES-VIDIGAL; KELLY, 2006)
Co-2	Cornel 49-242	Pv11	(MASTENBROEK, 1960)
Co-3	México 222	Pv04	(BANNEROT, 1965)
Co-3^2	México 226	Pv04	(FOUILLOUX, 1976, 1979)
Co-3^3 *(Co-9*)	BAT 93	Pv04	(GEFFROY et al. 2008)
Co-3^4 Co-10	Ouro Negro	Pv04	(VALENTINI et al., 2017)
Co-4	TO	Pv08	(FOUILLOUX, 1976, 1979)
Co-4^2	G2333	Pv08	(YOUNG et al., 1998)
Co-4^3	PI 207.262	Pv08	(ALZATE-MARIN et al., 2007)
Co-5	TU	Pv07	(ALZATE-MARIN et al., 2007)
Co-5^2	G2333	Pv07	(VALLEJO; KELLY, 2009)
Co-6	AB 136	Pv07	(KELLY, J.D.; YOUNG, 1996)
co-8	AB 136	-	(ALZATE-MARIN et al., 1997)
Co-11	Michelite	-	(GONÇALVES-VIDIGAL et al., 2007)
Co-12	Jalo Vermelho	-	(GONÇALVES-VIDIGAL et al., 2008)
Co-13	Jalo Listras Pretas	Pv03	(LACANALLO; GONÇALVES-VIDIGAL, 2015)
Co-14	Pitanga	-	(GONÇALVES-VIDIGAL et al., 2012)
Co-15	Corinthiano	Pv04	(SOUSA et al., 2015)
Co-16	Crioulo 159	Pv04	(COIMBRA-GONÇALVES et al., 2016)
Co- 17	Paloma	Pv01	(CASTRO et al., 2017)
Co-u	BAT 93	Pv02	(GEFFROY et al., 2008)
Co-v	BAT 93	Pv07	GEFFROY, 1997)
Co-x	Jalo EEP 558	Pv01	(RICHARD et al., 2014)
Co-w	Jalo EEP 558	Pv01	(GEFFROY et al., 2008)
Co-y	Jalo EEP 558	Pv04	(GEFFROY et al., 1999)
Co-z	Jalo EEP 558	Pv04	(GEFFROY et al., 1999)

Fonte: Dados da autora (2019).

O controle genético da resistência de cultivares de feijoeiro a seis diferentes isolados pertencentes à raça 65 de *C. lindemuthianum* foi recentemente estudado por Costa et al. (2017). O diferente padrão da reação de cultivares de feijoeiro, quando inoculadas com os diferentes isolados da raça 65 e as segregaçãoes de 15R:1S observadas na geração F_2 de vários cruzamentos, indicaram que alelos dominantes, provenientes de genes duplicados poderiam

estar conferindo resistência específica a cada um dos isolados utilizados. Esse resultado evidencia que a especificidade da interação patógeno-hospedeiro ocorre também à nível de isolado, dentro de raça. Portanto, uma grande quantidade de alelos, provenientes de diferentes genes, estão envolvidos na resistência à antracnose, dificultando a obtenção de cultivares com resistência durável. Ademais, os alelos de resistência, já identificados em algumas linhagens de feijoeiro, conferindo resistência a essa raça, tais como *Co*-12, na linhagem Jalo Vermelho (GONÇALVES-VIDIGAL; LACANALLO; VIDIGAL FILHO, 2008), *Co*-13, na linhagem Jalo Listras Pretas (LACANALLO; GONÇALVES-VIDIGAL, 2015), *Co*-14 na linhagem Pitanga (GONÇALVES-VIDIGAL et al., 2012), *Co*-15 na linhagem Corinthiano (SOUSA et al., 2015) e *Co*-17 na linhagem Paloma (CASTRO et al., 2017) provavelmente não conferem resistência a todos os isolados pertencentes a essa raça. Consequentemente, os marcadores moleculares ligados a esses genes podem não ser úteis em todas as regiões onde o feijão é cultivado.

O fato de uma cultivar apresentar resistência qualitativa, não exclui a possibilidade da resistência quantitativa estar presente. Ao contrário da resistência qualitativa, a resistência quantitativa ou resistência horizontal, é governada por muitos genes de pequeno efeito, não específicos a raças do patógeno, podendo, portanto, propiciar uma resistência mais duradoura (POLAND et al., 2008). Por gerar uma distribuição contínua de classes fenotípicas, que vai desde máxima resistência a máxima susceptibilidade, esse tipo de resistência, é também conhecida como resistência parcial. Na última década, *quantitative trait loci* (QTLs) conferindo resistência parcial também tem sido identificados no patossistema *P. vulgaris – C. lindemuthianum*. Nesses estudos, dois ou mais QTLs envolvidos na resistência a um único isolado de *C. lindemuthianum* têm sido identificados (FERREIRA; CAMPA; KELLY, 2013; GEFFROY et al., 2000; OBLESSUC et al., 2014; PERSEGUINI et al., 2016; ZUIDERVEEN et al., 2016). Interações epistáticas entre QTLs envolvidos na resistência do feijoeiro a *C. lindemuthianum*, também têm sido relatadas (GONZÁLEZ et al., 2015), o que evidencia que a resistência a antracnose no feijoeiro também envolve relações gênicas mais complexas.

2.5 Mapeamento de genes de resistência à antracnose

Para melhor caracterização dos genes de resistência, assim como de todos os outros genes de uma espécie, é muito importante saber a sua posição no genoma da espécie, assim como a sua relação com outros genes. A determinação da posição relativa dos genes nos cromossomos e a distância entre eles é realizada por meio da contrução de mapas genéticos

moleculares (CARNEIRO; VIEIRA, 2002). Para isso, é requerida uma população de mapeamento, que, no caso de plantas autógamas, como o feijoeiro, podem ser utilizadas: Populações F_2, Retrocruzamentos, ou, preferencialmente, Linhagens Endogâmicas Recombinantes (*Recombinant Imbreed Lines – RILs*). Em todos esses casos, as populações devem ser oriundas do cruzamento entre linhagens contrastantes para uma ou mais características de interesse. Uma vez que as populações RILs são derivadas de várias gerações de autofecundação, o que favorece a quebra dos blocos de ligação, estas propiciam uma melhor resolução de mapeamento e são, portanto, as mais adequadas para esse tipo de estudo. No entanto, são necessários vários anos para a obtenção dessas populações, o que pode dificultar a utilização das mesmas (SEMAGN; BJORNSTAD; NDJIONDJOP, 2007).

Os marcadores moleculares disponíveis para a cultura do feijoeiro, especialmente os microssatélites (*Simple Sequence Repeats - SSRs*) (BLAIR et al., 2009, 2012) e *Single Nucleotide Polymorphism* (SNPs) (GALEANO et al., 2009) têm sido largamente utilizados na construção de mapas moleculares para a identificação de genes, controlando diversas características, incluindo resistência a doenças. Os SNPs são as formas mais abundantes de polimorfismo de DNA em genomas de eucariotos (BROOKES, 1999). Com o advento das tecnologias de sequenciamento de nova geração, o número de SNPs identificados tem aumentado substancialmente (BLAIR et al., 2013), oferecendo a oportunidade para saturar regiões genômicas específicas e aumentar a resolução de mapeamento dos genes de interesse.

Atualmente, vários genes de resistência à antracnose, identificados em várias cultivares, conferindo resistência raça-específica, já foram mapeados em diferentes grupos de ligação do feijoeiro (TABELA 1), sendo frequentemente localizados nas extremidades dos cromossomos. Evidências moleculares têm revelado que, na verdade, esses genes de resistência são organizados em complexos blocos gênicos *(clusters*), dentro dos quais, genes individuais conferem resistência a um isolado/raça específica (CAMPA et al., 2014; CAMPA; TRABANCO; FERREIRA, 2017; FERREIRA; CAMPA; KELLY, 2013; MEZIADI et al., 2016; MURUBE; CAMPA; FERREIRA, 2019; OBLESSUC; FRANCISCO; MELOTTO, 2015).

Em 2014, o primeiro genoma de *P. Vulgaris* foi publicado, o qual pertence a linhagem andina G19833, com um genoma relativamente pequeno, de cerca de 587Mb (SCHMUTZ et al., 2014), comparado, por exemplo, com o tamanho do genoma da soja de 1.1Gb (SHULTZ et al., 2006). Em 2016, a linhagem mesoamericana BAT93 também teve o seu genoma sequenciado englobando 549.6Mb (VLASOVA et al., 2016). Com o sequenciamento do genoma, informações a respeito da posição física dos marcadores no genoma, bem como

informações sobre as sequências flanqueadoras desses marcadores, podem ser obtidas, levando ao desenvolvimento de marcadores moleculares estreitamente ligados aos genes de interesse. Utilizando o genoma de referência G19833, o mapeamento do alelo *Co*-x foi refinado para uma região de apenas 58Kb no cromossomo Pv01 (RICHARD et al., 2014). Mapeamento fino também tem sido realizado para os alelos *Co*-4 (BURT et al., 2015), *Co*-3[4] (VALENTINI et al., 2017) e, mais recentemente, para dois novos alelos mapeados no cluster *Co*-3 no cromossomo Pv04 (MURUBE; CAMPA; FERREIRA, 2019). Além disso, a grande disponibilidade de SNPs, aliada à sequência do genoma de referência do feijoeiro, tem permitido o desenvolvimento de chips de genotipagem de alto rendimento, para a construção de mapas de ligação (SONG et al., 2015). A anotação do genoma do feijeoiro também tem permitido a busca por genes candidatos dos alelos mapeados.

A maioria dos alelos de resistência caracterizados em várias espécies de plantas codificam proteínas conhecidas como NBS-LRR (*Nucleotide-Binding Site- Leucine-rich repeat*) (NELSON et al., 2018). Essas proteínas estão envolvidas no reconhecimento de diversos patógenos e pragas, incluindo fungos, bactérias, vírus e nematoides e os genes codificadores de tais proteínas encontram-se, frequentemente, agrupados em clusters (SCHMUTZ et al., 2014). Em particular, dois grandes clusters foram identificados nos grupos de ligação Pv04 e Pv11, contendo vários genes para proteínas NBS-LRR que se colocalizam com alguns alelos de resistência à antracnose, previamente mapeados nesses respectivos grupos de ligação (MEZIADI et al., 2016).

A resistência quantitativa também tem sido relatada no patossistema *P.vulgaris – C. lindemuthianum*. QTL's de grande e pequeno efeito conferindo resistência a diferentes raças de *C. lindemuthianum* têm sido mapeados nos 11 cromossomos de feijoeiro (GONZÁLEZ et al., 2015; OBLESSUC et al., 2014; PERSEGUINI et al., 2016). Nesses estudos, além do mapeamento tradicional de QTL, utilizando populações segregantes biparentais, estudos de associação genômica ampla (*Genome-Wide Association Studies* - GWAS), também conhecido por mapeamento associativo ou mapeamento por desequilíbrio de ligação, também têm sido utilizados no mapeamento de genes de resistência a antracnose do feijoeiro (ZUIDERVEEN et al., 2016). Por essa abordagem de mapeamento, a população de mapeamento não precisa ser sintetizada, de forma que pode ser utilizado um painel composto por um conjunto diverso de indivíduos, que representem algum germoplasma interessante, que esteja sofrendo eventos de recombinações históricas há muitas gerações. Dessa forma, espera-se que a resolução do mapeamento para identificação de QTLs seja maior em GWAS do que a obtida utilizando populações segregantes biparentais, as quais acumulam um número limitado de eventos de

recombinação, principalmente quando são utilizadas populações em gerações iniciais de endogamia (MOGHADDAM et al., 2016; ZHU et al., 2008).

A resolução com a qual um QTL pode ser mapeado é uma função do decaimento do desequilíbrio de ligação, o qual geralmente pode se estender por mais de 100kb em espécies autógamas, a exemplo do arroz e feijão (GARRIS et al., 2005; PERSEGUINI et al., 2016). Dessa forma, para que se tenha uma alta resolução de mapeamento em GWAS, também é necessário que se tenha uma grande saturação de marcas, cobrindo todo o genoma. Após o sequenciamento do genoma do feijoeiro (SCHMUTZ et al., 2014) e também o desenvolvimento de sistemas de genotipagem de alto rendimento (SONG et al., 2015), vários estudos de mapeamento associativo têm sido realizados para várias características em feijoeiro (HOYOS-VILLEGAS; SONG; KELLY, 2017; KAMFWA; CICHY; KELLY, 2015; MOGHADDAM et al., 2016; TOCK et al., 2017). Perseguini et al. (2016) utilizaram essa abordagem de mapeamento para identificar QTLs associados à resistência a raça 4 de *C. lindemuthianum* e a raça 39 de *Pseudocercospora griseola*. Neste trabalho, foi utilizado um conjunto diverso de 180 acessos de feijoeiro pertencentes ao banco de germoplasma do Instituto Agronômico de Campinas (IAC), dos quais 24 eram de origem andina e 156 de origem mesoamericana. Utilizando 384 SNPs e 103 SSRs, 38 e 28 QTLs foram associados a *C. lindemuthianum* e *P. griseola*, respectivamente, sendo a maioria de pequeno efeito, distribuídos em todo o genoma do feijão, o que indica um padrão de herança quantitativa e complexa para a resistência a ambas as doenças. Zuiderveen et al. (2016) utilizaram um painel de 230 linhagens pertencentes ao pool gênico andino de feijoeiro, as quais foram genotipadas com *Illumina BARCBean6K_3 BeadChip* (SONG et al., 2015) com 5398 SNPs para idenficar QTLs relacionados à resistência a diferentes raças de *C. lindemuthianum*. Foram identificados QTLs de grande efeito explicando até 31%, 36%, 37%, 28%, 33% e 36% da variação fenotípica, considerando as raças 7, 39, 65, 73, 109, 3481, respectivamente, em diferentes cromossomos do feijoeiro.

Comparado ao mapeamento tradicional de QTL, um painel de GWAS possui maior variação genética para mapeamento de várias características de interesse, além de maior resolução de mapeamento e não há necessidade de se construir uma população de mapeamento. Com esse painel, também é possível explorar um maior número de alelos, quando são utilizados marcadores multialélicos, como os SSRs. No entanto, é importante ressaltar que em populações biparentais, todo o desequilíbrio de ligação entre a marca e o QTL é atribuído a ligação física, originado pelo desequilíbrio gamético. Já em GWAS, outros fatores, que não a ligação física entre a marca e o QTL podem contribuir para o desequilíbrio

de ligação, tais como: migração, seleção, deriva genética, estrutura populacional e parentesco, podendo causar associações espúrias entre a marca e o QTL. A abordagem de modelos mistos que leva em consideração a estrutura populacional e parentesco podem minimizar a ocorrência de falsos-positivos, mas não os elimina completamente (ZHANG et al., 2010). Dessa forma, o mapeamento tradicional utilizando populações biparentais e GWAS são dois métodos frequentemente utilizados para complementar um ao outro (LI et al., 2016; NELSON et al., 2018; TOCK et al., 2017).

2.6 Estratégias de melhoramento visando resistência à antracnose

Como o fungo *C. lindemuthianum* é um patógeno que apresenta grande capacidade de recombinação, a resistência baseada em um único gene é, na maioria das vezes, facilmente superada pela evolução da população do patógeno. Uma alternativa para aumentar a vida útil de resistência a doenças, em várias espécies, tem sido introgredir dois, três ou mais alelos de resistência a diferentes raças do patógeno em uma mesma cultivar, ou seja, construir uma pirâmide de diferentes alelos de resistência (CONSORTIUM, 2016; MIKLAS et al., 2006; MUNDT, 2014; NELSON et al., 2018). Essas pirâmides são obtidas, por meio de cruzamentos entre linhagens portadoras de diferentes alelos de resistência com alguma linhagem suscetível, mas bem adaptada e com características agronômicas e comerciais desejáveis. Como as cultivares portadoras de alelos de resistência são, em geral, linhagens ou cultivares mal adaptadas e com vários fenótipos indesejáveis, são necessárias várias gerações de retrocruzamento com o genitor recorrente, a linhagem adaptada, de forma que, ao final do processo, se tenha uma linhagem com todas as características favoráveis (BOTELHO et al., 2011).

Na construção de uma pirâmide, a escolha de um genitor recorrente que apresente perspectiva de longa vida útil, ou seja, que apresente ampla adaptabilidade e estabilidade genotípica, é importante. Isso ocorre porque todos os esforços são concentrados em uma única cultivar e, muitas vezes, ao final de todo o processo, o qual é demorado, já existem outras linhagens superiores para outras características desejáveis e a linhagem com os alelos piramidados já se encontra em desuso (BOTELHO et al., 2011). Essa estratégia tem sido comumente empregada para introduzir diferentes alelos de resistência a várias raças de *C. lindemuthianum* em cultivares de feijoeiro. Em muitas ocasiões, o uso de marcadores moleculares tem facilitado a identificação de indivíduos que apresentem os alelos de resistência piramidados e também grande proporção do genoma do genitor recorrente em

gerações precoces de retrocruzamentos (FERREIRA; CAMPA; KELLY, 2013; GARZÓN; LIGARRETO; BLAIR, 2008; HEGAY et al., 2014; MARCONDES; PEREIRA; SANTOS, 2010; PEREIRA; SANTOS, 2004). No entanto, com o uso extensivo de uma cultivar resistente, ocorre uma forte pressão de seleção na população do patógeno. Como consequência, novas raças patogênicas, capazes de superar os alelos de resistência da linhagem com os alelos piramidados, surgem e são selecionadas, o que faz com que a pirâmide seja menos efetiva (McDONALD; LINDE, 2002; MUNDT, 2014).

A existência de coevolução entre a origem geográfica de *C. lindemuthianum* e a de seu hospedeiro tem sido verificada (CHIORATO et al., 2006). Cultivares de origem andina são mais resistentes às raças mesoamericanas de *C. lindemuthianum*e, da mesma maneira, cultivares mesoamericanas são mais resistentes às raças andinas. Esse fato, sugere que a transferência de alelos de resistência entre conjuntos gênicos, andinos e mesoamericanos, pode proporcionar resistência mais durável à antracnose (MIKLAS et al., 2006; SINGH; SCHWARTZ, 2010).

Uma outra alternativa que tem sido sugerida para o controle da antracnose, por meio da utilização da resistência genética das cultivares, é o emprego de multilinhas. Esse método consiste na utilização de mistura, em proporções previamente estabelecidas, de linhagens fenotipicamente semelhantes, porém, que apresentem alelos de resistência diferentes às diferentes raças do patógeno. Essa estratégia tem sido recomendada para o manejo de muitas doenças em várias culturas (McDONALD, 2014). Espera-se que, com o uso de multilinhas, ocorra redução na densidade de plantas suscetíveis, diante da redução de inóculo efetivo do patógeno no campo, o que resulta em uma maior estabilidade de produção e aumento da durabilidade da resistência em relação a uma única linhagem com vários alelos piramidados (MUNDT, 2014).

Botelho et al. (2011) avaliaram o potencial da utilização de multilinhas, compostas por sete linhagens de feijão tipo carioca, com características fenotípicas semelhantes, mas com diferentes reações às várias raças de *C. lindemuthianum.* Foi observado que a mistura das linhagens proporcionou a redução do progresso da doença no campo, sendo que a nota média de severidade da antracnose foi similar àquelas obtidas pelas linhagens resistentes, mesmo que na constituição da mistura também houvesse linhagens suscetíveis. Além disso, a multilinha apresentou produção similar a das linhagens mais produtivas. Resultados semelhantes foram obtidos, recentemente, por Carvalho (2018). Sendo assim, a mistura de linhagens controla o avanço de antracnose e, assim, diminui a perda de produção quando comparada ao uso de linhas puras.

No entanto, algumas questões podem dificultar a utilização de multilinhas e devem ser consideradas: 1) Necessidade de identificar um conjunto de linhagens que apresente diferença quanto à reação às raças do patógeno mas que tenha uniformidade para as demais características agronômicas, como, por exemplo, ciclo, porte, tamanho e coloração dos grãos. Isso porque, para a proteção de uma nova cultivar, esta deve ser submetida ao teste DHE (Distinguibilidade, Homogeneidade e Estabilidade). 2) Necessidade de recompor a mistura a cada safra para garantir as proporções adequadas da multilinha. 3) Ocorrência de competição intergenotípica entre as linhagens da mistura, o que pode afetar a produtividade de grãos.

2.7 Seleção Recorrente

A seleção recorrente é uma estratégia dinâmica de melhoramento que possibilita aumentar, gradativamente, a frequência dos alelos favoráveis, para uma determinada característica quantitativa, sem exaurir a variabilidade genética, por meio de sucessivos ciclos de avaliação, seleção e recombinação (GERALDI, 2005; NELSON et al., 2018; RAMALHO, 2012).

A seleção recorrente é realizada em três etapas. A primeira etapa compreende a obtenção da população base que, segundo Ramalho (2012), deve ser composta por 10 a 20 genitores, os quais devem apresentar o melhor desempenho possível para o caráter sob seleção. A segunda etapa envolve a seleção dos melhores indivíduos/progênies. Nesse caso, quando se tem um caráter de elevada herdabilidade, pode-se optar pela seleção massal. No entanto, quando o caráter apresenta herdabilidade baixa, a seleção a partir da avaliação de progênies, em experimentos com repetição, é mais recomendada. A última etapa envolve a recombinação dos indivíduos/progênies selecionadas para formar a próxima geração. Como esse é um processo dinâmico, o método ainda permite que novas linhagens, com características desejáveis, sejam inseridas em qualquer ciclo de intercruzamento (GERALDI, 1997; RAMALHO, 2012).

Em razão da sua eficiência em aumentar a média do caráter sob seleção, a seleção recorrente tem sido amplamente utilizada no melhoramento de várias culturas, autógamas e alógamas (EDWARDS, 2010; HALLAUER; CARENA; MIRANDA FILHO, 2010; MORAIS et al., 2003). Na cultura do feijoeiro, esse método tem sido utilizado no melhoramento de várias características tais como: produtividade; porte; tipo de grão e florescimento precoce (CUNHA; RAMALHO; ABREU, 2005; MODA-CIRINO; BURATTO; FONSECA JUNIOR, 2016; PIRES et al., 2014; RAMALHO et al., 2005). Além disso, o método tem sido eficiente

para aumentar a frequência dos alelos favoráveis para resistência à mancha angular e ao mofo branco do feijoeiro (ARANTES; ABREU; RAMALHO, 2010; LEITE et al., 2016; REZENDE et al., 2014).

Leite et al. (2016) avaliaram a eficiência da seleção recorrente visando à resistência fisiológica do feijoeiro ao mofo branco e porte ereto, o qual também contribui para a redução da doença no campo, utilizando progênies $S_{0:1}$, $S_{0:2}$ e $S_{0:3}$ dos ciclos III, IV, V e VI. Foi observado um progresso genético de cerca de 11% e 15% ao ano para resistência ao mofo branco e porte ereto, respectivamente. Os autores observaram, ainda, a existência de variabilidade genética entre as famílias, o que pode permitir ganhos futuros adicionais.

Amaro et al. (2007), ao avaliar cinco ciclos de seleção recorrente visando à resistência à mancha angular, obtiveram estimativa de ganho de 6,4% por ciclo e uma resposta indireta na produção de grãos de 8,9%. Arantes, Abreu e Ramalho (2010) também verificaram a eficiência do método em oito ciclo seletivos, com incremento expressivo na resistência à mancha angular e ganho indireto de 2,3% na produção de grãos. Pereira et al. (2015) ao avaliarem a agressividade de isolados de *Pseudocercospora griseola*, agente causal da mancha angular do feijoeiro, em diferentes linhagens de feijão, observaram um maior nível de resistência nas linhagens provenientes do programa de seleção recorrente, visando à resistência à mancha angular. Esse resultado revelou que esse método propicia a fixação de alelos que conferem resistência vertical e horizontal e, portanto, pode otimizar a obtenção de resistência durável a essa doença.

A seleção recorrente visando à resistência à mancha angular do feijoeiro também tem sido realizada com sucesso, através da inoculação artificial de *P. griseola*, em casa de vegetação (LIBRELON, 2016; PÁDUA, 2017). Para otimizar os ganhos com a seleção, os autores recomendam que a seleção das progênies resistentes em casa de vegetação deve ser associada à seleção realizada no campo, em época de maior ocorrência natural do patógeno. Isso porque no patossistema *P.vulgaris – P. griseola* há predominância de resistência quantitativa. Nesse caso, as plantas apresentam a chamada resistência de "planta adulta" a qual não é expressa em estágio de plântulas (COSTA et al., 2006). Isso explica o motivo do maior índice de coincidência da reação das linhagens a *P. griseola* no estágio V3 e no campo, em relação a reação das linhagens em estágio V2, uma vez que nesse estágio, os genes de resistência de "planta adulta" ainda não estão se expressando (PEREIRA, 2017).

É importante ressaltar que, em programas de seleção recorrente, o método bulk dentro de progênies é amplamente utilizado para a condução de populações segregantes (MOREIRA et al., 2010). Esse método consiste em selecionar plantas individuais nas gerações F_2 ou F_3 de

forma que cada planta selecionada origine uma progênie $F_{2:3}$ ou $F_{3:4}$. Essas progênies são individualmente avançadas em bulk por algumas gerações e a seleção é feita apenas entre progênies. Ao final do processo, as cultivares obtidas são uma mistura de linhas puras, o que lhes confere maior estabilidade, assim como numa multilinha formada por misturas de diferentes linhagens.

REFERÊNCIAS

ABREU, A. F. B. et al. 'BRSMG Talismã': common bean cultivar with Carioca grain type. **Crop Breeding and Applied Biotechnology**, Londrina, v. 4, p. 372–374, 2004.

ALZATE-MARIN, A. L. et al. Characterization of the anthracnose resistance gene present in Ouro Negro (Honduras 35) common bean cultivar. **Euphytica**, Wageningen, v. 133, n. 2, p. 165–169, Aug. 2003.

_____. Genetic characterization of anthracnose resistance genes *Co*-4^3 and *Co*-9 in common bean cultivar tlalnepantla 64 (PI 207262). **Euphytica**, Wageningen, v. 154, n. 1/2, p. 1–8, 1 Feb. 2007.

_____. Inheritance of anthracnose resistance in common bean differential cultivar AB 136. **Plant Breeding**, Saint Paul, v. 81, n. 9, p. 996–998, Sept. 1997.

AMARO, G. B. et al. Phenotypic recurrent selection in the common bean (*Phaseolus vulgaris* L) with carioca-type grains for resistance to the fungi *Phaeoisariopsis griseola*. **Genetics and Molecular Biology**, São Paulo, v. 30, n. 3, p. 584–588, 2007.

ARANTES, L. O.; ABREU, A. F. B.; RAMALHO, M. A. P. Eight cycles of recurrent selection for resistance to angular leaf spot in common bean. **Crop Breeding and Applied Biotechnology**, Viçosa, v. 10, n. 3, p. 232–237, set. 2010.

BALARDIN, R. S.; JAROSZ, A.; KELLY, D. Virulence and molecular diversity in *Colletotrichum lindemuthianum* from South, Central and North America. **Phytopathology,** Saint Paul, v. 87, n. 12, p. 1184–1191, Sept. 1997.

BANNEROT, H. Résults de I' infection d'une collection de haricots par six races physiologiques d'anthracnose. **Annales de l 'Amelioration des Plantes**, Paris, v. 15, n. 2, p. 201–222, 1965.

BARBOSA, F. R.; GONZAGA, A. C. O. **Informações técnicas para o cultivo do feijoeiro-comum na Região Central-Brasileira:** 2012-2014. Santo Antônio de Goiás, GO: Embrapa Arroz e Feijão, 2012. 247 p.

BARCELOS, Q. L. et al. Characterization of *Glomerella* strains recovered from anthracnose lesions on common bean plants in Brazil. **PLoS One**, San Francico, v. 9, n. 3, p. 1–15, Jan. 2014.

_____. Investigation of sources of resistance to antracnose disease in *Phaseolus vulgaris* germplasm collection in Brazil. **Annual Report of the Bean Improvement Cooperative**, Geneva, v. 56, p. 37–38, 2013.

BARRUS, M. F. Variation of varieties of bean in their susceptibility to anthracnose. **Phytopathology,** Saint Paul, v. 1, n. 6, p. 190–195, 1911.

BLAIR, M. W. M. et al. Development of microsatellite markers for common bean (*Phaseolus vulgaris* L.) based on screening of non-enriched, small-insert genomic libraries. **Genome**, Ottawa, v. 52, n. 9, p. 772–782, Sept. 2009.

_____. A high-throughput SNP marker system for parental polymorphism screening, and diversity analysis in common bean (*Phaseolus vulgaris* L.). **Theoretical and Applied Genetics**, Berlin, v. 126, n. 2, p. 535–548, Feb. 2013.

_____. New gene-derived simple sequence repeat markers for common bean (*Phaseolus vulgaris* L.). **Molecular Ecology Resources**, Oxford, v. 12, n. 4, p. 661–668, July 2012.

BOTELHO, F. B. S. et al. Multiline as a strategy to reduce damage caused by *Colletotrichum lindemuthianum* in common bean. **Journal of Phytopathology**, Berlin, v. 159, n. 3, p. 175-180, Mar. 2011.

BROOKES, A. J. The essence of SNPs. **Gene**, Amsterdam, v. 234, n. 2, p. 177–186, July 1999.

BROUGHTON, W. et al. Beans (Phaseolus spp.) - model food legumes. **Plant and Soil**, The Hague, v. 252, n. 1, p. 55–128, May 2003.

BULKHOLDER, W. H. The production of na anthracnose resistant White Marrow bean. **Phytopathology,** Saint Paul, v. 8, p. 353–359, 1918.

BURT, A. J. et al. Candidate Gene Identification with SNP Marker-Based Fine Mapping of Anthracnose Resistance Gene *Co*-4 in Common Bean. **PLoS One**, San Francisco, v. 10, n. 10, p. 1–19, 2015.

CAMPA, A. et al. Genetic analysis of the response to eleven *Colletotrichum lindemuthianum* races in a RIL population of common bean (*Phaseolus vulgaris* L.). **BMC Plant Biology**, London, v. 14, p. 115, Jan. 2014.

CAMPA, A.; TRABANCO, N.; FERREIRA, J. J. Identification of clusters that condition resistance to anthracnose in the common bean differential cultivars AB136 and MDRK. **Phytopathology,** Saint Paul, v. 107, n. 12, p. 1515–1521, Dec. 2017.

CARBONELL, S. A. M. et al. **Melhoramento genético do feijoeiro-comum e prevenção de doenças.** Viçosa: Epamig, 2012. 157 p.

_____. Raças fisiológicas de *Colletotrichum lindemuthianum* e reação de cultivares e linhagens de feijoeiro no Estado de São Paulo. **Fitopatologia Brasileira**, Brasília, v. 24, n. 1, p. 60–65, 1999.

CARNEIRO, J. E. S.; PAULA JÚNIOR, T. J.; BORÉM, A. (Ed.). **Feijão:** do plantio a colheita. Viçosa: Ed. UFV, 2015. 384 p.

CARNEIRO, M. S.; VIEIRA, M. L. C. Mapas genéticos em plantas. **Bragantia**, Campinas, v. 61, n. 2, p. 89–100, maio/ago. 2002.

CARVALHO, S. G. M. **Misturas de feijão carioca como estratégia na obtenção de resistência durável a diferentes raças de *Colletotrichum lindemuthianum.*** 2018. 58 p. Dissertação (Mestrado em Genética e Melhoramento de Plantas) – Universidade Federal de Lavras, Lavras, 2018.

CASTRO-PRADO, M. A. A. et al. Vegetative compatibility and parasexual segregation in *Colletotrichum lindemuthianum*, a fungal pathogen of the common bean. **Genetics and Molecular Research**, Ribeirao Preto, v. 6, n. 3, p. 634–642, set. 2007.

CASTRO, S. A. L. et al. Genetics and mapping of a new anthracnose resistance locus in Andean common bean Paloma. **BMC Genomics**, London, v. 18, n. 1, p. 1–12, Apr. 2017.

CHIORATO, A. F. et al. Co-evolução entre raças fisiológicas de *Colletotrichum lindemuthianum* e feijoeiro. **Bragantia**, Campinas, v. 65, n. 3, p. 381–388, 2006.

COELHO, R. T. et al. Characterization of the anthracnose resistance gene in the mesoamerican common bean cultivar Crioulo 159. **Annual Report of the Bean Improvement Cooperative**, Geneva, v. 56, p. 43–44, 2013.

COIMBRA-GONÇALVES, K. et al. Characterization and mapping of anthracnose resistance gene in mesoamerican common bean cultivar Crioulo 159. **Crop Science**, Madison, v. 56, n. 6, p. 2904-2915, Jan. 2016.

COMISSÃO TÉCNICA SUL-BRASILEIRA DE FEIJÃO (CTSBF). **Informações técnicas para o cultivo de feijão na Região Sul brasileira**. Florianópolis: Epagri, 2012. 157 p.

COMPANHIA NACIONAL DE ABASTECIMENTO (CONAB). Disponível em: <http://www.conab.gov.br>. Acesso em: 20 dez. 2019.

CONSORTIUM, R. E. X. Combining selective pressures to enhance the durability of disease resistance genes. **Frontiers in Plant Science**, Lausanne, v. 7, p. 1–8, Dec. 2016.

COSTA, I. F. D. et al. Resistance of six soybean cultivars to *Colletotrichum truncatum* (Schwein) in two phenologic stages. **Ciência Rural**, Santa Maria, v. 36, n. 6, p. 1684-1688, nov./dez. 2006.

COSTA, L.C. et al. Are duplicated genes responsible for anthracnose resistance in common bean? **PLoS One**, San Francisco, v. 12, n. 3, p. 1–15, Mar. 2017.

CUNHA, W. G.; RAMALHO, M. A. P.; ABREU, A. F. B. Selection aiming at upright growth habit common bean with carioca type grains. **Crop Breeding and Applied Biotechnology**, Londrina, v. 5, n. 4, p. 379-386, dez. 2005.

DAVIDE, L. M. C.; SOUZA, E. A. Pathogenic variability within race 65 of *Colletotrichum lindemuthianum* and its implications for common bean breeding. **Crop Breeding and Applied Biotechnology**, Londrina, v. 9, n. 1, p. 23–30, mar. 2009.

EDWARDS, J. Testcross response to four cycles of half-sib and S2 recurrent selection in the BS13 Maize (*Zea mays* L.) population. **Crop Science**, Madison, v. 50, n. 5, p. 1840-1847, Sept. 2010.

FERREIRA, J. J.; CAMPA, A.; KELLY, J. D. Organization of genes conferring resistance to anthracnose in common bean. In: VARSHNEY, R. K.; TUBEROSA, R. (Org.). **Translational genomics for crop breeding**. New York: John Wiley & Sons, 2013. p. 151–176.

FERREIRA, J. J. et al. Reaction of a bean germplasm collection against five races of *Colletotrichum lindemuthianum* identified in northern spain and implications for breeding. **Plant Disease**, Saint Paul, v. 92, n. 5, p. 705–708, May 2008.

FLOR, H. H. The complementary genic systems in flax and flax rust. **Advances in Genetics**, New York, v. 8, p. 29–54, 1956.

FOUILLOUX, G. Bean anthracnose: new genes of resistance. **Annual Report of the Bean Improvement Cooperative**, Geneva, v. 19, p. 36–37, 1976.

_____. New races of bean anthracnose and consequences on our breeding programs. In: INTERNATIONAL SYMPOSIUM ON DISEASES OF TROPICAL FOOD CROPS, 1979, Louvain-la-Neuve. **Proceedings…** Louvain-la-Neuve: Universite Catholique de Louvain,, 1979. p. 221–235.

GALEANO, C. H. A. C. et al. Single strand conformation polymorphism based SNP and Indel markers for genetic mapping and synteny analysis of common bean (*Phaseolus vulgaris* L.). **BMC Genomics**, London, v. 10, p. 629, Dec. 2009.

GARRIS A. J. et al. Genetic structure and diversity in Oryza sativa L. **Genetics**, Austin, v. 169, n. 3, p. 1631–1638, Mar. 2005.

GARZÓN, L. N.; LIGARRETO, G. A.; BLAIR, M. W. Molecular marker-assisted backcrossing of anthracnose resistance into Andean Climbing beans (*Phaseolus vulgaris* L.). **Crop Science**, Madison, v. 48, n. 2, p. 562–570, Mar. 2008.

GEFFROY, V. **Dissection génétique de la résistance à *Colletotrichum lindemuthianum*, agente de l' anthracnose, chez deux génotypes représentatifs des pools géniques de Phaseolus vulgaris L.** 1997. 263 p. Thesis (PhD) - Institut National Agronomique Paris Grignon, France, 1997.

GEFFROY, V. et al. Identification of an ancestral resistance gene cluster involved in the coevolution process between *Phaseolus vulgaris* and its fungal pathogen *Colletotrichum lindemuthianum*. **Molecular Plant-Microbe Interactions**, Saint Paul, v. 12, n. 9, p. 774–784, Sept. 1999.

_____. Inheritance of partial resistance against *Colletotrichum lindemuthianum* in *Phaseolus vulgaris* and co-localization of quantitative trait loci with genes involved in specific resistance. **Molecular Plant-Microbe Interactions**, Saint Paul, v. 13, n. 3, p. 287–296, Mar. 2000.

_____. et al. Resistance to *Colletotrichum lindemuthianum* in *Phaseolus vulgaris*: a case study for mapping two independent genes. **Theoretical and Applied Genetics**, Berlin, v. 116, n. 3, p. 407–415, Feb. 2008.

GERALDI, I. O. Por que realizar seleção recorrente? In: SIMPÓSIO DE ATUALIZAÇÃO

EM GENÉTICA E MELHORAMENTO DE PLANTAS, 9., 2005, Lavras. **Anais...** Lavras: Ed. UFLA, 2005. p. 97.

_____. Selección recurrente en el mejoramiento de plantas. In: GUIMARÃES, E. P. (Ed.). **Seleccion recurrente en arroz.** Cali: CIAT, 1997. p. 3-11.

GONÇALVES, P. R. C. **Cariotipagem de linhagens de *Colletotrichum lindemuthianum* e *Glomerella* spp. isolados de lesões de antracnose no feijoeiro.** 2016. 96 p. Tese (Doutorado em Genética e Melhoramento de Plantas) - Universidade Federal de Lavras, Lavras, 2016.

GONÇALVES-VIDIGAL, M. C. et al. Allelic relationships of anthracnose (*Colletotrichum lindemuthianum*) resistance in the common bean (*Phaseolus vulgaris* L .) cultivar Michelite and the proposal of a new anthracnose resistance gene , Co-11. **Genetics and Molecular Biology**, São Paulo, v. 30, n. 3, p. 589–593, 2007.

_____. Genetic analysis of anthracnose resistance in 'Pitanga' dry bean cultivar. **Plant Breeding**, Saint Paul, v. 131, n. 3, p. 423–429, June 2012.

_____. Linkage mapping of the Phg-1 and Co-1(4) genes for resistance to angular leaf spot and anthracnose in the common bean cultivar AND 277. **Theoretical and Applied Genetics**, Berlin, v. 122, n. 5, p. 893–903, Mar. 2011.

GONÇALVES-VIDIGAL, M. C. Inheritance of anthracnose resistance in common bean genotypes P.I. 207262 and AB 136. **Brazilian Journal of Genetics**, Ribeirão Preto, v. 20, n. 1, p. 1–4, 2015.

GONÇALVES-VIDIGAL, M. C.; KELLY, J. D. Inheritance of anthracnose resistance in the common bean cultivar Widusa. **Euphytica**, Wageningen, v. 151, n. 3, p. 411–419, Sept. 2006.

GONÇALVES-VIDIGAL, M. C.; LACANALLO, G. F.; VIDIGAL FILHO, P. S. A new gene conferring resistance to anthracnose in Andean common bean (*Phaseolus vulgaris* L.) cultivar 'Jalo Vermelho'. **Plant Breeding**, Saint Paul, v. 127, n. 6, p. 592–596, Dec. 2008.

GONZÁLEZ, A. M. et al. Uncovering the genetic architecture of *Colletotrichum lindemuthianum* resistance through QTL mapping and epistatic interaction analysis in common bean. **Frontiers in Plant Science**, Lausanne, v. 6, p. 141, Mar. 2015.

GONZÁLEZ-CHAVIRA, M. Analysis of pathotypes of *Colletotrichum lindemuthianum* found in the central region of Mexico and resistance in elite germ plasm of *Phaseolus vulgaris*. **Plant Disease**, Saint Paul, v. 88, n. 2, p. 152-156, Feb. 2004.

HABGOOD, R. M. Designation of physiological races of plant pathogens. **Nature**, London, v. 227, n. 5264, p. 1268–1269, Sept. 1970.

HALLAUER, A. R.; CARENA, M. J.; MIRANDA FILHO, J. B. **Quantitative genetics in maize breeding**. New York: Springer, 2010. 663 p.

HEGAY, S. et al. Introducing host-plant resistance to anthracnose in Kyrgyz common bean through inoculation-based and marker-aided selection. **Plant Breeding**, Saint Paul, v. 133, n. 1, p. 86–91, Feb. 2014.

HOYOS-VILLEGAS, V.; SONG, Q.; KELLY, J. D. Genome-wide Association Analysis for Drought Tolerance and Associated Traits in Common Bean. **The Plant Genome**, Madison, v. 10, n. 1, p. 1–17, Mar. 2017.

ISHIKAWA, F. H. et al. *Colletotrichum lindemuthianum* exhibits different patterns of nuclear division at different stages in its vegetative life cycle. **Mycologia**, New York, v. 105, n. 4, p. 795-801, July/Aug. 2013.

_____. Investigating variability whitin race 81 of *Colletotrichum lindemuthianum* strains from Brazil. **Annual Report of the Bean Improvement Cooperative**, Geneva, v. 55, p. 141–142, 2012.

_____. Levantamento de raças de *Colletotrichum lindemuthianum* de regiões produtoras de feijão. In: CONGRESSO NACIONAL DE PESQUISA DE FEIJÃO, 8., 2005, Goiânia. **Anais...** Santo Antônio de Goiás: Embrapa Arroz e Feijão, 2005. v. 2, p. 501–504.

ISHIKAWA, F. H.; RAMALHO, M. A. P.; SOUZA, E. A. Common bean lines as potential diffetential cultivars for race 65 of *Colletotrichum lindemuthianum*. **Journal of Plant Pathology**, Oxford, v. 93, n. 2, p. 461–464, 2011.

KAMFWA, K.; CICHY, K. A.; KELLY, J. D. Genome-wide association study of agronomic traits in common bean. **The Plant Genome**, Madison, v. 8, n. 2, p. 1–12, July 2015.

KELLY, J. D.; VALLEJO, V. A. A comprehensive review of the major genes conditioning resistance to anthracnose in common bean. **HortScience**, Alexandria, v. 39, n. 6, p. 1196–1207, 2004.

KELLY, J. D.; YOUNG, R. A. Proposed symbols for anthracnose resistance genes. **Annual Report of the Bean Improvement Cooperative**, Geneva, v. 39, p. 20–24, 1996.

KIMATI, H. et al. **Manual de fitopatologia**: doenças das plantas. 3. ed. São Paulo: Agronômica Ceres, 1997. v. 2, 705 p.

KIMATI, H.; GALLI, F. *Glomerella* cingulata f. sp. phaseoli, fase ascógena do agente causal da anthracnose do feijoeiro. **Escola Superior de Agricultura Luiz de Queiróz**, São Paulo, v. 27, p. 411–437, 1970.

LACANALLO, G. F.; GONÇALVES-VIDIGAL, M. C. Mapping of an andean gene for anthracnose resistance (Co-13) in common bean (*Phaseolus vulgaris* L .) Jalo Listras Pretas landrace. **Australian Jornal of Crop Science**, Lismore, v. 9, n. 5, p. 394–400, Jan. 2015.

LEITE, M. E. et al. Increasing the resistance of common bean to *White mold* through recurrent selection. **Scientia Agricola**, Piracicaba, v. 73, n. 1, p. 71–78, jan./fev. 2016.

LI, X. et al. Combined linkage and association mapping reveals QTL and candidate genes for plant and ear height in maize. **Frontiers in Plant Science**, Lausanne, v. 7, p. 1–11, June 2016.

LIBRELON, S. S. **Seleção recorrente visando resistência à mancha angular no feijoeiro com inoculação artificial precoce.** 2016. 70 p. Tese (Doutorado em Genética e Melhoramento de Plantas) - Universidade Federal de Lavras, Lavras, 2016.

LIMA, P. R. A. et al. Eficiência de fungicidas no controle da antracnose e da mancha angular do feijoeiro comum. **Revista Agrociências**, Pato de Minas, v. 1, n. 1, p. 54–59, 2010.

MAHUKU, G. S.; RIASCOS, J. J. Virulence and molecular diversity within *Colletotrichum lindemuthianum* isolates from Andean and Mesoamerican bean varieties and regions. **European Journal of Plant Pathology**, Dordrecht, v. 110, n. 3, p. 253–263, Mar. 2004.

MARCONDES, E. H. K.; PEREIRA, H. S.; SANTOS, J. B. Seleção de linhagens de feijoeiro com tipo de grão carioca e com os alelos Co-4 e Co-5 de resistência à antracnose. **Ciência e Agrotecnologia**, Lavras, v. 34, n. 4, p. 975–982, jul./ago. 2010.

MASTENBROEK, C. A breeding programme for resistance to anthracnose in dry shell haricot beans, based on a new gene. **Euphytica**, Wageningen, v. 9, n. 2, p. 177–185, July 1960.

McDONALD, B. A.; LINDE, C. The population genetics of plant pathogens and breeding strategies for durable resistance. **Euphytica**, Wageningen, v. 124, n. 2, p. 163–180, Mar. 2002.

McDONALD, B. A. Using dynamic diversity to achieve durable disease resistance in agricultural ecosystems. **Tropical Plant Pathology**, Brasília, v. 39, n. 3, p.191-196, maio/jun. 2014.

MCROSTIE, G. P. Inheritance of anthracnose resistance as indicated by a cross between a resistant and a susceptible bean. **Phytopathology,** Saint Paul, v. 9, p. 141–148, 1919.

MELOTTO, M.; KELLY, J. D. An allelic series at the Co-1 locus conditioning resistance to anthracnose in common bean of Andean origin. **Euphytica**, Wageningen, v. 116, n. 2, p. 143–149, Nov. 2000.

MEZIADI, C. et al. Development of molecular markers linked to disease resistance genes in common bean based on whole genome sequence. **Plant Science**, Shannon, v. 242, p. 351–357, Jan. 2016.

MIKLAS, P. N. et al. Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. **Euphytica**, Wageningen, v. 147, n. 1/2, p. 105–131, Jan. 2006.

MODA-CIRINO, V.; BURATTO, J. S.; FONSECA JUNIOR, N. S. Recurrent selection to development of carioca type common beans cultivars at IAPAR. **Annual Report of the Bean Improvement Cooperative**, Geneva, v. 1, n. 59, p. 73-74, Apr. 2016.

MOGHADDAM, S. M. et al. Genome-wide association study identifies candidate loci underlying agronomic traits in a middle american diversity panel of common bean. **The Plant Genome**, Madison, v. 9, n. 3, p. 1–21, Nov. 2016.

MORAIS, O. P. M. et al. Performance da população CG1 em seu terceiro ciclo de seleção. In: CONGRESSO BRASILEIRO DE MELHORAMENTO DE PLANTAS, 2., 2003, Porto Seguro. **Anais...** Porto Seguro: SBMP, 2003. p. 1-5.

MOREIRA, S. O. et al. Breeding methods and history of bean cultivars released in CBAB - Crop Breeding and Applied Biotechnology. **Crop Breeding and Applied Biotechnology**, Viçosa, v. 10, n. 4, p. 345-350, dez. 2010.

MOTA, S. F. et al. Variability of Colletotrichum spp in common bean. **Genetics and Molecular Research**, Ribeirao Preto, v. 15, n. 2, p. 22, Apr. 2016.

MUNDT, C. C. Durable resistance: a key to sustainable management of pathogens and pests. **Infection, Genetics and Evolution**, Amsterdam, v. 27, p. 446–455, Oct. 2014.

MURUBE, E.; CAMPA, A.; FERREIRA, J. J. Integrating genetic and physical positions of the anthracnose resistance genes described in bean chromosomes Pv01 and Pv04. **PLoS One**, San Francisco, v. 14, n. 2, p. 1–17, Feb. 2019.

NELSON, R. et al. Navigating complexity to breed disease-resistant crops. **Nature Reviews Genetics**, London, v. 19, n. 1, p. 21–33, Jan. 2018.

OBLESSUC, P. R. et al. Quantitative analysis of race-specific resistance to *Colletotrichum lindemuthianum* in common bean. **Molecular Breeding**, Dordrecht, v. 34, n. 3, p. 1313–1329, Oct. 2014.

OBLESSUC, P. R.; FRANCISCO, C.; MELOTTO, M. The Co-4 locus on chromosome Pv08 contains a unique cluster of 18 COK-4 genes and is regulated by immune response in common bean. **Theoretical and Applied Genetics**, Berlin, v. 128, n. 6, p. 1193–1208, June 2015.

O'CONNELL, R. J.; BAILEY, J. A.; RICHMOND, D. V. Cytology and physiology of infection of *Phaseolus vulgaris* by *Colletotrichum lindemuthianum*. **Physiological Plant Pathology**, London, v. 27, n. 1, p. 75–98, July 1985.

OLIVEIRA, B. G. et al. Caracterizaçao de raças de *Colletotrichum lindemuthianum* no estado do Paraná. **Iniciação Científica CESUMAR**, Maringá, v. 16, n. 2, p. 205–211, jul./dez. 2014.

O'SULLIVAN, D. et al. Variation in genome organization of the plant pathogenic fungus *Colletotrichum lindemuthianum*. **Current Genetics**, New York, v. 33, n. 4, p. 291–298, Apr. 1998.

PADDER, B. A. et al. *Colletotrichum lindemuthianum*, the causal agent of bean anthracnose. **Journal of Plant Pathology**, Oxford, v. 99, n. 2, p. 317–330, Jan. 2017.

PÁDUA, P. F. **Viabilidade da condução de três ciclos de seleção recorrente visando à resistência a *Pseudocercospora griseola* no feijoeiro.** 2017. 70 p. Tese (Mestrado em Genética e Melhoramento de Plantas) - Universidade Federal de Lavras, Lavras, 2017.

PASTOR-CORRALES, M. A. Estandarizacion de variedades diferenciales y de designacion de razas de *Colletotrichum lindemuthianum*. **Phytopathology,** Saint Paul, v. 81, p. 694, 1991.

PAULA JÚNIOR, T. J.; WENDLAND, A. **Melhoramento genético do feijoeiro-comum e prevenção de doenças**. Viçosa: EPAMIG, 2012. 157 p.

PAULA JÚNIOR, T. J.; ZAMBOLIM, L. Doenças. In: VIEIRA, C.; PAULA JÚNIOR, T. J.; BORÉM, A. (Org.). **Feijão**. 2. ed. Viçosa: Ed. UFV, 2006. p. 359–414.

PEREIRA, H. S.; SANTOS, J. B. Genetic constitution of anthracnose-resistance in common bean lines. **Crop Breeding and Applied Biotechnology**, Londrina, v. 4, n. 4, p. 422–426, 2004.

PEREIRA, R. **Diversidade genética e reação de linhagens de feijoeiro inoculados com mistura de raças de *Pseudocercospora griseola* em diferentes estádios e ambientes**. 2017. 94 p. Tese (Doutorado em Genética em Melhoramento de Plantas) - Universidade Federal de Lavras, Lavras, 2017.

PEREIRA, R. et al. Aggressiveness of *Pseudocercospora griseola* strains in common bean genotypes and implications for genetic improvement. **Genetics and Molecular Research**, Ribeirao Preto, v. 14, n. 2, p. 5044–5053, jul. 2015.

_____. Occurence of anthracnose in common bean cultivars collected in the state of Minas Gerais, Brazil. **Annual Report of the Bean Improvement Cooperative**, Geneva, v. 53, p. 224–225, 2010.

PERFECT, S. E. et al. *Colletotrichum*: a model genus for studies on pathology and fungal-plant interactions. **Fungal Genetics and Biology**, Orlando, v. 27, n. 2/3, p. 186–198, July/Aug. 1999.

PERFECT, S. E.; GREEN, J. R.; O'CONNELL, R. J. Surface characteristics of necrotrophic secondary hyphae produced by the bean anthracnose fungus, *Colletotrichum lindemuthianum*. **European Journal of Plant Pathology**, Dordrecht, v. 107, n. 8, p. 813–819, Oct. 2001.

PERSEGUINI, J. M. K. C. et al. Genome-wide association studies of anthracnose and angular leaf spot resistance in common bean (*Phaseolus vulgaris* L.). **PLoS One**, San Francisco, v. 11, n. 3, p. 1–19, 2016.

PINTO, A. C. S. et al. Análise do padrão espacial e do gradiente da antracnose do feijoeiro em duas épocas de cultivo. **Summa Phytopathologica**, Botucatu, v. 27, p. 392–398, 2001.

PINTO, J. M. A. et al. Investigating phenotypic variability in *Colletotrichum lindemuthianum* populations. **Phytopathology,** Saint Paul, v. 102, n. 5, p. 490–497, May 2012.

PIRES, L. P. M. et al. Recurrent mass selection for upright plant architecture in common bean. **Scientia Agricola**, Piracicaba, v. 71, n. 3, p. 240-243, maio/jun. 2014.

POLAND, A. J. et al. c. **Trends in Plant Science**, Kidlington, v. 14, n. 1, p. 21-29, Jan. 2008.

RAMALHO, M. A. P. **Aplicações da genética quantitativa no melhoramento de plantas autógamas**. Lavras: Ed. UFLA, 2012. 522 p.

RAMALHO, M. A. P. et al. Progresso genético do quinto ao sétimo ciclo de seleção recorrente no melhoramento genético do feijoeiro. In: CONGRESSO NACIONAL DE FEIJÃO, 8., Goiânia. **Anais...** Santo Antônio de Goiânia: Embrapa Arroz e Feijão, 2005.

REIS, E. M.; CASA, R. T.; BIANCHIN, V. Controle de doenças de plantas pela rotação de culturas. **Summa Phytopathologica**, Botucatu, v. 37, n. 3, p. 85–91, jul./set. 2011.

REZENDE, B. A. et al. Severity evaluation methods in common bean recurrent selection programme for resistance to angular leaf spot. **Journal of Phytopathology**, Berlin, v. 162, n. 10, p. 643–649, Oct. 2014.

RIBEIRO, T. et al. Classification of *Colletotrichum lindemuthianum* races in differential cultivars of common bean. **Acta Scientiarum. Agronomy**, Maringá, v. 38, n. 2, p. 179–184, abr./jun. 2016.

RICHARD, M. M. S. et al. Fine mapping of Co-x, an anthracnose resistance gene to a highly virulent strain of *Colletotrichum lindemuthianum* in common bean. **Theoretical and Applied Genetics**, Berlin, v. 127, n. 7, p. 1653–1666, July 2014.

ROBINSON, R. A. Vertical resistance. **Review of Plant Pathology**, Farnham Royal, v. 50, n. 5, p. 233–239, 1971.

RODRÍGUEZ-SUÁREZ, C. et al. Anthracnose resistance spectra of breeding lines derived from the dry bean landrace Andecha. **Annual Report of the Bean Improvement Cooperative**, Geneva, v. 48, p. 48–49, 2005.

RON, A. M. et al. History of the common bean crop : its evolution beyond its areas of origin and domestication. **Arbor:** ciencia, pensamiento y cultura, Madrid, v. 192, n. 779, p. 1–11, 2016.

RUAS, J. F. Feijão. **Perspectivas para Agropecuária**, Brasília, v. 5, p. 45–55, 2017.

SALA, G. M.; ITO, M. F.; CARBONELL, S. A. M. Reação de genotipos de feijoeiro comum a quatro raças de Fusarium oxysporum f. sp. phaseoli. **Summa Phytopathologica**, Botucatu, v. 32, n. 3, p. 286–287, jul./set. 2006.

SANTOS, J. et al. Virulência das raças 65, 73 e 81 de *colletotrichum lindemuthianum* (sacc. & magn.) scrib. e determinação de fontes de resistência em *Phaseolus vulgaris* L. **Revista Brasileira de Agrociência**, Pelotas, v. 14, n. 3, p. 115–124, jul./set. 2008.

SARTORATO, A. sensibilidade “in vitro” de isolados de Colletotrichum lindemuthianum A FUNGICIDAS. **Pesquisa Agropecuária Tropical**, Goiânia, v. 36, n. 3, p. 211–213, set./dez. 2006.

SCHMUTZ, J. et al. A reference genome for common bean and genome-wide analysis of dual domestications. **Nature Genetics**, New York, v. 46, n. 7, p. 707–713, July 2014.

SEMAGN, K.; BJØRNSTAD, Å.; NDJIONDJOP, M. N. Principles, requirements and prospects of genetic mapping in plants. **African Journal of Biotechnology**, Nairobi, v. 525, n. 25, p. 2569–2587, Jan. 2007.

SHARMA, P. N. et al. Pathological and molecular diversity in *Colletotrichum lindemuthianum* (bean anthracnose) across Himachal Pradesh, a north-western Himalayan state of India. **Australasian Plant Pathology**, Clayton, v. 36, n. 2, p. 191-197, Mar. 2007.

SHEAR, C. L.; WOOD, A. K. **Studies of fungal parasites belonging to the genus *Glomerella*.** Washington: Government Printing, 1913. 110 p.

SHULTZ, J. L. et al. The Soybean Genome Database (SoyGD): a browser for display of duplicated, polyploid, regions and sequence tagged sites on the integrated physical and genetic maps of Glycine max. **Nucleic Acids Research**, London, v. 34, p. 758-765, Jan. 2006.

SILVA, K. J. D.; SOUZA, E. A.; ISHIKAWA, F. H. Characterization of *Colletotrichum lindemuthianum* Isolates from the State of Minas Gerais, Brazil. **Journal of Phytopathology**, Berlin, v. 155, n. 4, p. 241–247, Apr. 2007.

SINGH, S. P.; SCHWARTZ, H. F. Breeding common bean for resistance to diseases: a review. **Crop Science**, Madison, v. 50, n. 6, p. 2199–2223, Nov. 2010.

SONG, Q. et al. SNP assay development for linkage map construction, anchoring whole-genome sequence, and other genetic and genomic applications in common bean. **G3**, Bethesda, v. 5, n. 11, p. 2285–2290, Aug. 2015.

SOUSA, L. L. et al. Genetic characterization and mapping of anthracnose resistance of common bean landrace cultivar corinthiano. **Crop Science**, Madison, v. 55, n. 5, p. 1900-1910, Sept. 2015.

SOUZA, R. S.; WANDER, A. E. Aspectos econômicos da produção de feijão no Brasil. **Revista de Política Agrícola**, Brasília, v. 23, n. 3, p. 43–54, jul./set. 2014.

TOCK, A. J. et al. Genome-wide linkage and association mapping of halo blight resistance in common bean to Race 6 of the globally important bacterial pathogen. **Frontiers in Plant Science**, Lausanne, v. 8, p. 1–17, July 2017.

VALENTINI, G. et al. High-resolution mapping reveals linkage between genes in common bean cultivar Ouro Negro conferring resistance to the rust, anthracnose, and angular leaf spot diseases. **Theoretical and Applied Genetics**, Berlin, v. 130, n. 8, p. 1705–1722, Aug. 2017.

VALLEJO, V.; KELLY, J. D. New insights into the anthracnose resistance of common bean landrace G 2333. **The Open Horticulture Journal**, Kyoto, v. 2, n. 1, p. 29–33, Apr. 2009.

VANDERPLANK, J. E. **Plant diseases**: epidemics and control. New York: Academic Press, 1963. 349 p.

VLASOVA, A. et al. Genome and transcriptome analysis of the Mesoamerican common bean and the role of gene duplications in establishing tissue and temporal specialization of genes. **Genome Biology**, London, v. 17, n. 32, p. 1–18, Feb. 2016.

YOUNG, R. A. et al. Marker-assisted dissection of the oligogenic anthracnose resistance in the common bean cultivar, "G 2333". **Theoretical and Applied Genetics**, Berlin, v. 96, n. 1, p. 87–94, Jan. 1998.

ZHANG, Z. et al. Mixed linear model approach adapted for genome-wide association studies. **Nature Genetics**, New York, v. 42, n. 4, p. 355–360, 2010.

ZHU, C. et al. Status and prospects of association mapping in plants. **The Plant Genome**, Madison, v. 1, n. 5, p. 5–20, July 2008.

ZUIDERVEEN, G. H. et al. Genome-wide association study of anthracnose resistance in andean beans (*Phaseolus vulgaris*). **PloS One**, San Francisco, v. 11, n. 6, p. 1–17, Jan. 2016.

SEGUNDA PARTE – ARTIGOS

ARTIGO 1 - GENETIC RESISTANCE TO DIFFERENT STRAINS OF RACE 65 OF *Colletotrichum lindemuthianum* IS CONFERRED BY DIFFERENT LOCI IN COMMON BEAN GENOME

Normas da Revista Theoretical and Applied Genetic.

(VERSÃO PRELIMINAR)

ABSTRACT

It is challenging to obtain common bean cultivars with durable resistance to *Colletotrichum lindemuthianum* due to the high pathogenic variability of this pathogen. There is variability even within strains classified as the same race, using the current standard set of anthracnose common bean differentials. Combining linkage and association analyses, using the BARCBEAN6K_3 Illumina SNP chip, we aimed at identifying genomic regions associated with resistance to different strains of race 65, an important and widespread race of *C. lindemuthianum* in Brazil. The linkage analysis using a large F_2 population, derived from the Estilo x Ouro Vermelho cross, revealed two major genes on Pv04 associated with resistant to two different strains of race 65. Association mapping using a diverse panel of 189 common common bean accessions from the Germplasm Bank of Federal University of Lavras, Brazil and five different strains of race 65 also revealed major QTL in different positions of Pv04 and also on Pv01, Pv05, Pv10 and Pv11. Besides that, the previously reported race 65 resistant common bean genotypes, Jalo vermelho (*Co-12*), Jalo listras pretas (*Co-13*), Pitanga (*Co-14*) and Corinthiano (*Co-15*), were evaluated and they were not resistant to all strains of race 65 used in the present study. Our results revealed that genomic regions controlling resistance to *C. lindemuthianum*, race 65 in common bean varied according to the strain used for inoculation. Therefore, sources of resistance to race 65 should be thoroughly evaluated by breeding programs in order to deploy a complementary combination of resistance genes to obtain durable resistance.

Keywords: *Colletotrichum lindemuthianum.* Race 65. Mapping of resistance genes. *Phaseolus vulgaris.* Plant breeding. Association mapping.

Key message: Linkage and association analyses using high-throughput SNPs genotyping revealed that there are different loci in common bean genome conferring resistance to specific strains of race 65 of *Colletotrichum lindemuthianum.*

Introduction

Common bean (*Phaseolus vulgaris L.*) is one of the most important grain legumes for direct consumption in many parts of the world (Broughton et al. 2003; De Ron et al. 2013). Particularly in developing countries, its grains are the sources of proteins and minerals with greater social accessibility (Broughton et al. 2003; De Ron et al. 2013). Unfortunately, the incidence of numerous pests and pathogens can often greatly reduce the yield of the crop (De Ron et al. 2013; Kelly and Bornowski 2018). Anthracnose, caused by the seed-borne hemibiotrophic fungus *Colletotrichum lindemuthianum*, stand out as one of the most economically important disease of common bean (Padder et al. 2017). Although sources of genetic resistance have been identified, being the most cost-effective strategy to control the disease, this disease has remained as one of the major factors that limits common bean production worldwide, especially in tropical areas of Latin America and Eastern Africa, where climatic conditions favor disease development (Pastor-Corrales 1988; Schwartz and Pastor-Corrales 2005; Ferreira et al. 2013). The main reason for breaking resistance or lack of durable genetic resistance is the extensive pathogenic variability displayed by *C. lindemuthianum.* To date, the study of host-pathogen interaction allowed the identification of over 180 different pathogenic races globally using a host differential set (Kelly and Vallejo 2004; Ferreira et al. 2013; Padder et al. 2017).

Understanding how genes influence the phenotypic variation is one of the major goals in evolutionary biology. Due to the high specific interaction between *P. vulgaris* and *C. lindemuthianum,* this pathosystem has been considered as a reference model for studying race-specific resistance in plants (Campa et al. 2017). Several anthracnose resistance genes, whose dominant allele promote resistance to different races of *C. lindemuthianum* have already been identified in many common bean varieties. Those alleles are identified by *Co-*symbol and, at present, more than 20 *Co-* alleles have been described (Ferreira et al. 2013; Castro et al. 2017). Molecular markers linked to those alleles have also been identified, enabling the use of marker-assisted selection (MAS) (Meziadi et al. 2016; Kelly and Bornowski 2018). Many of those alleles have been mapped in various linkage groups of common bean and they are often organized in complex clusters comprised of many tightly linked genes, especially at the end of the chromosomes (David et al., 2009; Ferreira et al. 2013; Meziadi et al. 2016; Murube et al., 2019).

Recently, the development of inexpensive next-generation sequencing technologies has led to an unprecedented era of genomic studies (Poland 2015). Moreover, the common

bean genome sequence (Schmutz et al. 2014; Vlasova et al. 2016) and the high-throughput genotyping technologies (Song et al. 2015) offer the opportunity for the development of increasingly saturated linkage maps and consequently, development of markers tightly linked to resistant alleles. A number of *Co*-alleles, such as, *Co*-x, *Co*-4 and *Co*-3^{4}, *Co*-3^{38B}, have been fine-mapped (Richard et al. 2014; Burt et al. 2015; Valentini et al. 2017; Murube et al., 2019). In addition, quantitative trait loci (QTL), conferring partial resistance to different races of *C. lindemuthianum* have also been identified (Oblessuc et al. 2014; González et al. 2015; Choudhary et al. 2018).

The most studies about mapping of anthracnose resistance genes has been accomplished through linkage analysis, using biparental populations (Ferreira et al. 2013). However, by this mapping approach, only a limited number of recombination events can be explored, especially if the population used are in early stages of development. Another approach that have been used to identify genomic regions associated with interest traits is the Genome Wide Association Study (GWAS). In this case, a diverse set of individuals from a germplasm bank, for example, can be used and the recombination events that have occurred over several generations can be explored (Zhu et al. 2008). Recently, QTLs for resistance to different anthracnose races have been identified using this mapping approach (Perseguini et al. 2016; Zuiderveen et al. 2016). Compared with traditional QTL mapping using biparental population, GWAS can increase mapping resolution, reduce research time (do not have to synthesize a population for mapping) and offer broader genetic variation. However, in biparental populations all the existing linkage disequilibrium (LD), between a marker and a QTL, comes from the physical linkage, while different factors, such as, selection, genetic drift, relatedness or population substructure can also contribute to the LD in a GWAS analysis, which may cause spurious associations.

C. lindemuthianum, race 65 is a cosmopolitan Mesoamerican race widespread in North, Central and South America. In Brazil, this race has been frequently found in all states where common bean is grown. Therefore, the race 65 has been a major target in common bean breeding programs that aim at incorporating anthracnose resistance (Silva et al. 2007; Gonçalves-Vidigal et al. 2008b; Pinto et al. 2012; Ribeiro et al. 2016). However, the achieving of durable anthracnose resistance is even more difficult due to the high genetic and pathogenic variability that have been recorded within strains of this race (Rodríguez-Suárez et al. 2005; Ishikawa et al. 2008, 2011; Davide and Souza 2009).

Ishikawa et al. (2011) proposed a complementary set of differentials, using Brazilian commercial common bean lines, in order to do a better discrimination of different strains of *C.*

lindemuthianum, race 65. More recently, this set of differentials was used in a study about genetic control of resistance to different strains of *C. lindemuthianum*. 15R:1S segregations observed in the F_2 generation of several crosses of this study indicated that duplicate genes could be conferring specific resistance to each of the strains of race 65 used (Costa et al. 2017). That conclusion suggested that the specificity of the interaction between host resistance genes and pathogen avirulence genes may be greater than originally thought. From the perspective of a common bean breeding program, this is highly relevant because some cultivars can be resistant to some strains but susceptible to others of the same race. In addition, the resistance genes already identified in some common bean lines against some specific races (Gonçalves-Vidigal et al. 2008a, 2012; Lacanallo and Gonçalves-vidigal 2015; Sousa et al. 2015), might not confer resistance to all strains of those races. Consequently, the molecular markers linked to those genes cannot be useful in all regions where common bean is grown.

In this context, the present study combined linkage and GWAS analyses, using high-throughput genotyping technologies in order to identify genomic regions associated with resistance to different strains of *C. lindemuthianum*, race 65. The use of these two strategies allows the validation, in different populations, of associations between the markers and QTLs, increasing reliability of the results obtained. This work is the first report about mapping of resistance genes in *P. vulgaris* using different strains of a same *C. lindemuthianum* race. Additionally, common bean lines with already described anthracnose resistance alleles were evaluated with different strains of *C. lindemuthianum*, race 65 in order to verify if they present variability for their reaction to these different strains. That information will provide insights about *C. lindemuthianum – P. vulgaris* interaction and it will contribute to the development of more effective strategies to obtain common bean cultivars with more durable anthracnose resistance.

Materials and Methods

Development of bi-parental population and phenotypic evaluation of anthracnose

A population of 400 F_2 plants derived from Estilo x Ouro Vermelho cross was developed at Federal University of Lavras (Universidade Federal de Lavras - UFLA), Minas Gerais, Brazil. Both cultivars are market available Brazilian common bean lines. Estilo is a carioca grain type and Ouro Vermelho is a red grain type. The intermediate color observed in F_2 seeds coat confirmed that they are hybrids (Figure 1). The rationale to choose these bean lines was their inconsistent reaction when inoculated with two different strains (Lv134 and Lv238) of *C. lindemuthianum* which were previously classified as race 65. Estilo is resistant to Lv134 and susceptible to Lv238 whereas Ouro Vermelho is susceptible to Lv134 and resistant to Lv238.

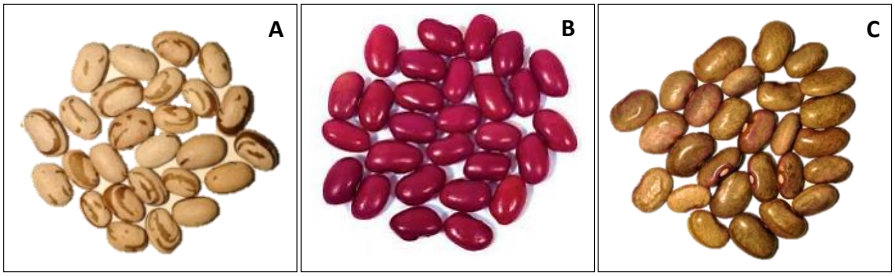

Fig.1: Common bean seeds from Estilo (A) and Ouro Vermelho (B) Cultivars and from F_2 generation derived from Estilo x Ouro Vermelho cross (C).

All F_2 plants and parents were sown in polystyrene trays containing Plantmax® substrate. The conidial suspensions of each strain were prepared as described in Costa et al (2017). When the plants are very susceptible to a specific strain, few days after inoculation, they often present very severe symptoms or die. For this reason and also to avoid contamination and unequivocally ensure the phenotype of each strain, among the 400 F_2 plants, 200 were separately inoculated with Lv134 strain and the remaining 200 were inoculated with Lv238 strain of *C. lindemuthianum*. Six plants of each parental common bean line were also inoculated separately with each strain. Inoculations were done by spraying the inoculum at a concentration of 1.2×10^6 conidia ml^{-1} onto the primary leaves and stems of all bean plants. Plants were then kept under high humidity (around 90%) for 72 hours in a mist chamber. Plants were later transferred and kept under greenhouse conditions. Symptoms were observed on susceptible plants 7-10 days after inoculation and the disease severity was evaluated according to the descriptive score scale from 1 to 9 (Schoonhoven and Pastor-

Corrales 1987). Plants with no symptoms or small lesions (1 to 3) were classified as resistant and plants with larger lesions or dead (4 to 9) were classified as susceptible.

Genotyping analysis using the BARCBEAN6K_3 SNP Chip

Newly emerged trifoliate leaves from each of the 400 F_2 plants and three samples from each parent were collected and total genomic DNA was extracted using a modified CTAB (Hexadecyltrimethyl ammonium bromide) extraction protocol (Doyle and Doyle 1987). DNA concentration was measured using Nanodrop spectrophotometer and quality was checked on 1.5% agarose gel. DNA samples from each of the F_2 plants and parents were then screened with 5398 Single nucleotides polymorphism (SNPs) (Song et al. 2015) on the BARCBEAN6K_3 Illumina BeadChip by following the Infinium HD Assay Ultra Protocol (Illumina, Inc. San Diego, CA, USA) at the Soybean Genomics and Improvement Laboratory of the USDA-ARS Beltsville Agricultural Research Center, Beltsville, MD, USA. The Illumina BeadArray Reader was used to measure the intensity fluorescence signal. Automatic allele calling was performed using Illumina GenomeStudio v. 1.9.4 and allele call data was visually inspected for each locus. Polymorphic SNPs between parents were selected to construct the linkage map.

Construction of genetic linkage map and mapping of regions associated with resistance to Lv134 and Lv238 strains of *C. lindemuthianum,* race 65

Polymorphic SNPs between the parents were selected to construct the genetic linkage map of *P. vulgaris*. SNP markers and individuals with more than 5% and 20% of missing data respectively, were removed. SNP markers with segregation distortion from expected Mendelian segregation pattern (1:2:1) (p-value < 0.05), calculated using a Chi-square test and corrected for Bonferroni's multiple tests, were also eliminated. After applying the filters mentioned above, the genetic linkage map for the 11 chromosomes of common bean was determined using OneMap software (Margarido et al. 2007). A multipoint recombination analysis was used to define the recombination fraction among markers and Kosambi map function was used to set up the genetic map distance (cM). For mapping SNPs located in unmapped scaffolds, two-point analysis was used to identify the most probable chromosomes in which those markers should be located. Then, these markers were placed in the regions of greatest likelihood and multipoint distances were recalculated. Linkage map was graphically

obtained using MapChart 2.3 (Voorrips 2002). Mapping of regions associated with the genetic resistance to the specific strains of races 65 for the anthracnose pathogen (Lv134 and Lv238), was done by multiple interval mapping method using qtl package, version 1.42-8 (Broman et al. 2003) implemented in R software (RCore Team 2018). Significant LOD thresholds were determined based on permutation test with 1000 permutations.

Population used for Genome-Wide Association study of anthracnose resistance

In order to validate the results of linkage analysis, a set of 189 common bean lines from the work collection of UFLA that was screened with the same strains of *C. lindemuthianum*, race 65, Lv134 and Lv238, that were used to evaluate the biparental population from Estilo x Ouro Vermelho. Besides that, these common bean lines were assessed for the reaction to three other strains of *C. lindemuthianum*, race 65, Cl1532, Cl1614 e Cl1740. The large majority of these accessions are carioca grain type and also include some commercial cultivars from other breeding institutions, some cultivars from the international set of anthracnose common bean differentials and four common bean lines, Pitanga, Corinthiano, Jalo vermelho and Jalo listras pretas, which present already identified resistance genes that confer resistance to race 65 of *C. lindemuthianum* (Gonçalves-Vidigal et al. 2008a, 2012; Lacanallo and Gonçalves-vidigal 2015; Sousa et al. 2015) in order to know if the reported resistance alleles confer resistance to different strains of this race.

Seeds of these 189 common bean lines were sown in polystyrene trays containing Plantmax® substrate. The experiment was carried out in a greenhouse in a randomized complete blocks design with 3 replicates, being 4 seedlings per plot. The inoculum preparation, inoculation, evaluation and genotyping with BARCBEAN6K_3 BeadChip followed the same procedures described above for the F_2 population assessment.

GWAS analysis

After filtering for low quality and monomorphic SNPs as well as for minor allele frequency (MAF > 0.05), 3095 SNPs were retained for association analysis. As population structure and relatedness can result in spurious associations, population structure analysis was conducted using STRUCTURE (Pritchard et al. 2000). A Q matrix was developed to describe the percent subpopulation for each common bean line in analysis. The assumed number of

subpopulations was simulated from k = 1 to k = 10 for an initial assessment of the most likely number of subpopulations with 10,000 iterations of burnin followed by 100,000 Markov chain Monte Carlo (MCMC) iterations. The ideal number of subpopulations was found by examining the optimal Delta K value (Evanno et al. 2005) in STRUCTURE Harvester (Earl and vonHoldt 2012) and the population structure was graphically represented using the online application CLUMPAK (Kopelman et al. 2015) available at http://clumpak.tau.ac.il/. Relatedness was determined by calculating the kinship matrix using the NTSYS software v.2.2 (Rohlf 2009). Then, a Mixed Linear Model (MLM) (Yu et al. 2006) was run in TASSEL software (Bradbury et al. 2007) to identify SNP-trait-association. The following MLM equation was used: $Y = X\alpha + P\beta + K\mu + \varepsilon$, where: Y is the phenotype of an individual; X is the incidence matrix that relates α and Y; α is the fixed effect of SNP; P is the matrix of population structure which relates β and Y; β is the fixed effect of population structure; K is an identity matrix relating μ and Y; μ is the background random effect related to the genotypes and their relative kinship; ε is the error term and is assumed to be normally distributed with a mean of zero.

Conservative Bonferroni corrected $p = 3.23 \times 10^{-6}$ (for $\alpha = 0.01$ and 3095 SNPs) was used to determine the significance threshold for SNPs and the Manhattan plots were constructed using the qqman package (Turner 2014) implemented in R software (RCore Team 2018).

The linkage disequilibrium (LD) was estimated as the square of the correlation coefficient between two loci (r^2) and were calculated for all pairs of SNPs within a 10kb genomic window using TASSEL software.

Identification of candidate genes

To identify putative candidate genes associated with significant SNPs derived from linkage analysis or GWAS, physical position of the SNPs were visualized through the genome browser hosted in the NCBI website for the version 1 of the common bean genome sequence (https://www.ncbi.nlm.nih.gov/genome/genomes/380). A genomic region was delimited considering the physical position of significant SNPs. Thereafter the functional annotation for those genes was recorded in order to infer their possible role in conferring anthracnose resistance.

Results

Inheritance of anthracnose resistance in common bean Estilo and Ouro vermelho

From 400 F_2 plants of the Estilo x Ouro Vermelho cross, half of them were evaluated for their reaction to Lv134 strain and another half were evaluated for the reaction to Lv238 strain of race 65 of *C. lindemuthianum*. As expected, Estilo was resistant to Lv134 strain (scores 2, 2, 2) and susceptible to Lv238 strain (scores 8, 7, 6), whereas Ouro Vermelho was susceptible to Lv134 strain (scores 7, 7, 6) and resistant to Lv238 strain (scores 1, 2, 1). According to the reaction of 200 F_2 plants inoculated with Lv134 strain, the inheritance of anthracnose resistance study exhibited segregation that fitted a ratio of 3 resistants to 1 susceptible according with chi-square test ($\chi 2$) (p-value > 0.05), confirming that anthracnose resistance to Lv134 strain in Estilo is conferred by one single gene, whose dominant allele confers resistance. Regarding the reaction of 200 F_2 plants inoculated with Lv238 strain, the segregation also showed a good fit of 3 resistants to 1 susceptible (p-value > 0.05), that means that anthracnose resistance to strain Lv238 in Ouro Vermelho is also conferred by one single gene, whose dominant allele confers resistance (Table 1).

Table 1 Reaction of parents, F_1 and F_2 generation of Estilo x Ouro Vermelho cross to strains Lv134 and Lv238 of race 65 of *Colletotrichum lindemuthianum*

Parents and cross	Strain	Observed frequency		Expected frequency		□²	P-value
		R[1]	S[2]	R	S		
Estilo	LV134	3	-				
OV[3]		-	3				
Estilo x OV (F_1)		6	-				
Estilo x OV (F_2)		145	55	150	50	0.027	0.87
Estilo	LV238	-	3				
OV		3	-				
Estilo x OV (F_1)		6					
Estilo x OV (F_2)		149	51	150	50	0.667	0.41

[1]Resistant plants; [2]Susceptible plants. [3]Ouro Vermelho

Genetic linkage map of Estilo and Ouro Vermelho population

A total of 400 F_2 plants from Estilo and Ouro Vermelho cross and three plants of each parent were genotyped using an Illumina BARCBean6K_3 BeadChip with 5398 SNPs. Among those SNPs, 1053 were polymorphic between Estilo and Ouro Vermelho, which

represent 19.5% of the SNPs present in the BeadChip. In the set of 400 individuals, four had more than 10% of missing data and were taken out. After filtering for low quality and for SNP markers with more than 95% of missing data, 18 SNPs of the original 1053 polymorphic SNPs were removed. A set of 36 SNP's that deviated from the expected 1:2:1 expect ratio ($p = 4.83 \times 10^{-5}$; for $\alpha = 0.05$ and 1,035 SNPs) were also removed. Therefore, a total of 999 SNPs was retained for linkage analysis. Among those SNPs, eight were described to be located in unmapped scaffolds (Song et al. 2015). Using the F_2 population of the cross between Ouro Vermelho and Estilo, it was possible to map all these markers, being three in the Pv04 and Pv08 and one in the Pv05 and Pv10 chromosomes (Supplementary Figure S1). Besides that, two markers previously mapped in the Pv08 and Pv10 (Song et al. 2015) were reordered for a location of greatest likelihood in the same chromosomes (Fig. S1).

SNP markers associated with anthracnose resistance genes in Estilo and Ouro Vermelho

SNPs associated with resistance to Lv134 and Lv238 strains of *C. lindemuthianum*, race 65, was found in linkage group Pv04. However, given the extensive linkage disequilibrium that exist in F_2 populations, many SNPs associated with these strains were identified in a large region of this chromosome (Fig. 2). Among these SNPs, the genetic linkage analysis revealed SNPs 100% associated with Lv134 and Lv238 strains (Table 2). With respect to physical position, the markers most associated ($r^2 = 100\%$) with the regions that control resistance were between 1.1345Mb and 1.1657Mb for Lv238 and between 0.0111Mb and 0.2270 Mb for Lv134 (Table 2). According to multiple interval mapping analysis the most probable position of the gene associated with resistance to strains Lv134 and Lv238 were 0.2553cM and 2.0457cM, respectively, that is, 1.80cM from each other (Fig. 3).

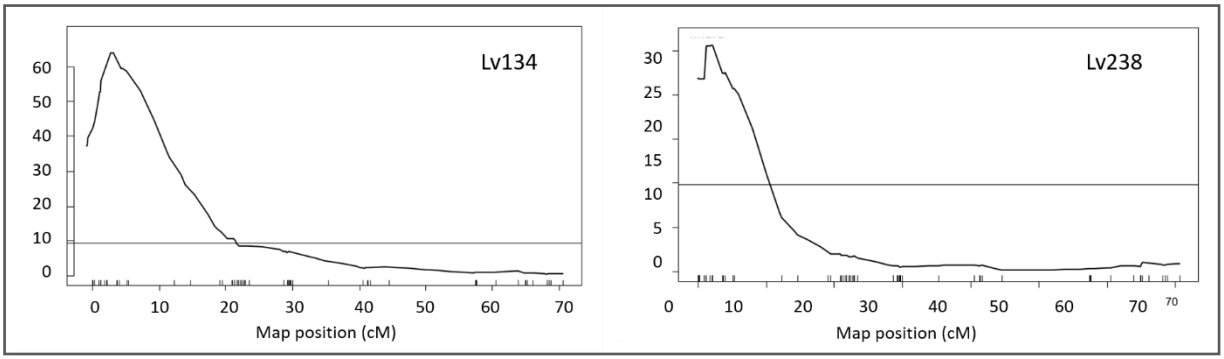

Fig. 2 LOD profile of Linkage Group Pv04 for the reaction of the F_2 population of Estilo x Ouro Vermellho cross to Lv134 and Lv238 strains. The horizontal line represents the LOD threshold (Alpha = 0.05), calculated based on permutation

Table 2 SNP markers on chromosome Pv04 of *Phaseolus vulgaris s* (v1.0 assembly) completely associated with resistance to strain Lv134 and Lv238 of *Colletotrichum lindemuthianum*, race 65 according to linkage analysis using F_2 population derived from Estilo x Ouro Vermelho cross and 999 polymorphic SNPs on the Illumina BARCBEAN_6K_3 BeadChip.

Strain	SNP ID (NCBI ss#)	Physical position (Pb)	p-value	R^2 (%)
Lv134	ss715649768	11168	1.46E+03	100
	ss715649777	46027	1.46E+03	100
	ss715649776	55042	2.60E+03	100
	ss715649774	70941	2.62E+03	100
	ss715649773	80687	2.01E+03	100
	ss715649772	90666	2.67E+03	100
	ss715649771	96165	3.09E+03	100
	ss715640025	212864	2.94E+03	100
	ss715640024	226600	2.36E+03	100
	ss715648681	227060	1.70E+03	100
Lv238	ss715646889	1134467	1.67E+03	100
	ss715646891	1147939	2.33E+03	100
	ss715646892	1155846	2.30E+03	100
	ss715646893	1165722	1.64E+03	100

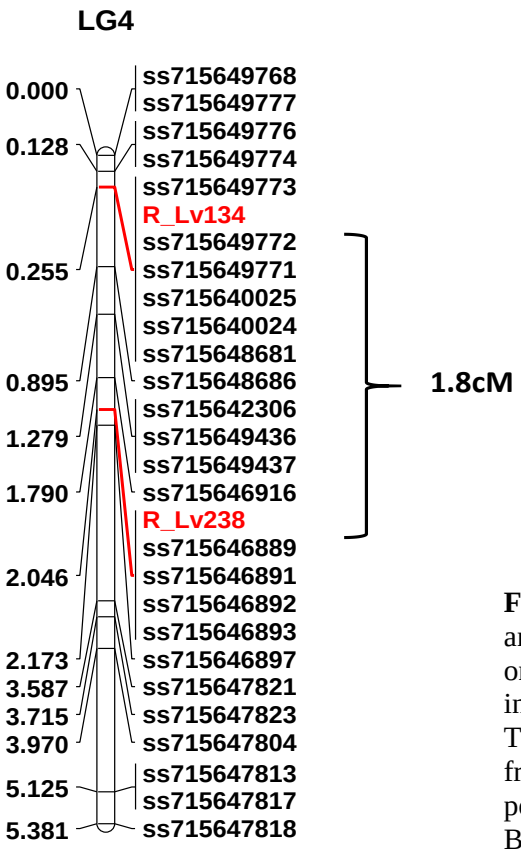

Fig. 3 Genetic map of resistant genes to strain Lv134 and Lv238 of *Colletotrichum lindemuthianum* race 65 on the short arm of Pv04. Map distances are expressed in centimorgans using the Kosambi mapping function. The map was constructed using F_2 population derived from Estilo x Ouro Vermelho cross and 999 polymorphic SNPs on the Illumina BARCBEAN_6K_3 BeadChip.

Phenotypic data of the common bean lines from germplasm bank of UFLA to different strains of *C. lindemuthianum* race 65

The average scores to reaction to each five strains of *C. lindemuthianum*, race 65 of the 189 common bean lines from the germplasm bank of UFLA shows high variability for the reaction of the host to the different strains used (Table S1). Among the five strains of *C. lindemuthianum*, the lines were more susceptible to strains Lv238 and Cl1532 and more resistant to strain Cl1614 (Fig. 4). As all the strains belong to race 65, among the international set of anthracnose common bean differentials, only the cultivars, Michelite (2^0) and Mexico 222(2^6) were susceptible to the strains used. Except the differential cultivars, only 25 common bean lines were resistant to the five strains of *C. lindemuthianum* race 65 used. Regarding the strains Lv134 and Lv238, used to map the anthracnose resistance genes present in Estilo and Ouro Vermelho, there were common bean lines resistant or susceptible to both of these strains and common bean lines resistant to strains Lv134 and susceptible to strain Lv238 and vice versa, highlighting that different genes confer resistance to these two strains of *C. lindemuthianum*, race 65 (Fig. 5).

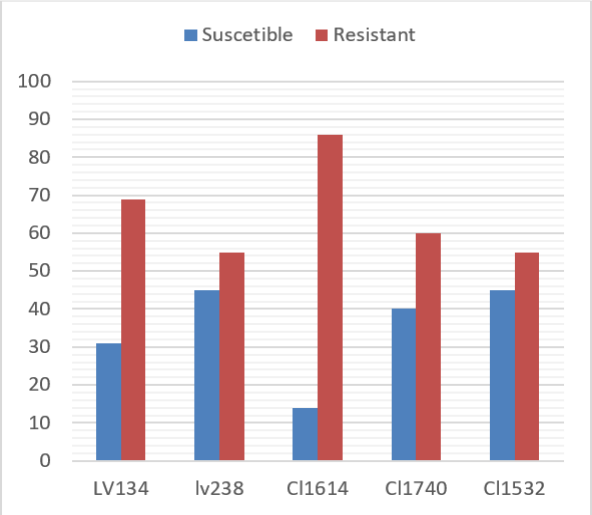

Fig.4 Percentage of resistant and susceptible common bean lines to different strains of *Colletotrichum lindemuthianum* race 65 among 189 common bean lines of the germplasm bank of Universidade Federal de Lavras.

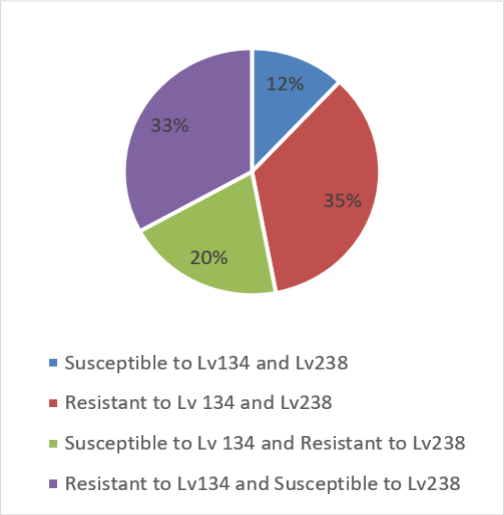

Fig. 5 Reaction of common bean lines from germplasm bank of Universidade Federal de Lavras to strain Lv134 and Lv238 of *C. lindemuthianum* race 65.

The common bean lines, Jalo vermelho (*Co*-12), Jalo listras pretas (*Co*-13), Pitanga (*Co*-14) and Corinthiano (*Co*-15), are described in the literature as resistant to race 65 of *C. lindemuthianum* (Gonçalves-Vidigal et al. 2008a, 2012; Lacanallo and Gonçalves-vidigal 2015; Sousa et al. 2015). On the other hand, Ouro Negro (*Co*-3^4) is described as susceptible to this race (Alzate-marin et al. 2003; Valentini et al. 2017). However, in the present study, considering the five strains of race 65 of *C. lindemuthianum* used, these lines were resistant to some strains but susceptible to others (Table 3). Besides that, our data showed that, the common bean line Corinthiano was susceptible to all five strains. Just Jalo Vermelho was resistant to the five strains.

Table 3 Mean scores of Pitanga, Corinthiano, Ouro Negro, Jalo Vermelho and Jalo Listras pretas to five strains of *Colletotrichum lindemuthianum* race 65 according to the descriptive scale from 1 to 9 (Schoonhoven and Pastor-Corrales 1987).

Strains	Common bean lines				
	Pitanga	Corinthiano	Ouro Negro	Jalo vermelho	Jalo listras pretas
Lv134	2.1	9.0	9.0	1.0	6.0
Lv238	9.0	9.0	2.0	1.4	6.2
Cl1614	5.2	9.0	2.4	1.0	4.4
Cl1532	9.9	9.0	2.4	1.6	1.4
Cl1740	6.0	9.0	5.6	1.4	2.8

GWAS

After applying filters, 3095 SNPs were retained for GWAS analysis. The Evanno method suggested that the most likely number of subpopulations in the panel was K = 2, indicated by the largest change in Delta K among assumed K values (Fig. S2). The population structure can also be graphically visualized (Fig. S3). A total of eight SNPs was significantly associated with resistance to Lv134 strain and six were associated with resistance to Lv238 strain (Table 4, Fig. 6), all of them in Pv04. The SNPs most associated with Lv238 (ss715646893) and Lv134 (ss715649771) were also associated with resistance to the same strains using the F_2 population of Estilo x Ouro Vermelho cross (Table 2). Some of the SNPs associated with strain Lv238 were also associated with resistant to strain Cl1532. Besides that, it was found significant association on Pv05 and Pv11 for this strain. For the strain Cl1614, significant associations were also found in Pv04 but in different regions compared with the strains Lv134, Lv238 and Cl1532 and one significant but smaller association on Pv01. Lastly for the strain Cl1740 it was found significant association on Pv10 (Table 3, Fig. 6).

Table 4 SNP markers (v1.0 assembly) associated with resistance to strains Lv134, Lv238, Cl1532, Cl1614 and Cl1740 of *Colletotrichum lindemuthianum*, race 65 according to association analysis using 189 common bean lines and 3095 polymorphic SNPs on the Illumina BARCBEAN_6K_3 SNP ship.

Strain	SNP ID (NCBI ss#)	Chromosome	Physical position	p-value	R^2
Lv134	ss715649777	Pv04	46027	4.09E-15	0.42988
	ss715649776	Pv04	55042	1.21E-17	0.52218
	ss715649774	Pv04	70941	3.50E-18	0.54375
	ss715649771	Pv04	96165	1.03E-20	0.64441
	ss715640025	Pv04	212864	4.93E-12	0.32342
	ss715648681	Pv04	226600	1.03E-14	0.41459
	ss715640024	Pv04	227060	1.11E-12	0.34499
	ss715642306	Pv04	447225	6.94E-07	0.16477
Lv238	ss715649437	Pv04	575006	1.48E-06	0.15635
	ss715646889	Pv04	1134467	3.60E-08	0.21004
	ss715646891	Pv04	1147939	2.63E-17	0.52424
	ss715646892	Pv04	1155846	1.55E-18	0.55979
	ss715646893	Pv04	1165722	1.65E-22	0.72185
	ss715646247	Pv04	2147821	1.12E-10	0.29712
Cl1532	ss715646891	Pv04	1147939	4.07E-07	0.18002
	ss715646892	Pv04	1155846	8.99E-08	0.19825
	ss715646893	Pv04	1165722	3.29E-11	0.30732
	ss715646247	Pv04	2147821	1.13E-08	0.23749
	ss715650069	Pv05	3452997	1.32E-06	0.17072
	ss715648093	Pv11	47800050	3.67E-08	0.2141
Cl1614	ss715649433	Pv04	547509	3.35E-08	0.23575
	ss715649434	Pv04	554477	2.93E-09	0.23619
	ss715646910	Pv04	1503482	1.15E-14	0.48372
Cl1740	ss715639777	Pv10	6738210	2.99E-09	0.27056

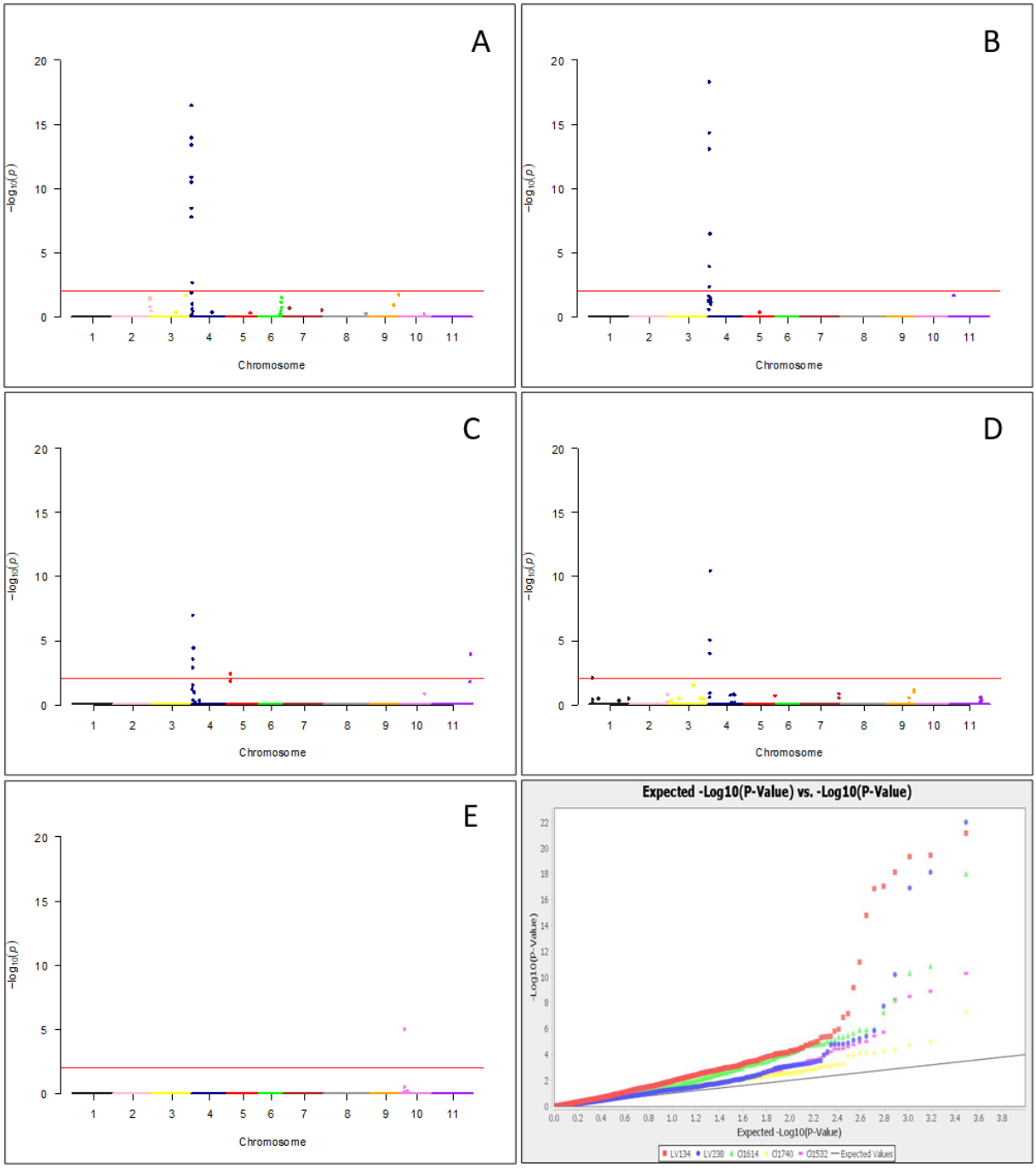

Fig. 6 Manhattan Plots showing significant SNPs for anthracnose resistance to strains LV134 (A), LV238 (B), Cl1532(C), Cl1614(D) and Cl1740(E) of *Colletotrichum lindemuthianum*, race 65. Redline on Manhattan Plot is the significant threshold of 3.23 x 10^{-6} after Bonferonni correction (α = 0.01)

Linkage disequilibrium

Regarding linkage disequilibrium, as common bean is a self-pollinating species, it was observed that, in general, the decay was low and extended to several megabases (Fig S4).

Candidate genes for anthracnose resistance

Using the reference genome of common bean, through the genome browser hosted in the NCBI website for the version 1 of the common bean genome sequence, significant SNPs were located within or next to candidate genes which are annotated as hypothetical proteins. The SNP ss715649771, associated with resistant to Lv134 of *C. lindemuthianum* (Table 2,4) is located within one exon of the Phvul.004G001500 gene. This gene codes for an elongation factor and its involvement in resistance against powder powdery mildew in common bean has been reported (Campa and Ferreira 2017). It was also identified that the SNP ss715648093 in Pv11, associated with resistance to CL1532 is located near to the gene Phavu_011G200300, whose a hypothetical protein is a member of a family of resistant proteins. Besides that, different functions could be assigned to the genes where the other significant SNPs were located, including three kinases in Pv04. The complete table with the predicted function of the genes detected in the candidate region according to the physical position is displayed on Table S2.

Discussion

One of the main problems that limit bean production is the occurrence of diseases. Host-plant resistance is the cornerstones of ecologically safe management systems of disease control. In this way, more insights into plant defense against pathogens are necessary to understand how plants deal with those organisms. For instance, breeders can choose to pyramid resistance genes that have different molecular basis because they can stop the pathogen at different levels of its cycle (Brown 2015). Besides that, the knowledge about the variability of the pathogens and plant-pathogens interaction is fundamental for developing effective strategies of durable resistance.

The genetics of anthracnose resistance has been studied for a long time in common bean. Many genes conferring resistance to different races of *C. lindemuthianum* have already been mapped and tagged with molecular markers (Ferreira et al. 2013; Valentini et al. 2017; Kelly and Bornowski 2018). However, in these studies, only one strain of a specific race has been used. This work is the first report of gene mapping of anthracnose resistance using different strains of a single race of *C. lindemuthianum*. The characterization of races of *C. lindemuthianum* is carried out based on the reaction in an international set of twelve differential cultivars (Pastor-Corrales 1991). Although that system is very effective and has

been allowed the exchange of information among researchers from different parts of the world, it has some limitations that need to be considered, especially in common bean breeding. The main reason for that is the high pathogenic variability of *C. lindemuthianum* that cannot be completely discriminated by using this set of differential cultivars. Several studies have been reported the existence of pathogenic variability among strains previously classified as the same race according with these differential cultivars (Rodríguez-Suárez et al. 2005; Santos et al. 2008; Ishikawa et al. 2008, 2011, 2012a; Davide and Souza 2009; Costa et al. 2017). In other words, it means that some cultivars may be resistant to some strains but susceptible to others of the same race. Indeed, from the point of view of common bean breeding, this issue is very important because when a new common bean cultivar is released as resistant to a specific race, it is expected that this cultivar will be resistant to all strains belonging to that race, otherwise, this could compromise the credibility of the breeding program.

Using 400 plants of F_2 population from the cross Estilo x Ouro Vermelho, half inoculated with strain Lv134 and another half inoculated with strain Lv238, both classified as *C. lindemuthianum*, race 65, it was confirmed that Estilo presents a dominant allele of a single gene conferring resistance to Lv238 strain and Ouro Vermelho presents a dominant allele of a single gene conferring resistance to Lv134 strain. The mapping of those genes from the high-throughput SNP genotyping with the BARCBEAN6K_3 BeadChip revealed SNPs markers associated with the resistance to the strains Lv238 and Lv234 on the short arm of Pv04. The SNPs markers most associated to each one of these two strains, although tightly linked, were different, which means that there are different genes conferring resistance to specific strains of a same *C. lindemuthianum* race.

At the molecular level, the most resistance genes, against all types of pathogens, encode intracellular proteins containing a nucleotide - binding (NB) site and a C - terminal leucine rich - repeat (LRR) domain. The genes of these NB-LRR proteins are often organized in complex clusters of tightly linked genes located in distal regions of the chromosomes (Meziadi et al. 2016). The candidate region on short arm of Pv04, containing the most associated SNPs to resistance to strain Lv134 and Lv238, has one of the most important and well characterized cluster of resistance genes in common bean (Ferreira et al. 2013; Meziadi et al. 2016; Valentini et al. 2017). Besides anthracnose resistance, a lot of resistance genes against different pathogens such as *Uromyces appendiculatus* (Rust), *Pseudocercospora griseola* (Angular leaf spot) and *Pseudomonas syringae* (Halo blight) had been identified in this region (De Souza et al. 2007; Miklas et al. 2014; Keller et al. 2015; Souza 2016).

The first anthracnose resistance gene identified in the Pv04 of common bean was the *Co*-3 (Bannerot 1965). This gene was identified in the anthracnose differential cultivar Mexico 222. As the race 65 is the result of the susceptibility reaction in the differential cultivars Michelite (2^0) and Mexico 222 (2^6), ($2^0 + 2^1 = 65$), the *Co-3* allele identified in Mexico 222 does not confer resistance to race 65 of *C. lindemuthianum*. To date, five different alleles of this gene have already been described in different common bean cultivars: *Co*-3^2 (Fouilloux 1976, 1979), *Co*-3^3, previously described as *Co-9* (Geffroy et al. 1999; Méndez-Vigo et al. 2005; Alzate-Marin et al. 2007), *Co*-3^4, previously described as *Co-10* (Valentini et al. 2017) and *Co*-3^5, previously described as *Co-7* (Sousa et al. 2014). However, the lack of information of accurate physical position of many markers linked to the major resistance genes in the literature prevents a final determination of co-localization with the regions found in the present study.

It is currently known that the *Co-3*, as well as some other major *C. lindemuthianum* resistance genes, like *Co-2*, it is not a unique gene, it is, actually, a large cluster of several resistance genes conferring resistance to specific strains of the pathogen. The SNPs associated with resistance to Lv134 and Lv238 of race 65 in this work were found in the region of *Co-3* cluster. More recently, (Murube et al. 2019) using a recombinant inbred line population Xana/BAT93 did a fine mapping of a gene conferring resistance to race 73 and 48 of *C. lindemuthianum* in BAT93 whitin this cluster. Interestingly, in the same work the resistance to race 65 in Xana was mapped in Pv01 and BAT93 was susceptible to this race. The Mesoamerican common bean cultivar Ouro Negro has been reported to have the *Co*-3^4 allele conferring resistance for a wide range of *C. lindemuthianum* races. Valentini et al (2017) found Kompetitive allele specific PCR (KASP) markers tightly linked to this gene in the short arm of Pv04 chromosome. These markers are located close to the SNPs associated with resistance to the two strains used in the present work, however, the *Co*-3^4 is probably different from the gene that confers resistance to strains Lv134 and Lv238 of race 65 of *C. lindemuthianum*. This fact can be proven because Ouro Negro is susceptible to Lv134 (Costa et al. 2017), for which Ouro Vermelho cultivar is resistant. Indeed, according to the literature, Ouro Negro is considered susceptible to race 65 of *C. lindemuthianum* (Alzate-marin et al. 2003). However, Ouro Negro is resistant to strain Lv238, emphasizing that there is pathogenic variability among *C. lindemuthianum* strains classified as race 65. Costa et al (2017) found a segregation that fitted a ratio of 15R:1S in F_2 population of Ouro Negro x Estilo cross, which demonstrates that the genes that confer resistant to strain Lv238 in Estilo and Ouro Negro are different.

Other independent genes conferring resistance to different *C. lindemuthianum* races have been identified in different positions of Pv04 in common bean: *Co*-15 (Sousa et al. 2015), *Co*-16 (Castro et al. 2017), and the closed *Co*-y and *Co*-z genes (Geffroy et al. 1999, 2008) . It is important to highlight that the gene *Co*-15, identified in Corinthiano common bean line, is supposed to confer resistance to race 65 of *C. lindemuthianum* (Sousa et al. 2015), however, Corinthiano was susceptible to all six different strains of race 65 used in this study, including the strains Lv134 and Lv238. Other common bean cultivars with already described anthracnose resistance genes such as Jalo Listras Pretas (*Co*-13) in the Pv-02 (Lacanallo and Gonçalves-vidigal 2015) and Pitanga (*Co*-14) in the Pv-03 (Gonçalves-Vidigal et al. 2012) were resistant to some strains of race 65 and susceptible to others (Table 4). As a community, common bean breeders have widely adopted marker-assisted selection (MAS) (Kelly and Bornowski 2018). Therefore, if these genes do not confer resistance to all strains classified as race 65, before using the markers associated with those genes in MAS it is important to know if these markers are associated with the specific and most frequent strains of this race in a particular growing region of common bean. Otherwise this could cause serious economic losses. Interestingly, the common bean cultivar Jalo Vermelho that present the *Co*-12 resistance gene (Gonçalves-Vidigal et al. 2008a) was resistant to the six strains used in present study and it appears to be a good source of resistance to race 65 of *C. lindemuthianum*, even though the mapping of this gene has not yet been performed.

The 999 polymorphic and no distorted markers (19% of the markers present in the BeadChip), identified in the cross between Estilo and Ouro Vermelho, generated a linkage map with a total length of 982.18 cM comprising the 11 chromosomes of common bean. As Estilo and Ouro Vermelho both Mesoamerican lines, the low polymorphism rate is probably due to the use of two lines belonging to the same gene pool. Populations derived from cross between Andean and Mesoamerican lines could provide higher polymorphism (Song et al. 2015). However, the total length of linkage map found in the present work corroborated with the genetic linkage map reported by (Song et al. 2015) that found a length of 1042.2 cM using a F_2 population derived from the Stampede x Red Hawk cross. A similar length of 999.8 cM was also related by Valentini et al. (2017) using a F_2 population derived from Rudá x Ouro Negro cross. In addition, in the present study it was possible to map a set of 8 SNPs in different chromosomes that were reported to be located in unmapped scaffolds (Supplementary Fig. S1).

It was verified that the resistance to each one of the two strains of race 65 of *C. lindemuthianum* used in this study is conferred by different genes, tightly linked. In order to

validate this result, a diverse set of common bean lines from germplasm bank of Universidade Federal de Lavras, that presents different reaction to these *C. lindemuthianum* strains, was used in GWAS analysis. The combination of linkage and association analyses increasing reliability of the results obtained. Using GWAS approach, it was found SNPs strongly associated with resistance to strains Lv238 and Lv134 of *C. lindemuthianum*, race 65 also on Pv04 (Table 3). Interestingly, the most associated SNPs for resistance to these strains were the same found in the linkage analysis using F_2 population between Estilo x Ouro Vermelho cross. This correspondence between the results of two different approaches evidence that this regions on Pv04 could contain genes associated with resistance to these strains of race 65 of *C. lindemuthianum* used in the present study.

In the present study, using F_2 population derived from Estilo x Ouro Vermelho cross it was found SNPs markers completely associated with resistance to Lv134 and Lv238 in extensive linkage blocks (Fig. 2). One advantage of association mapping compared with linkage analysis consists in exploring historical and evolutionary recombination events at the population level (Zhu et al. 2008). However, as common bean is a predominant selfing species, the extended LD and low rates of effective recombination are expected (Perseguini et al., 2016). Indeed, in general, the LD in the present study was very high (high values of r^2) in the panel of GWAS, probably also due the degree of kinship among the common bean lines. Nevertheless, this panel present great variability for resistant to different strains of *C. lindemuthianum*, race 65 (Table S1) and the results indicated that there were recombination events in the regions where the significant SNPs were found.

The same set of common bean lines from germplasm bank of UFLA were screened with three more different strains classified as *C. lindemuthianum*, race 65. The data from GWAS analysis indicate that resistance to anthracnose strain Cl1614 of race 65 also resides in Pv04, but, differently to the other strains used, the most significant SNP for this strain was ss715646910, located at 1.50 Mb. For the strain Cl1740 it was found significant SNP (ss715639777) on Pv10 at 6.74 Mb. Some significant SNPs markers for Lv238 strain was also significant for the strain Cl1532. However, the strain Cl1532 also showed additional levels of resistance on Pv05 and Pv11 chromosomes (Table 3). According to Meziadi et al (2016), the cluster of resistance genes on Pv04 originated from the *Co*-2 (Pv11) cluster by ectopic recombination between non-homologous chromosomes in the subtelomeric regions. In the common bean, these regions are rich in Knobs (heterochromatic blocks) containing Khipu satellite DNA tandem repeat, which favor the amplification of resistance genes through unequal exchange (David et al. 2009).

In most cases it was found more than one SNP associated with resistance response to each strain of *C. lindemuthianum*, race 65 used. As stated above, Costa et al (2017) observed segregations that fitted a ratio of 15R:1S in different F_2 populations inoculated with the same strains used in the present study, suggesting that duplicate genes would be involved in the genetic control of resistance to these strains.

Significant SNPs were located within genes whose protein domain is known to be involved in pathogen resistance response in plants, like kinases (Table S2). Kinases have previously been identified as playing an important role in the COK-4 anthracnose resistance locus on Pv08 (Burt et al. 2015). The ss715642306 SNP, associated with resistance to Lv134 in the present study were located in a region of the gene Phavu004G005800, whose hypothetical protein have the Cytochrome P450 domain, involved in the oxidative degradation. Furthermore, ss715649771 SNP significantly associated with resistance response to Lv134, was located in a region that there is a candidate gene, Phvul.004G001500, annotated as an elongation factor. Although it is not a typical gene related to recognition of specific pathogen in plants, Campa and Ferreira 2017 in a study combining genetic, genomic and transcriptomic analysis verified that this gene was significantly upregulated in a genotype resistant to powdery mildew, suggesting its involvement in the resistance response.

In association mapping a higher genetic variation can be explored and therefore a larger number of genes associated with the interest trait can be identified. This approach is more cost effective and less time consuming compared with the traditional linkage analysis because it does not necessarily develop a segregating population. However, it is important to use a large population size and to consider the population structure and avoid spurious marker–trait associations (Kloth et al. 2012). The identification of only two subpopulations (K=2) in the panel of this present study results in a subtle level of population stratification for association studies (Zhu et al. 2008). These two subpopulations were probably due some Andean genotypes in the panel. Several candidate regions were identified involved in the resistance to different strains of race 65 of *C. lindemuthianum.* For strains Lv134 and Lv238 we used two different approaches, traditional QTL mapping and association mapping and these two approaches were quite consistent. For the other three strains evaluated in GWAS analysis we also have promising results and future researches are necessary to validate the significant identified SNPs.

GWAS analysis has been explored in common bean for identification of loci related to several important agronomic traits (Kamfwa et al. 2015a, b; Moghaddam et al. 2016; Hoyos-Villegas et al. 2017). For anthracnose resistance, Perseguini et al. (2016) found

positive associations for resistance to race 4 in different chromosomes explaining until 10% of the phenotypic variation. Zuiderveen et al. (2016) also find a positive association to different races of *C. lindemuthianum* explaining from 10 to 47% of the phenotypic variation. Regarding race 65 of *C. lindemuthianum*, the authors found a SNP that explained 39% of the phenotypic variation on Pv01.

In Brazil, there are three well defined growing season of common bean per year, where the crop is exposed to different environmental conditions as well as to different pests and pathogens. These factors can favor the dynamics of the sub telomeric genomic region of the common bean for the rapid evolution of resistance genes that may be conferring resistance to different strains of the race 65. In addition, the expression of resistance may also be involved with epigenetic mechanisms. In the same way, all these factors can also affect the rapid evolution of the virulence genes of the pathogen. The genetic variability among different races and strains of *C. lindemuthianum* have been studied for a long time. The pathogen presents high variability as result of sexual and asexual recombination mechanism (Souza et al. 2010; Ishikawa et al. 2012b; Barcelos et al. 2014). Confrontation between different strains of race 65 suggests that hyphal anastomosis happens within strains of this race (Ishikawa et al. 2008). Conidial anastomosis tubes (CATs) also has been observed among different *C. linemuthianum* strains belonging to race 65 (Pinto et al. 2012). In asexually reproducing fungi, CATs may also contribute to high level of genetic and pathogenic variability among and within races of *C. lindemuthianum*. Moreover, plasticity in the *C. lindemuthianum* genome has been observed especially among different strains of race 65 (Gonçalves 2016).

The understanding about the genetic basis of anthracnose resistance is essential to develop effective strategies for more durable resistance. Many anthracnose resistance genes have been identified in different common bean cultivars. Resistance to race 65 of *C. lindemuthianum* have been related in different linkage groups of common bean (Ferreira et al. 2013; Campa et al. 2014). However, in the majority of anthracnose resistance studies, resistance to a particular race of *C. lindemuthianum* is generalized based on the reaction to only one strain belonging to that race. Since there is pathogenic variability within strains classified as the same race, the location of resistance genes can vary not only according to the genotype used but also with the strain. Consequently, it is important that, in studies about anthracnose resistance, the strain used and the region where it was obtained should be informed. The recurrent selection breeding method using strains collected in the fields, where the common bean is growing, could be a good strategy to pyramid anthracnose resistance

alleles. The adoption of a dynamic, regional and complementary set of differential cultivars that discriminate the variability within strains of a same race of *C. lindemuthianum* is also important in order to select the most frequent and virulent strains to be used in breeding programs.

The findings of this work indicate a great potential value of anthracnose resistance genes in common bean lines of the germplasm bank of UFLA for breeders that aim resistance to race 65 of *C. lindemuthianum*. Since there are different loci conferring resistance to different strains of race 65 of *C. lindemuthianum*, common bean cultivars should be thoroughly evaluated by breeding programs with different strains of this race in order to obtain cultivars pyramided with different resistance genes. The future efforts will focus on fine mapping of these genes and to realize co-segregation studies combined with the genes that have already been identified in the literature.

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References

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

Alzate-Marin AL, Souza KA, Morais Silva MG, et al (2007) Genetic characterization of anthracnose resistance genes Co-4 3 and Co-9 in common bean cultivar tlalnepantla 64 (PI 207262). Euphytica 154:1–8.

Bannerot H (1965) Résults de I' infection d'une collection de haricots par six races physiologiques d'anthracnose. Ann l 'amelioration 15:201–222

Barcelos L, Pinto JMA, Vaillancourt LJ, Souza EA (2014) Characterization of *Glomerella* strains recovered from anthracnose lesions on common bean plants in Brazil. PLoS One 9:1–15.

Bradbury PJ, Zhang Z, Kroon DE, et al (2007) TASSEL: Software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633–2635.

Broman KW, Wu H, Sen Ś, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889–890.

Broughton W, Hernandez G, Blair M, et al (2003) Beans (Phaseolus spp.) - model food legumes. Plant Soil 252:55–128.

Brown JKM (2015) Durable Resistance of Crops to Disease: A Darwinian Perspective. Annu Rev Phytopathol 53:513–539.

Burt AJ, Manilal William H, Perry G, et al (2015) Candidate Gene Identification with SNP Marker-Based Fine Mapping of Anthracnose Resistance Gene Co-4 in Common Bean. PLoS One 10:1–19.

Campa A, Ferreira JJ (2017) Gene coding for an elongation factor is involved in resistance against powdery mildew in common bean. Theor Appl Genet 130:849–860.

Campa A, Rodríguez-Suárez C, Giraldez R, Ferreira JJ (2014) Genetic analysis of the response to eleven *Colletotrichum lindemuthianum* races in a RIL population of common bean (*Phaseolus vulgaris* L.). BMC Plant Biol 14:12.

Campa A, Trabanco N, Ferreira JJ (2017) Identification of Clusters that Condition Resistance to Anthracnose in the Common Bean Differential Cultivars AB136 and MDRK. Phytopathology 107:1515–1521.

Castro SA de L, Gonçalves-Vidigal MC, Gilio TAS, et al (2017) Genetics and mapping of a new anthracnose resistance locus in Andean common bean Paloma. BMC Genomics 18:1–12.

Choudhary N, Bawa V, Paliwal R, et al (2018) Gene / QTL discovery for Anthracnose in common bean (*Phaseolus vulgaris* L .) from North-western Himalayas. PLoS One 1–12

Costa LC, Nalin RS, Ramalho MAP, De Souza EA (2017) Are duplicated genes responsible for anthracnose resistance in common bean? PLoS One 12:1–15.

David P, Nicolas W., Chen NWG, et al (2009) A Nomadic Subtelomeric Disease Resistance Gene Cluster in Common Bean. Plant Physiol 151:1048–1065.

Davide LMC, Souza EA De (2009) Pathogenic variability within race 65 of *Colletotrichum lindemuthianum* and its implications for common bean breeding. Crop Breed Appl Biotechnol 9:23–30

De Ron AM, Papa R, Bitocchi E, et al (2013) Common Bean. In: De Ron AM (ed) Grain Legumes, Springer. New York, pp 5–36

De Souza TLPO, Alzate-Marin AL, Dessaune SN, et al (2007) Inheritance study and validation of SCAR molecular marker for rust resistance in common bean. Crop Breed Appl Biotechnol 7:11–15.

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet

Resour 4:359–361.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol Ecol 14:2611–2620

Ferreira JJ, Campa A, Kelly JD (2013) Organization of Genes Conferring Resistance to Anthracnose in Common Bean. In: Translational Genomics for Crop Breeding. pp 151–182

Fouilloux G (1976) Bean anthracnose: new genes of resistance. Annu Rep Bean Improv Coop 19:36–37

Fouilloux G (1979) New races of bean anthracnose and consequences on our breeding programs. In: International symposium on diseases of tropical food crops. Louvain-la-Neuve, pp 221–235

Geffroy V, Sévignac M, Billant P, et al (2008) Resistance to *Colletotrichum lindemuthianum* in *Phaseolus vulgaris*: a case study for mapping two independent genes. Theor Appl Genet 116:407–415.

Geffroy V, Sicard D, Oliveira JCF de, et al (1999) Identification of an ancestral resistance gene cluster involved in the coevolution process between *Phaseolus vulgaris* and its fungal pathogen *Colletotrichum lindemuthianum*. Mol plant-microbe Interact 12:774–784.

Gonçalves-Vidigal MC, Lacanallo GF, Vidigal Filho PS (2008a) A new gene conferring resistance to anthracnose in Andean common bean (*Phaseolus vulgaris* L.) cultivar 'Jalo Vermelho.' Plant Breed 127:592–596.

Gonçalves-Vidigal MC, Meirelles AC, Poletine JP, et al (2012) Genetic analysis of anthracnose resistance in 'Pitanga' dry bean cultivar. Plant Breed 131:423–429.

Gonçalves-Vidigal MC, Thomazella C, Vidigal Filho PS, et al (2008b) Characterization of *Colletotrichum lindemuthianum* isolates using differential cultivars of common bean in Santa Catarina state, Brazil. Brazilian Arch Biol Technol 51:883–888

Gonçalves PRC (2016) Cariotipagem de Linhagens de *Colletotrichum lindemuthianum* E *Glomerella* spp. Isoladas de Lesões De Antracnose no Feijoeiro. Univ Fed Lavras

González AM, Yuste-Lisbona FJ, Rodiño a P, et al (2015) Uncovering the genetic architecture of *Colletotrichum lindemuthianum* resistance through QTL mapping and epistatic interaction analysis in common bean. Front Plant Sci 6:13.

Hoyos-Villegas V, Song Q, Kelly JD (2017) Genome-wide Association Analysis for Drought Tolerance and Associated Traits in Common Bean. Plant Genome 10:1–17.

Ishikawa FH, Barcelos QL, Costa LC, Souza EA (2012a) Investigating variability whitin race 81 of *Colletotrichum lindemuthianum* strains from Brazil. Annu Rep Bean Improv Coop 55:141–142

Ishikawa FH, Ramalho MAP, Souza EA (2011) Common bean lines as potential diffetential cultivars for race 65 of *Colletotrichum lindemuthianum*. J Plant Pathol 93:461–464

Ishikawa FH, Souza EA, Davide LMC (2008) Genetic variability within isolates of

Colletotrichum lindemuthianum belonging to race 65 from the state of Minas Gerais, Brazil. Biologia (Bratisl) 63:156–161.

Ishikawa FH, Souza EA, Shoji J-Y, et al (2012b) Heterokaryon incompatibility is suppressed following conidial anastomosis tube fusion in a fungal plant pathogen. PLoS One 7:1–9.

Kamfwa K, Cichy KA, Kelly JD (2015a) Genome-Wide Association Study of Agronomic Traits in Common Bean. Plant Genome 8:1–12.

Kamfwa K, Cichy KA, Kelly JD (2015b) Genome-wide association analysis of symbiotic nitrogen fixation in common bean. Theor Appl Genet 128:1999–2017.

Keller B, Manzanares C, Jara C, et al (2015) Fine-mapping of a major QTL controlling angular leaf spot resistance in common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 128:813–826.

Kelly JD, Bornowski N (2018) Marker-Assisted Breeding for Economic Traits in Common Bean. In: Biotechnologies of Crop Improvement. pp 211–238

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

Kelly JD, Young RA (1996) Proposed symbols for anthracnose resistance genes. Annu Rep Bean Improv Coop 39:20–24

Kloth KJ, Thoen MPM, Bouwmeester HJ, et al (2012) Association mapping of plant resistance to insects. Trends Plant Sci 17:311–319.

Kopelman NM, Mayzel J, Jakobsson M, et al (2015) CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across k. Mol Ecol Resour 20:1179–1191.

Lacanallo GF, Gonçalves-vidigal MC (2015) Mapping of an andean gene for anthracnose resistance (Co-13) in common bean (*Phaseolus vulgaris* L .) Jalo Listras Pretas landrace. Aust J Crop Sci 9:394–400

Margarido GRA, Souza AP, Garcia AAF (2007) OneMap: Software for Genetic Mapping in Outcrossing Species. Hereditas 144:78–79.

Méndez-Vigo B, Rodríguez-Suárez C, Pañeda A, et al (2005) Molecular markers and allelic relationships of anthracnose resistance gene cluster B4 in common bean. Euphytica 141:237–245.

Meziadi C, Richard MMS, Derquennes A, et al (2016) Development of molecular markers linked to disease resistance genes in common bean based on whole genome sequence. Plant Sci 242:351–357.

Miklas PN, Fourie D, Trapp J, et al (2014) New loci including Pse-6 conferring resistance to halo bacterial blight on chromosome Pv04 in common bean. Crop Sci 54:2099–2108.

Moghaddam SM, Mamidi S, Osorno JM, et al (2016) Genome-Wide Association Study Identifies Candidate Loci Underlying Agronomic Traits in a Middle American Diversity

Panel of Common Bean. Plant Genome 9:1–21.

Murube E, Campa A, Ferreira J (2019) Integrating genetic and physical positions of the anthracnose resistance genes described in bean chromosomes Pv01 and Pv04. 1–17

Oblessuc PR, Baroni RM, da Silva Pereira G, et al (2014) Quantitative analysis of race-specific resistance to *Colletotrichum lindemuthianum* in common bean. Mol Breed 34:1313–1329.

Padder BA, Sharma PN, Awale HE, Kelly JD (2017) *Colletotrichum lindemuthianum*, the Causal Agent of Bean Anthracnose. J Plant Pathol 99:317–330

Pastor-Corrales MA (1988) La antracnosis del frijol comun, *Phaseolus vulgaris*, en America Latina. CIAT - Centro Internacional de Agricultura Tropical, Cali, Colombia

Pastor-Corrales MA (1991) Estandarizacion de variedades diferenciales y de designacion de razas de *Colletotrichum lindemuthianum*. Phytopathology 81:694

Perseguini JMKC, Oblessuc PR, Rosa JRBF, et al (2016) Genome-Wide Association Studies of Anthracnose and Angular Leaf Spot Resistance in Common Bean (*Phaseolus vulgaris* L.). PLoS One 11:1–19.

Pinto JMA, Pereira R, Mota SF, et al (2012) Investigating Phenotypic Variability in *Colletotrichum lindemuthianum* Populations. Phytopathology 102:490–497.

Poland J (2015) ScienceDirect. Curr Opin Plant Biol 24:119–124.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of Population Structure Using Multilocus Genotype Data. Genetics 155:945–959.

RCore Team (2018) R: A Language and Environment for Statistical Computing. R Found Stat Comput Vienna 1

Ribeiro T, Esteves JA de F, Silva DA, et al (2016) Classification of *Colletotrichum lindemuthianum* races in differential cultivars of common bean. Acta Sci Agron 38:179–184.

Richard MMS, Pflieger S, Sévignac M, et al (2014) Fine mapping of Co-x, an anthracnose resistance gene to a highly virulent strain of *Colletotrichum lindemuthianum* in common bean. Theor Appl Genet 127:1653–1666.

Rodríguez-Suárez C, Pañeda A, Campa A, et al (2005) Anthracnose resistance spectra of breeding lines derived from the dry bean landrace Andecha. Annu Rep Bean Improv Coop 48:48–49

Rohlf FJ (2009) NTSYSpc Numerical Taxonomy and Multivariate Analysis System. New York

Santos J, Antunes I, Rey M, Rossetto A (2008) Virulência das raças 65, 73 e 81 de *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib. e determinação de fontes de resistência em *Phaseolus vulgaris* L. R Bras Agrociência 14:115–124

Schmutz J, McClean PE, Mamidi S, et al (2014) A reference genome for common bean and

genome-wide analysis of dual domestications. Nat Genet 46:707–713. doi: 10.1038/ng.3008

Schoonhoven A van, Pastor-Corrales MA (1987) Standard system for the evaluation of bean germplasm. CIAT - Centro Internacional de Agricultura Tropical

Schwartz HF, Pastor-Corrales MA (2005) Anthracnose. In: Schwartz HF, Steadman JR, Hall R, Forster R (eds) Compendium of Bean Diseases, 1st edn. APS Press, pp 25–27

Silva KJD, Souza EA, Ishikawa FH (2007) Characterization of *Colletotrichum lindemuthianum* Isolates from the State of Minas Gerais, Brazil. Phytopathology 155:241–247.

Song Q, Jia G, Hyten DL, et al (2015) SNP Assay Development for Linkage Map Construction, Anchoring Whole-Genome Sequence, and Other Genetic and Genomic Applications in Common Bean. G3 Genes, Gonomes, Genet 5:2285–2290.

Sousa LL, Cruz AS, Filho PSV, et al (2014) Genetic mapping of the resistance allele Co-5 2 to *Colletotrichum lindemuthianum* in the common bean MSU 7-1 line. Aust J Crop Sci 8:317–323

Sousa LL, Gonçalves AO, Gonçalves-Vidigal MC, et al (2015) Genetic Characterization and Mapping of Anthracnose Resistance of Common Bean Landrace Cultivar Corinthiano. Crop Sci 55:1900.

Souza TLPO de (2016) Major angular leaf spot resistance loci in common bean(*Phaseolus vulgaris*). Bean Improv Coop Annu Rep 59:XV

Souza EA, Camargo Júnior OA, Pinto JMA (2010) Sexual recombination in *Colletotrichum lindemuthianum* occurs on a fine scale. Genet Mol Res 9:1759–1769.

Turner SD (2014) qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. bioRxiv 1–2.

Valentini G, Gonçalves-Vidigal MC, Gonzales OPH, et al (2017) High-resolution mapping reveals linkage between genes in common bean cultivar Ouro Negro conferring resistance to the rust, anthracnose, and angular leaf spot diseases. Theor Appl Genet 130:1705–1722.

Vlasova A, Capella-Gutiérrez S, Rendón-Anaya M, et al (2016) Genome and transcriptome analysis of the Mesoamerican common bean and the role of gene duplications in establishing tissue and temporal specialization of genes. Genome Biol 17:1–18.

Voorrips RE (2002) MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs. J Hered 93:77–78.

Yu J, Pressoir G, Briggs WH, et al (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38:203–208.

Zhu C, Gore M, Buckler ES, Yu J (2008) Status and Prospects of Association Mapping in Plants. Plant Genome 1:5–20.

Zuiderveen GH, Padder B a, Kamfwa K, et al (2016) Genome-Wide Association Study of Anthracnose Resistance in Andean Beans (*Phaseolus vulgaris*). PLoS One 11:1–17.

Supplementary Material

Table S1 Results from screening of common bean lines from the germplasm bank of Federal University of Lavras against five different strains of race 65 of *C. lindemuthianum.*

Genotype	Disease severity score to each strains of race 65 of *C. lindemuthianum*				
	Lv134	Lv238	Cl 1532	Cl 1614	Cl 1740
MAVll-127	1.37[a]	6.25	1.00	1.00	2.5
VC37	1.00	1.00	7.08	1.00	2.66
MAVI-24	1.60	7.25	7.33	1.62	1.12
MAIII-17.159	5.82	8.10	6.00	6.16	6.03
MAVII 129	6.50	7.25	5.00	6.02	8.62
MAVIII-128	1.50	7.00	4.69	1.00	3.02
MAI-2.10	6.66	4.00	4.79	5.05	1.25
MAVIII-78[b]	**1.00**	**2.00**	**1.83**	**1.00**	**2.37**
MAV-5.60	1.00	4.00	2.87	-	5.66
MAV-14.206	**1.62**	**1.00**	**2.16**	**1.00**	**1.33**
VC39	1.25	8.50	6.25	1.62	4.66
MAVIII-89	**1.62**	**1.67**	**1.00**	**1.00**	**2.29**
MAV-3.36	2.03	6.66	6.25	2.17	3.44
MAIII-9.91	7.30	8.25	4.86	4.66	3.50
CARIOCA	2.25	7.00	7.50	8.12	8.50
E 09/10-8	7.16	6.37	6.86	3.00	7.25
CXI-26	1.16	8.25	6.50	3.00	2.04
TALISMA	2.25	7.50	7.25	1.75	2.00
CNFCMG-11-06	2.25	2.69	4.00	1.87	1.50
BRS AMETISTA	5.87	1.00	1.00	1.00	1.00
CXII-1	3.00	7.75	6.25	1.00	3.08
CNFCMG 11-08	**2.50**	**2.83**	**1.87**	**1.00**	**1.00**
MBC1 17/5	2.70	4.00	2.75	1.00	1.12
CXII-23	3.00	3.00	6.75	1.33	2.50
MAIX 14	6.25	2.25	1.00	1.00	1.37
CXII-13	2.00	7.00	6.54	1.16	2.38
MBCO-22/3	**1.45**	**1.83**	**1.00**	**1.00**	**1.12**
MAIX 12	3.00	3.00	8.00	3.00	5.75
CXII-8	1.83	5.33	3.00	1.00	2.83
MAIX-4	2.12	8.10	6.12	8.00	5.29
MBC1-36/8	1.50	5.25	1.96	2.75	5.22
E09/10-28	4.50	1.91	3.00	3.00	3.95
MAV-1.7	5.87	1.41	2.90	4.7	1.16
MAVI-60	7.62	6.50	5.67	3.00	3.71
RPCVIII-4	1.00	4.00	2.25	1.00	1.00
CARIOCA MG	2.66	9.00	7.37	2.33	4.56
MAVI-39	2.04	7.12	7.87	2.25	4.41
MAIII-17.185	1.45	6.00	5.50	1.00	1.37
MAII-16	3.00	3.00	5.56	3.00	4.25
MAIV-8.102	1.25	2.66	4.65	1.00	2.11
CXII-15	6.12	2.50	3.84	1.00	2.62
MAI-8.9	5.16	1.00	-	-	1.25
MAIV-18.259	3.00	3.00	6.75	2.50	3.80
MAIV-15.524	2.41	2.35	3.50	3.00	4.25
MAVI-20	3.00	5.66	3.00	3.00	4.04

E09/10-7	1.50	6.83	4.65	1.00	1.25
VC 27	1.25	7.00	7.37	3.00	2.62
VC 25	1.50	6.87	7.62	1.00	3.29
CXII-6	1.12	8.87	8.00	1.00	4.57
MADREPEROLA	1.00	4.00	1.00	1.00	1.00
VC 24	2.25	8.00	6.75	1.00	2.75
CNFCMG 11-13	3.00	4.00	2.33	1.00	2.75
CXII 19	1.62	6.50	5.75	1.00	1.33
CNFC 11-07	7.12	8.37	6.62	8.41	8.37
E09/ 10-5	3.00	1.91	6.12	3.00	2.16
CNFC 10429	7.95	2.83	1.00	1.00	1.12
CNFC 11946	3.00	3.00	5.62	2.62	6.37
RPCVIII 13	1.00	7.00	2.25	1.00	1.00
RPCVIII 6	**2.00**	**1.00**	**1.20**	**1.00**	**1.00**
CXII 16	3.00	2.16	4.00	1.00	1.25
MAIX 10	5.00	1.00	1.00	1.00	1.00
RPCVIII-8	4.95	2.62	2.45	2.25	1.75
MBCI 32/14	3.00	6.62	7.37	2.50	2.58
MB 89	1.00	8.70	6.87	1.00	2.00
CXI 18	5.00	3.00	2.25	1.00	1.62
OURO NEGRO	8.75	2.25	1.00	1.00	1.00
CXI 1	3.00	6.29	3.00	1.12	1.83
RPCVIII 7	**1.00**	**1.62**	**1.00**	**1.00**	**1.00**
CXI 7	6.75	1.12	3.00	3.37	1.37
PEROLA	7.31	9.00	7.87	7.75	8.62
MAJESTOSO	3.50	4.00	3.00	1.00	1.62
VR 18	1.25	7.50	4.33	1.00	1.12
VR 15	1.00	2.00	1.00	4.00	1.12
RC2 RAD 155	1.00	1.83	4.33	4.00	5.37
CNFRX 15 275	7.85	1.87	1.00	1.00	1.16
VR 16	**1.00**	**2.25**	**1.83**	**1.16**	**1.37**
VR 17	1.00	3.00	1.16	4.00	1.87
CNFJ 15288	9.97	3.00	1.00	4.00	1.00
RADIANTE	1.00	1.12	6.00	3.00	1.00
BRS TIMBÓ	1.62	8.37	7.12	1.45	2.25
VR 14	1.00	3.00	6.00	1.25	2.83
CNFP MG 11-18	4.00	1.00	1.00	1.00	1.00
CNFP MG 11-08	3.00	2.37	4.12	2.70	1.50
CNFPMG -1106	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
CNFPMG -11-21	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
BRS CAMPEIRO	3.00	2.50	3.66	3.66	1.37
BRS VALENTE	1.16	8.00	7.00	1.00	1.00
VP 30	1.00	6.25	6.75	1.00	1.00
VP 31	1.00	6.25	3.00	1.00	1.00
BRS ESTEIO	1.25	4.00	1.00	1.00	1.00
CNFP11978	**1.00**	**1.00**	**1.00**	**1.25**	**1.00**
BRS ESPLENDOR	2.90	3.00	2.00	2.00	7.41
VC20	7.16	2.87	1.00	1.00	1.00
VC 22	2.37	8.00	5.87	3.00	1.00
CNFC 11965	1.65	7.00	1.00	1.00	1.25
VC 21	3.00	7.83	8.41	3.00	6.37
VC 19	2.70	-	9.00	7.00	6.50
CV-6	5.16	7.00	7.12	8.37	6.00

EMB9	3.00	4.00	4.83	1.16	2.33
VC 18	2.70	5.33	7.00	1.00	1.00
BRS NOTAVEL	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
VC 17	1.62	7.00	6.83	1.62	1.75
CNFC 10108	**1.00**	**1.00**	**1.00**	**1.00**	**1.12**
IPR UIRAPURU	1.37	6.21	5.12	1.00	1.16
BRS COMETA	6.00	6.50	1.20	1.20	1.00
MAIX -9	7.75	7.58	6.50	3.00	4.79
MAIX-7	**1.62**	**2.70**	**1.87**	**2.87**	**1.33**
MAIX-2	5.66	3.00	5.12	3.00	6.29
MAIX-11	4.00	1.00	1.00	1.00	1.00
MAIX-8	4.00	1.00	1.00	1.00	2.33
MAIX-6	7.29	6.00	4.51	3.00	4.79
MAIX-4	4.00	1.00	1.00	2.25	1.00
MAIX -5	4.50	1.00	-	-	-
BRS SUBLIME	6.87	1.12	1.00	1.00	2.00
CXIII 4.484	3.00	7.62	6.67	3.00	1.50
CXIII 1.23	6.62	3.00	4.70	1.25	1.125
CXIII 6.84	9.00	2.88	3.00	3.00	3.91
CXIII 85.62	1.29	8.50	8.62	3.00	2.75
CXIII 1.1	1.50	7.75	8.37	2.87	1.87
CXIII 1.7A	3.00	3.00	3.59	1.33	3.00
CXIII1.7B	3.00	2.80	4.54	1.62	1.00
RPXI -1	7.12	2.25	1.00	1.00	1.25
RPXI -6	2.87	3.00	4.45	1.00	2.00
RPXI-42	3.00	2.75	4.50	2.37	1.00
RPXI-26	7.62	2.25	1.00	1.00	2.5
RPXI-7	8.16	1.62	1.00	2.25	1.00
RPXI-38	8.50	2.12	1.00	1.00	1.37
RPXI-18	9.00	1.00	1.00	1.62	1.25
RPXI-43	3.00	7.25	5.50	1.12	1.00
RPXI-14	8.66	2.25	1.00	1.62	1.83
MAXII- 22	3.00	8.12	6.00	6.95	6.50
MAX-1	7.12	1.75	1.00	8.75	3.00
MAX -2	5.50	2.62	2.62	3.00	4.80
MAX-7	1.87	3.00	3.50	3.50	4.41
MAII-23	3.00	3.00	4.79	2.87	1.75
MAX-5	4.50	1.00	1.00	1.00	1.00
MAX-9	7.83	8.00	5.58	3.00	5.33
MAX-6	7.87	6.12	6.00	3.00	5.62
MAXII-7	6.25	1.00	1.62	2.00	1.00
MAX-10	4.6	3.00	1.50	4.5	2.37
MAX-8	4.00	1.00	1.00	1.00	1.37
MAXII-20	8.20	8.37	6.12	-	6.62
MAXII-5	7.87	2.25	4.00	3.00	1.50
MAX-11	4.00	2.00	1.12	1.87	2.25
MAX-4	7.62	1.31	3.00	1.87	1.00
VP-33	**1.00**	**1.00**	**1.00**	**1.00**	**1.45**
VP-34	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
VR-19	4.50	6.00	1.12	3.00	4.72
VR-20	4.50	4.52	1.00	3.00	3.70
VR-21	1.33	3.00	1.00	5.87	1.62
CNFP 10794	6.5	3.00	3.00	3.00	6.62

CNFP 11979	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
CNFP 15194	**1.00**	**1.00**	**1.62**	**1.00**	**1.00**
CNFP 15677	9.00	1.62	1.00	1.00	1.00
CNFP 15680	8.50	1.00	1.00	2.25	1.00
VC36	1.12	5.50	3.00	1.75	3.33
VC35	3.00	4.00	1.00	7.37	2.00
VC34	**1.25**	**3.00**	**1.87**	**1.00**	**1.12**
VC38	2.5	7.83	8.00	3.00	3.25
BRS Horizonte	**1.37**	**2.50**	**1.87**	**1.25**	**2.00**
BRSPitanga	1.12	6.87	7.12	1.37	4.08
BRS Bentivi	1.75	6.43	4.30	1.37	5.40
BRS União	1.00	7.00	6.00	6.62	6.37
BRSUai	8.25	1.62	1.00	1.12	1.12
Roxo90	1.16	8.12	7.12	1.00	1.16
IPR Colibbri	5.29	8.25	7.25	9.00	9.00
BRSMG Tesouro	1.62	7.50	6.12	1.00	2.5
ESAL503	6.87	8.00	6.83	8.50	7.33
ESAL535	1.00	6.62	4.00	2.16	2.00
ESAL664	**1.00**	**1.00**	**1.00**	**1.00**	**1.12**
ESAL576	1.00	4.00	1.00	1.00	1.00
ESAL517	9.00	9.00	9.00	9.00	9.00
ESAL561	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
ESAL502	7.75	8.37	6.62	8.62	8.66
ESAL652	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
ESAL573	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
Jalo Vermelho	**1.00**	**1.40**	**1.60**	**1.00**	**1.40**
Jalo Listras Pretas	6.00	6.20	1.40	4.40	2.80
Corinthiano	9.00	9.00	9.00	9.00	9.00
Michelite[c]	7.50	8.20	6.80	8.00	7.50
MDRK[c]	**2.00**	**2.00**	**1.10**	**1.00**	**1.20**
Perry Marrow[c]	**2.50**	**1.00**	**1.12**	**1.00**	**1.00**
Cornell[c]	**1.50**	**2.10**	**1.00**	**1.12**	**1.12**
Widusa[c]	**1.00**	**2.20**	**1.00**	**1.00**	**1.00**
PI 207.262[c]	**2.00**	**1.10**	**2.00**	**1.25**	**1.00**
TO[c]	**1.00**	**1.12**	**1.16**	**1.12**	**1.25**
TU[c]	**1.12**	**1.11**	**1.14**	**1.00**	**1.00**
AB136[c]	**1.30**	**1.00**	**1.00**	**1.00**	**1.20**
G2333[c]	**1.20**	**1.13**	**1.00**	**1.00**	**1.00**

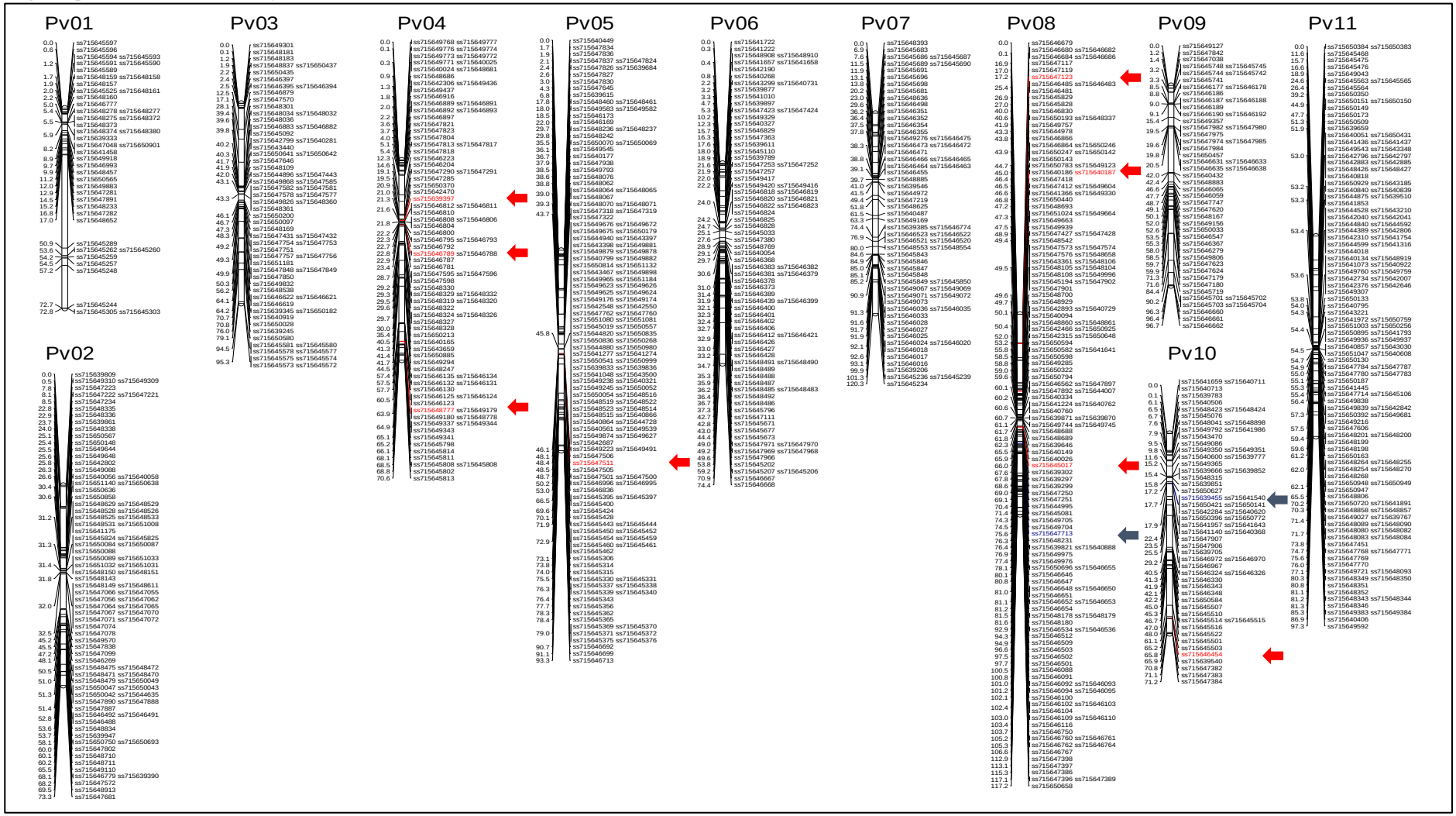
[a]Mean score according with the descriptive scale from 1 to 9 ((SCHOONHOVEN; PASTOR-CORRALES, 1987). Plants with no symptoms or small lesions (1 to 3) were classified as resistant and plants with larger lesions or dead (4 to 9) were classified as susceptible.

[b]Common bean lines resistant to the five strains of *C. lindemuthianum* evaluated are indicated in bold.

[c]Common bean lines differentials for anthracnose.

"-"no information of anthracnose severity

Fig. S1 Genetic linkage map of common bean based on the F_2 population derived from Estilo x Ouro Vermelho cross. The map was generated using MapChart 2.3. Distances between the loci (cM) are shown to the left and the names of the loci are shown to the right of the linkage groups. SNP markers described to be located in unmapped scaffolds (Song et al. 2015) were mapped using this population and they are indicated in red on Pv04, Pv05, Pv08 and Pv10. SNPs reordered from their original position (v1.0 assembly) associated5) are indicated in blue.



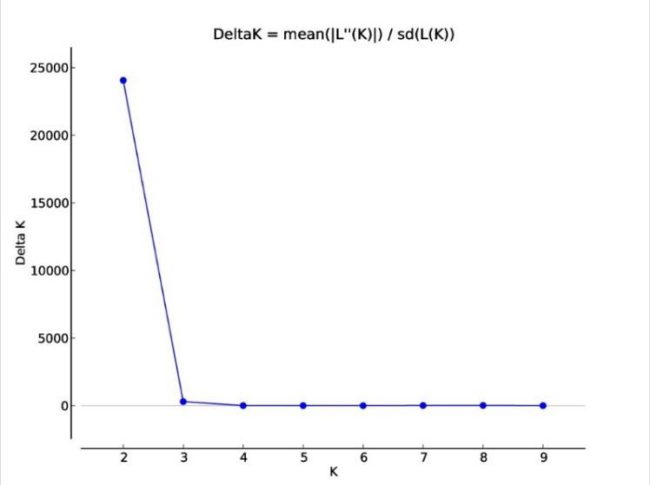


Fig S2 K values for STRUCTURE analysis according to the methodology of Evano et al (2005). The analysis was based on 3095 polymorphic SNPs and 189 common bean accessions from the Germplasm Bank of Federal University of Lavras, Brazil.

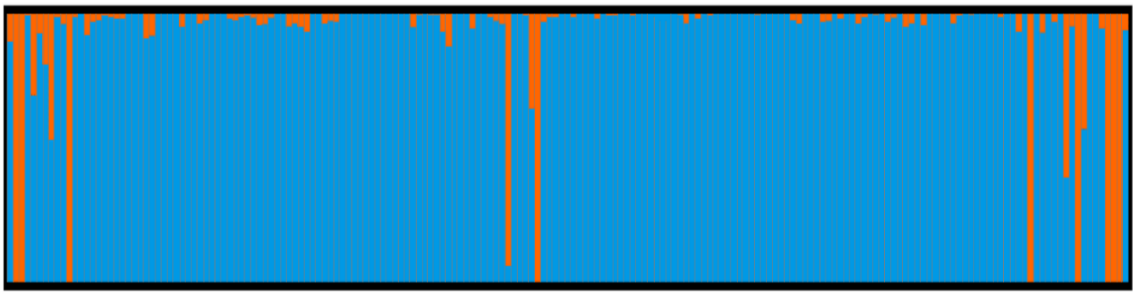


Fig S3 Population structure diagram of the 189 common bean genotypes according to 3095 SNPs and K=2. Bars with different colors represent genotypes belong to different subpopulations. The graphic was obtained using the online application CLUMPAK (Kopelman et al, 2015). Vertical lines have been added on the X-axis to make easy the visualization of a unique genotype.

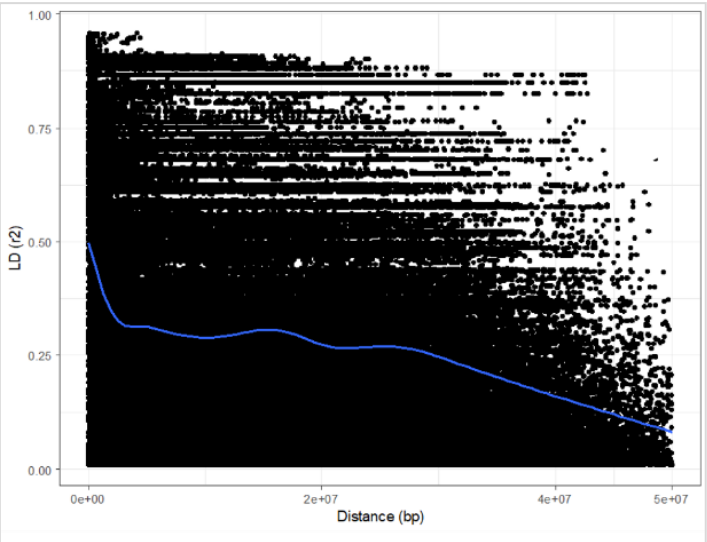


Fig. S4 Allele pair linkage disequilibrium (r^2) across the entire common bean genome for the 189 genotypes against genetic distance, against genetic distance with SNP markers.

Table S2. Predicted function of genes detected in the candidate region based on physical position of significant SNP

Gene	SNP ID (NCBIss#)	Chromosome	Position	Gene Length	Gene annotation and Predicted function based on protein domain
PHAVU_004G000500g	ss715649777	Pv04	41964-47578	5,615	Hypothetical protein with Diacylglycerol kinase catalytic domain that acts as a protein kinase C activator
PHAVU_004G000600g	ss715649776	Pv04	53.602-64.899	11,298	Hypothetical protein with WD40 domain that cover a wide variety of functions including adaptor/regulatory modules in signal transduction
PHAVU_004G000800g	ss715649774	Pv04	69.243-72.924	3,682	Hypothetical protein with Pyruvate kinase (PK) domain that regulates glycolysis through binding of the substrate
PHAVU_004G001500g	ss715649771	Pv04	95.644-100.091	4,448	Hypothetical protein coding for an elongation factor is involved in resistance against powdery mildew in common bean.
PHAVU_004G001700g	ss715640025	Pv04	206.436-215.551	9,116	Hypothetical protein with Mediator of RNA polymerase II domain, a coactivator involved in the regulated transcription of nearly all RNA polymerase II-dependent genes.
PHAVU_004G001900g	ss715648681	Pv04	224.350-227.563	3,214	Hypothetical protein with MATE_eukaryotic domain involved in exporting metabolites across the cell membrane and are responsible for multidrug resistance (MDR) and disease resistance in plants.
PHAVU_004G001900g	ss715640024	Pv04	224.350-227.563	3,214	Hypothetical protein with MATE_eukaryotic domain involved in exporting metabolites across the cell membrane and are responsible for multidrug resistance (MDR) and disease resistance in plants.
PHAVU_004G005800g	ss715642306	Pv04	446.146-448.147	2,002	Hypothetical protein with Cytochrome P450 domain, involved in the oxidative degradation
PHAVU_004G007000g	ss715649437	Pv04	569.144-575.259	6,116	Hypothetical protein with Branch domain which forms crucial side-chain branches in O-glycans
close to PHAVU_004G011500g	ss715646889	Pv04	1.134.959-1.138.016	3,058	Hypothetical protein with Cytochrome b5-like Heme/Steroid binding domain
PHAVU_004G011700g	ss715646891	Pv04	1.147.398-1.157.989	10,592	Hypothetical protein with AdoMet_MTases Superfamily which use S-adenosyl-L-methionine (SAM or AdoMet) as a substrate for methyltransfer and target atoms for methylation

Gene	SNP ID (NCBIss#)	Chromosome	Position	Gene Length	Gene annotation and Predicted function based on protein domain
PHAVU_004G011700g	ss715646892	Pv04	1.147.398-1.157.989	10,592	Hypothetical protein with AdoMet_MTases Superfamily which use S-adenosyl-L-methionine (SAM or AdoMet) as a substrate for methyltransfer and target atoms for methylation
PHAVU_004G011800g	ss715646893	Pv04	1.166.904-1.170.781	3,878	Hypothetical protein with Glycosyl hydrolases family 28 which is important in the cell wall metabolism
PHAVU_004G020900g	ss715646247	Pv04	2.145.561-2.154.213	8,653	Hypothetical protein with CRS1 / YhbY (CRM) domain containing protein in plant chloroplasts has been shown to have RNA binding activity
PHAVU_004G011700g	ss715646891	Pv04	1.147.398-1.157.989	4,716	Hypothetical protein with AdoMet_MTases Superfamily which use S-adenosyl-L-methionine (SAM or AdoMet) as a substrate for methyltransfer and target atoms for methylation
PHAVU_004G011700g	ss715646892	Pv04	1.147.398-1.157.989	4,716	Hypothetical protein with S-adenosylmethionine-dependent methyltransferases and target atoms for methylation
PHAVU_004G011800g	ss715646893	Pv04	1.166.904-1.170.781	3,878	Hypothetical protein with Glycosyl hydrolases family 28 which is important in cell wall metabolism
PHAVU_004G020900g	ss715646247	Pv04	2.145.561-2.154.213	8,653	Hypothetical protein with CRS1 / YhbY (CRM) domain containing protein in plant chloroplasts has been shown to have RNA binding activity
PHAVU_005G036300g	ss715650069	Pv05	3.452.375-3.459.013	6,639	Hypothetical protein with RNA recognition motif (RRM) is a highly abundant domain in eukaryotes found in proteins involved in post-transcriptional gene expression processes including mRNA and rRNA processing, RNA export, and RNA stability.
close to PHAVU_011G200300g	ss715648093	Pv11	47.805.798-47.809.484	3,687	Hypothetical protein with Coiled-coil domain of the potato virux X resistance protein which confers resistance against potato virus X. It is a member of a family of resistance proteins
PHAVU_004G006800g	ss715649433	Pv04	528.718-556.900	28,183	Hypothetical protein with Big_2 Superfamily which are Ig-like fold
PHAVU_004G006800g	ss715649434	Pv04	528.718-556.900	28,183	Hypothetical protein with Big_2 Superfamily which are Ig-like fold
PHAVU_004G014500g	ss715646910	Pv04	1.503.213-1.509.448	6,236	Hypothetical protein with protein prenyltransferase (PTase) domain which are required for catalytic activity and play critical roles in a variety of cellular processes including Ras/mitogen activated protein kinase signaling pathways

ARTIGO 2 - RECURRENT SELECTION AS AN ALTERNATIVE FOR DURABLE ANTRACNOSE RESISTANCE IN COMMON BEAN

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ABSTRACT

The evolutionary potential of the fungus *Colletotrichum lindemuthianum* for quickly overcome common bean resistance makes difficult the achievement of long-term anthracnose genetic resistance. The object of this study was to develop and evaluate the efficiency of a recurrent selection program as an alternative to obtain durable anthracnose resistance in common bean. The mixture of 45 F_2 populations (S_0 population) derived from the diallel cross of ten common bean lines, with variability for resistance to different *C. lindemuthianum* strains, was used to form the base population (Cycle 0). From Cycle 0, five recombination cycles among the S_0 plants, selected for resistance to different *C. lindemuthianum* strains, was carried out. The genetic gain for anthracnose resistance, after five selective cycles, estimated by the overall mean of anthracnose severity scores of $S_{0:2}$ progenies from each cycle, was 7.4% and 10.7% for two different strains of race 65 and 9.0%, 9.0%, 8.0% and 7.8% for strains of race 73, 81, 89 and for the mixture of eight different strains of *C. lindemuthianum*, respectively. There was an increase in the number of progenies resistant to a greater number of *C. lindemuthianum* strains from the first to the last selective cycle. Furthermore, the method offers the opportunity to develop commom bean cultivars composed by a mixture of pyramided pure lines with resistance genes against diffenrent strains of *C. lindemuthianum*. Therefore, the recurrent selection can be an effective and dynamic breeding method to provide greater stability and durability of anthracnose resistance.

Keywords: *Phaseolus vulgaris*. *Colletotrichum lindemuthianum*. Disease resistance. Recurrent selection

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the leguminous most widely grown and consumed, especially in tropical regions. The crop has been grown in distinct areas and in different seasons by subsistence-level farmers as well as by farmers that use high technologies. Besides economic and social importance, due the nutritional properties, the crop has a major role in human diets, especially for low-income populations (Broughton et al., 2003; De Ron et al., 2016). However, several factors, like the occurrence of fungal diseases, can cause serious damage in the crop and lead to substantial yield losses (Singh and Schwartz, 2010). Anthracnose, caused by specialized phytopathogen fungus *Colletotrichum lindemuthianum*, is one of the most destructive diseases that affect *P. vulgaris* worldwide. Disease outbreaks generally originate from contaminated seeds or infect plant debris. The use of susceptible cultivars in cool and humid conditions also favor disease development (Ferreira et al., 2013; Padder et al., 2017).

The management practices to prevent the disease include crop rotation, use of fungicides and use of genetic host resistance. The last one is considered the most economic and ecologically safe strategy to control the disease, mainly for not increasing production costs and also because it avoids environmentally damages caused by chemical control (Miklas et al., 2006; Ferreira et al., 2013). In fact, several major resistance genes (R genes), referred as *Co-* genes, have been identified in common bean, against specific strains of the pathogen (Ferreira et al., 2013; Castro et al., 2017; Valentini et al., 2017). These genes are frequently organized in wide resistance clusters, within which several genes have been mapped conferring specific-race-resistance (Ferreira et al., 2013; Murube et al., 2019). Nevertheless, as *C. lindemuthianum* is highly variable, evidenced by the numerous physiological races described in the literature, the use of these R genes has not provided durable resistance (Padder et al., 2017). The races of *C. lindemuthianum* are designated according with the reaction in an international set of twelve anthracnose differential cultivars (Pastor-Corrales, 1991). In Brazil, races 65, 73, 81 and 89 are the most frequent races in most regions (Silva et al., 2007; Pereira et al., 2010; Pinto et al., 2012; Ribeiro et al., 2016). Furthermore, the occurrence of variability within strains of a same race, especially for race 65, have been reported, contributing for the rapid resistance breakdown (Davide and Souza, 2009; Ishikawa et al., 2011; Costa et al., 2017). For these reasons, resistance breeding to anthracnose is an ongoing process, non - trivial and resistance must be managed strategically.

Different strategies, like gene rotation through time and space, pyramiding resistance

alleles and multiline with different resistance alleles, have been proposed aiming to increase the durability of genetic resistance (Mcdonald and Linde, 2002; Brown, 2015; Consortium, 2016). Among these strategies, breeding for anthracnose resistance has mainly focused in pyramid major resistance genes into a single cultivar, so that the pathogen will not be able to undergo a sequence of mutations corresponding to each resistance gene (Miklas et al., 2006; Marcondes et al., 2010). This strategy can be greatly facilitated by marker-assisted selection (MAS) (Miklas et al., 2006; Garzón et al., 2008; Tryphone et al., 2013). However, although pyramiding increases the durability of disease resistance, its effectiveness can also be reduced when pathogens undergoes regular recombination (Mcdonald and Linde, 2002; Mundt, 2014), as occurs in *C. lindemuthianum.* This pathogen presents sexual and asexual recombination mechanisms, which contribute to high level of variability among different strains of the species (Castro-Prado et al., 2007; Pinto et al., 2012; Padder et al., 2017).

The utilization of multiline with different resistance alleles against different races of *C. lindemuthianum* is an interesting alternative to control anthracnose in common bean (Botelho et al., 2011; Carvalho et al, 2018). This strategy may provide greater resistance stability when compared to the use of a single line with pyramided resistance alleles (Mundt, 2014). However, the use of multiline presents some issues that need to be taken into account, such as: It is necessary to mix the common bean lines to establish the multiline at each crop season; Identifying common bean lines that have different resistance alleles and that also exhibit uniformity for agronomic traits, such as, maturation time, growth habit, seeds characteristics (size, shape and color), cannot be an easy task. Beyond that, there is the effect of intergenotypic competition among the lines of the multiline, with reflection on the grain yield (Nelson et al., 2018).

A reliable method for accumulating favorable alleles of several traits is the recurrent selection. It consists in a dynamic approach of successive cycles of evaluation, selection and recombination, being useful especially in breeding programs involving traits of quantitative inheritance, which present low heritability and are controlled by a large number of genes of small effect (Nelson et al., 2018). In common bean crop, this method has been successfully used to improve several traits, including resistance to diseases (Arantes et al., 2010). Genetic progress for resistance to *Pseudocercospora griseola* (Angular Leaf Spot) (Rezende et al., 2014), and also to *Sclerotinia sclerotiorum* (White mold) (Leite et al., 2016) has been achieved after some selective cycles. In those cases, common ben cultivars coming from the different selective cycles that associate desirable agronomic traits, good level of resistance and grain yield have also been obtained.

Despite the fact that the response of common bean plants to *C. lindemuthianum* usually exhibits a qualitative inheritance, there are a large number of different genes conferring race-specific-resistance. Costa et al. (2017) observed that different genes in common bean are involved in the response to different strains of *C. lindemuthianum* belong to the same race and that duplicate genes may be conferring resistance to specific strains. Quantitative Resistance Loci (QRL) have also been reported conferring partial resistance to different races of the pathogen (Oblessuc et al., 2014; Perseguini et al., 2016; Zuiderveen et al., 2016). Moreover, significant epistatic interactions between QRLs have also been reported in quantitative resistance against *C. lindemuthianum*, suggesting that resistance to anthracnose may involve more genetic complexity (González et al., 2015).

Due to the large number of major resistance genes that have been identified involved in genetic control of the anthracnose in common bean and also the evidence of genes of small effect, the object of this study was to develop a recurrent selection program as an alternative to get durable anthracnose resistance. The use, implications and effectiveness of this method for obtaining anthracnose resistant cultivars has never been studied so far for this disease and the outcomes of this research may contribute for the development of common bean lines with more stable and durable anthracnose resistance.

Materials and Methods

Experiments were performed in the Laboratory of Plant Resistance to Diseases, as well as in the greenhouse of the Biology Department and in the experimental farm, both from the Universidade Federal de Lavras, Minas Gerais, Brazil.

Common bean lines of the base population and *C. lindemuthianum* strains used in the present study

The base population was formed by ten Brazilian common bean lines. Among then, eight are market cultivars, of which five are carioca grain type (BRS Estilo, BRSMG Majestoso, BRSMG Madrepérola, BRS Cometa and BRS Uai) and three are black grain type (Ouro Negro, BRS Valente and BRS Esplendor). The other two common bean lines were M20 and MAIII-16.159, both are carioca grain type and came from the recurrent selection program aiming White mold resistance and angular leaf spot resistance, respectively. The choice of these cultivars was based on their different resistance spectrum for eight different *C. lindemuthianum* strains, four belonging to race 65 and one of each 73, 81 and 89 races (Table 1).

Table 1 – Origin of *C. lindemuthianum* strains used in the present study, obtained in different regions of Brazil

Strains	Race	Origin	Year
Cl1532	65	Ponta Grossa/PR	2011
Cl1610	65	Anápolis/GO	2011
Cl1740	65	Lambari/MG	2012
Lv238	65	Patos de Minas/MG	2010
Lv134	65	Lambari/MG	2009
1172	73	Ponta Grossa/PR	2012
Lv201	81	Lambari/MG	2009
Lv228	89	Lambari/MG	2010

Reaction of the common bean lines to different *C. lindemuthianum* strains

The ten common bean lines that formed the base population were separately evaluated for the reaction to the eight *C. lindemuthianum* strains (Table 1). All the common bean lines were also evaluated for an equitable mixture of conidial suspension (1.2×10^6 conidia.ml^{-1}) of the eight *C. lindemuthianum s*trains. The seeds of those ten common bean lines were sown in polystyrene trays containing Plantmax® substrate. The experiment was carried out in a greenhouse in a completely randomized design with three replicates, being nine seedlings per plot. For the inoculation, each *C. lindemuthianum* strains were grown on Petri dishes containing M3 culture medium. Posteriorly, small pieces of mycelium were transferred to sterile common-bean pods placed in test tubes for an incubation period of about 12 days at 22°C, in the dark. Subsequently, the conidia suspensions (1.2×10^6 conidia.ml^{-1}) were inoculated in the primary fully expanded leaves of common bean lines by spraying both leaf surfaces and the stem. After inoculations, seedlings were kept in the greenhouse with 95% relative humidity, at 24°C for three days and 75% relative humidity for more seven days. Disease severity was visually assessed according to the descriptive scale (1 to 9) (Schoonhoven and Pastor-Corrales, 1987), where score "1" indicates absence of symptoms and score "9" indicates dead plants.

Obtaining the base population (Cycle 0) and progenies of cycle I to V

Biparental crosses among the ten common bean lines, in all possible combinations were performed in a greenhouse in June of 2015 winter season. F_1 seeds from the 45 hybrids obtained were sown in November of the same year to obtain the seeds of F_2 generation of

each hybrid. These F_2 seeds from all hybrids were mixed equally to form the base population or population of Cycle 0 of recurrent selection program aiming anthracnose resistance. Eight samples of 250 seeds from the bulk of F_2 seeds (S_0) were sown in polystyrene trays with individualized cells containing Plantmax® substrate. The seedlings of each sample were inoculated with one different *C. lindemuthianum* strain (Table 1). The inoculum preparation and inoculation were performed as previously mentioned for the evaluation of the common bean lines that formed the base population. Ten days after inoculation, about eight plants from each eight samples of 250 S_0 plants, with few or no symptoms of anthracnose, were selected and transplanted to 10-liter pots containing previously fertilized soil and Rohrbacher® substrate in the 2:1 ratio, respectively. Three or two plants were transplanted per pot. The plants selected for resistance to each C. *lindemuthianum* strain were then intercrossed with the resistant plants selected to the other strains. The seeds from self-fertilization of the selected plants were individually harvested and formed the $S_{0:1}$ progenies of cycle I (CI).

The hybrid seeds from intercross of the selected resistant plants were then divided in eight samples of about 60 "F_1" seeds. In this case, the separation of the seeds was not completely random. Hybrids seeds from different crosses were distributed among the eight samples to be inoculated with the eight strains of *C. lindemuthianum* (Table 1). Eight individual plants in each sample, with few or no symptoms of anthracnose, from hybrids that were also resistant to most strains, were selected, transplanted and recombined. The seeds from self-fertilization formed the $S_{0:1}$ progenies of cycle II (CII) and the "F_1" seeds from the recombination of selected plants were evaluated and selected for resistance to the eight *C. lindemuthianum* strains to posteriorly form the cycle III (CIII). The same procedure was adopted to obtain the progenies of cycle IV (CIV) and cycle V (CV). Seeds of about 50 $S_{0:1}$ progenies from each five selective cycles were sown under field conditions in July (2016), October (2016), February (2017), July (2017), and October (2017) to obtain the $S_{0:2}$ progenies of CI, CII, CIII, CIV and CV, respectively. As the selection and recombination were performed using F_1 (S_0) seeds, it was possible to obtain three selective cycles per year. A flowchart of the methodology used to obtain the base population and progenies to cycle I to V can be found in Figure S1.

Evaluation of $S_{0:2}$ progenies of cycles I to V

The $S_{0:2}$ progenies of the five cycles were separately evaluated for the reaction to five *C. lindemuthianum* strains, being two belonging to race 65 (Lv134 and Lv238) and one of

each 73, 81 and 89 races (Table 1). Furthermore, all progenies were also evaluated for an equitable mixture of conidial suspension (1.2 x 10^6 conidia.ml^{-1}) of the eight *C. lindemuthianum s*trains (Table 1). To do so, it was performed six different experiment for evaluation of the progenies to the five *C. lindemuthianum* strains and for the mixture of the eight strains. It was used 40 $S_{0:2}$ progenies from each cycle, except for the CIV for which it was obtained only 38 progenies, totaling 198 treatments. All six experiments were performed in a randomized complete blocks design with 3 replicates in time, being 9 seedlings per plot. The experiments were carried out under greenhouse conditions with temperature and humidity control system (95% relative humidity, at 24°C for three days after inoculation and 75% relative humidity for more seven days before evaluation). The inoculum preparation, inoculation and evaluation followed the same procedures already described for the evaluation of the ten lines that formed the base population. Common bean cultivar Pérola was used as susceptible control in all the six experiments.

Data analysis

For the six experiments, the mean of disease severity scores of the plots of each $S_{0:2}$ progeny from the five recurrent cycles were submitted to variance analysis, considering all the effects as random, except the mean. The genetic variance ($\hat{\sigma}_G^2$), phenotypic variance ($\hat{\sigma}_F^2$) and broad-sense heritability (h^2) were estimated according to Ramalho et al. (1993). The h^2 confidence interval was calculated according to the equations proposed by Knapp et al. (1985). As measure of experimental quality, the selective accuracy ($\hat{r}_{\hat{g}g}$) was also calculated for all experiments (Resende and Duarte, 2007). The joint variance analysis considering all the six experiments was carried out to measure the effect of progenies x strains interaction.

The adjusted mean of disease severity scores of $S_{0:2}$ progenies from cycle I to cycle V for each experiment were used to obtain linear regressions equations, considering the selection cycles as independent variable (x) and the mean of $S_{0:2}$ progenies from each cycle as dependent variable (y). The percentage genetic progress (GP) achieved, for resistance to each *C. lindemuthianum* strain, per cycle, compared to the mean of $S_{0:2}$ progenies from CI was then estimated as follows: $PG(\%) = \left(\frac{b_1}{\overline{X}_{CI}}\right) * 100$, where b_1 is the linear regression coefficient and $\overline{X}_{CI}$ is the mean of $S_{0:1}$ progenies of CI.

The frequency of favorable alleles was estimated in each cycle considering the percentage of resistant $S_{0:2}$ progenies to none, one, two, three, four and five strains of *C. lindemuthianum*. The effect in the disease severity scores of the progenies inoculated with the

mixture of different *C. lindemuthianum* strains, compared with inoculations of one strain at a time was also evaluated.

Results

Reaction of the common bean lines that formed the base population and the $S_{0:2}$ progenies to different *C. lindemuthianum* strains

Great variability for resistance to *C. lindemuthianum* strains used was observed among the common bean lines that formed the base population of this recurrent selection program. Some cultivars were resistant to several strains but none of them was resistant to all the strains used. In fact, all cultivars were susceptible when inoculated with the mixture of the eight *C. lindemuthianum* strains. However, Madrepérola and Cometa showed milder anthracnose symptoms (Table 2).

Table 2 Mean of Anthracnose severity scores of the common bean lines that formed the base population of the recurrent selection program to different *C. lindemuthianum* strains

Bean Lines	Race 65 (Lv134)	Race 65 (Lv238)	Raca 65 (Cl1532)	Raça 65 (Cl1610)	Raça (Cl1614)	Race 73	Race 81	Race 89	Mix[2]
BRS Cometa	7.1[1]	2.0	1.0	1.0	1.0	2.0	9.0	7.0	4.5
BRSEsplendor	1	1	2.1	9.0	1	8.3	4.2	6.5	5.5
Madrepérola	1.0	7.0	1.0	1.0	7.0	1.0	6.0	1.0	4.0
Valente	1.1	9.0	8.0	3.0	1.2	8.0	1.0	9	8.2
Ouro Negro	9.0	1.0	1.0	1.2	1.3	9.0	8.5	5.0	7.5
Majestoso	9.0	7.4	1.0	1.0	1.0	1.0	1.0	9.0	7.4
BRS Estilo	8.2	1.0	1.3	1.0	7.0	1.0	9.0	1.6	7.0
BRS Uai	8.0	1.0	6.0	1.0	3.0	2.0	9.0	4	7.2
M20	4.0	7.0	3.0	6.0	6.0	7.0	9.0	3.0	9.0
MAIII 16.159	2.0	2.0	6.0	7.0	6.0	9.0	1.0	1.0	6.5

[1]Anthracnose severity score according to the descriptive scale from 1 to 9 (Schoonhoven, Pastor-Corrales, 1987) where score "1" indicates absence of symptoms and score "9" indicates dead plant.
[2] Equitable mixture of conidial suspension from each of the five *C. lindemuthianum* strains.

The $S_{0:2}$ progenies from the five selective cycles were individually evaluated in six different experiments for the reaction to the strains of races 73, 81 and 89 and for the reaction to strains Lv134 and Lv238 of race 65 of *C. lindemuthianum*. The progenies were also evaluated for the reaction to the mixture of all eight strains used in this study (Table 1). There was significant difference ($P<0.01$) among the $S_{0:2}$ progenies for anthracnose severity scores in all cycles for all of the five strains inoculated separately and also for the mixture of the eight *C. lindemuthianum* strains. The estimates of genetic and phenotypic parameters confirm

the existence of variability among the progenies in all selective cycles for the reaction to each *C. lindemuthianum* strain used (Table S2). Although the disease severity scores of the $S_{0:2}$ progenies ranged to 1 to more than 7 in almost all cycles for all strains (Table S2), the general mean of the $S_{0:2}$ progenies were less than 5 since the first selective cycle to all strains and tended to decrease over the selective cycles due to the increase in the number of resistant cultivars (Table 3, Figure 1). The mean of anthracnose severity scores of the susceptibility control (Pérola) was always higher than 7 in all experiments, confirming that the inoculation was effective. The estimates of heritability (h^2) associated with the mean of progenies ranged from 86 to 97% in all the six experiments carried out (Table 3) and the lower limits, even within each cycle (Table S2), were always positive (Table 3), indicating that the estimates obtained are different from zero at the 95% confidence level. The short range between the lower and upper limits indicates that the h^2 values obtained were well estimated. Moreover, the selective accuracy estimates ($\hat{r}_{\hat{g}g}$) obtained in all experiments was close to 1, which highlights the good quality of the experiments (Table 3).

The joint variance analysis was carried out to the six experiments and the effect of progenies x strains interaction was also significant, therefore, the reaction of the $S_{0:2}$ progenies were not consistent when they were inoculated with the different strains (Table S3).

Table 3 Means of anthracnose severity scores of the $S_{0:2}$ progenies from the five recurrent selection cycles against different *C. lindemuthianum* strains and for the mixture of eight different *C. lindemuthianum* strains and estimates of genetic variance ($\hat{\sigma}_G^2$), phenotypic variance ($\hat{\sigma}_F^2$) broad-sense heritability (h^2) and selective accuracy ($\widehat{r}_{\hat{g}g}$) of the six experiments performed.

	Race 65 (Lv134)	Race 65 (Lv238)	Race 73	Race 81	Race89	Mixture
	Mean of anthacnose severity score					
Progenies	3.77	2.65	2.83	2.67	2.74	4.20
CI	4.36	3.45	3.67	3.36	3.37	4.80
CII	3.73	2.91	2.89	2.84	2.89	4.44
CIII	4.28	2.59	2.79	2.61	2.64	4.43
CIV	3.81	2.4	2.74	2.51	2.64	4.41
CV	2.66	1.87	2.09	2.01	2.14	2.94
$\hat{\sigma}_G^2$	4.8	2.49	3.13	2.53	2.63	3.99
$\hat{\sigma}_F^2$	5.08	2.76	3.63	2.82	2.89	4.12
h^2	94.63 (93.1-95.8)[1]	90.34 (87.7-92.5)	86.09 (82.3-89.1)	89.76 (87.0-92.0)	91.09 (88.7-92.1)	96.85 (96.0-97.5)
$\hat{r}_{\hat{g}g}$	97.28	95.05	92.78	94.74	95.44	98.41

[1]Lower and upper limits of heritability confidence interval

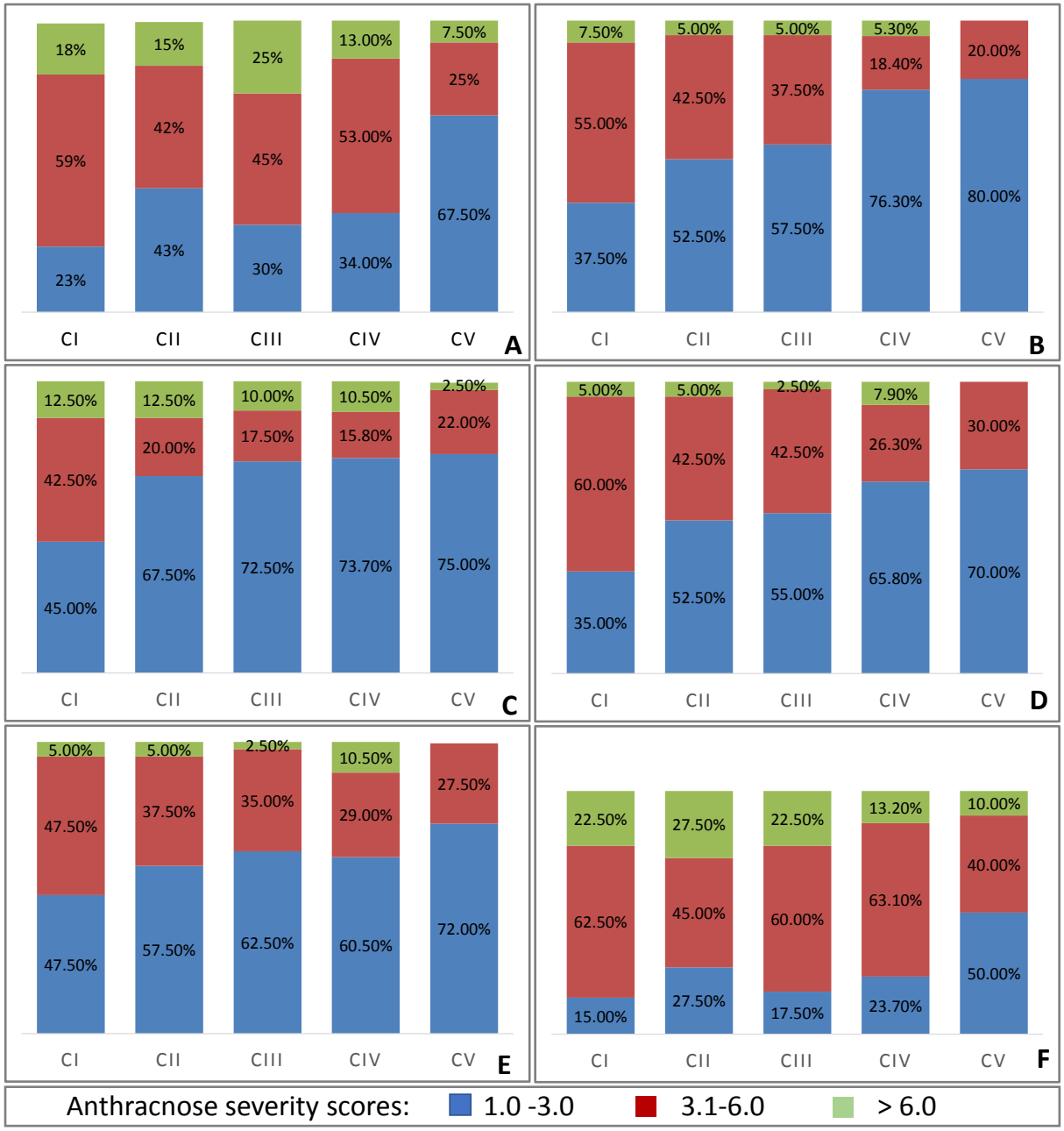

Figure 1. Frequency of $S_{0:2}$ progenies in five recurrent selection cycle according to the range of anthracnose severity scores. *C. lindemuthianum* strains: A) Race 65 (Lv134); B) Race 65 (Lv238); C) Race 73; D) Race 81; E) Race 89; F) Mixture of eight different *C. lindemuthianum* strains.

Genetic progress in the five recurrent selection cycles aiming anthracnose resistance

The negative value of linear regression coefficient (b_1) of the linear regression equations confirms that the mean of anthracnose severity scores of the $S_{0:2}$ progenies decreased over the five recurrent selection cycles for all *C. lindemuthianum* strains and for the mixture of eight strains, that is, the resistance level increased with selection (Figure 2). Taking

as reference the $S_{0:2}$ progenies of the first cycle, the estimates of selection gain per cycle was 7.47%, 10.68%, 9.04%, 8.99%, 8.03% and 7, 81% for the Race 65 (Lv134), Race 65 (Lv238), Race 73, Race 81, Race 89 and for the mixture of different *C. lindemuthianum* strains, respectively. Except for one strain of the race 65, Lv134 and for the mixture of different strains, in general the coefficients of determination (R^2) were close to 1, which shows that the data had a good fit in the linear regression model (Figure 2).

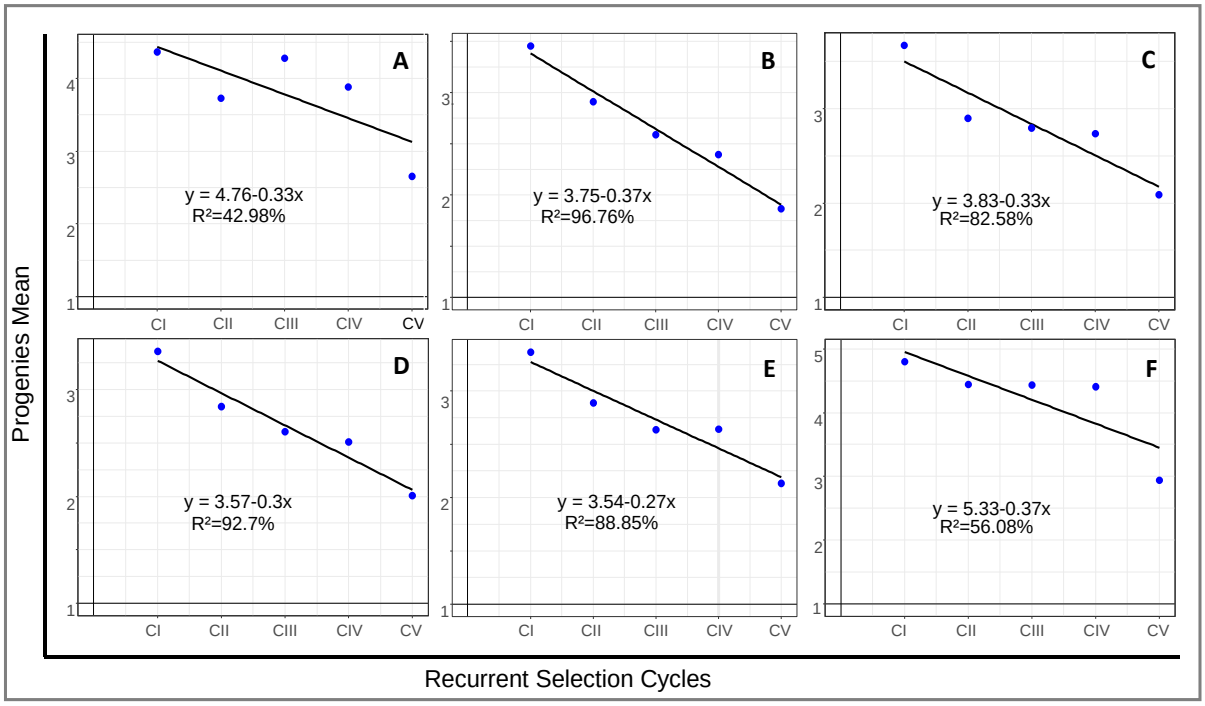

Figure 2. Linear regression for the mean of anthracnose severity scores of the $S_{0:2}$ progenies from selective cycles I to V of recurrent selection. *C. lindemuthianum* strains: A) Race 65 (Lv134); B) Race 65 (Lv238); C) Race 73; D) Race 81; E) Race 89; F) Mixture of eight different *C. lindemuthianum* strains.

Frequency of progenies with favorable alleles in the five recurrent selection cycle

Considering as resistant the $S_{0:2}$ progenies that present the mean of anthracnose severity scores lower or equal to 3 according to the descriptive scale from 1 to 9 (Schoonhoven and Pastor-Corrales, 1987), in the present study there was an increase in the number of resistant $S_{0:2}$ progenies to more strains over the selective cycles (Figure 3). In the first recurrent selection cycle, 13 $S_{0:2}$ progenies were susceptible for all strains used. However, in the fifth cycle most of the progenies were resistant to the five strains. In other words, there was an increase in the number of progenies with higher frequency of favorable alleles for anthracnose resistance from cycle I to cycle V. From CII to CIII there was decrease

in a number of $S_{0:2}$ progenies resistant to five strains. This happened because in the CIII there was a decrease in a number of resistant cultivars to strain Lv134 of race 65 (Figure 1).

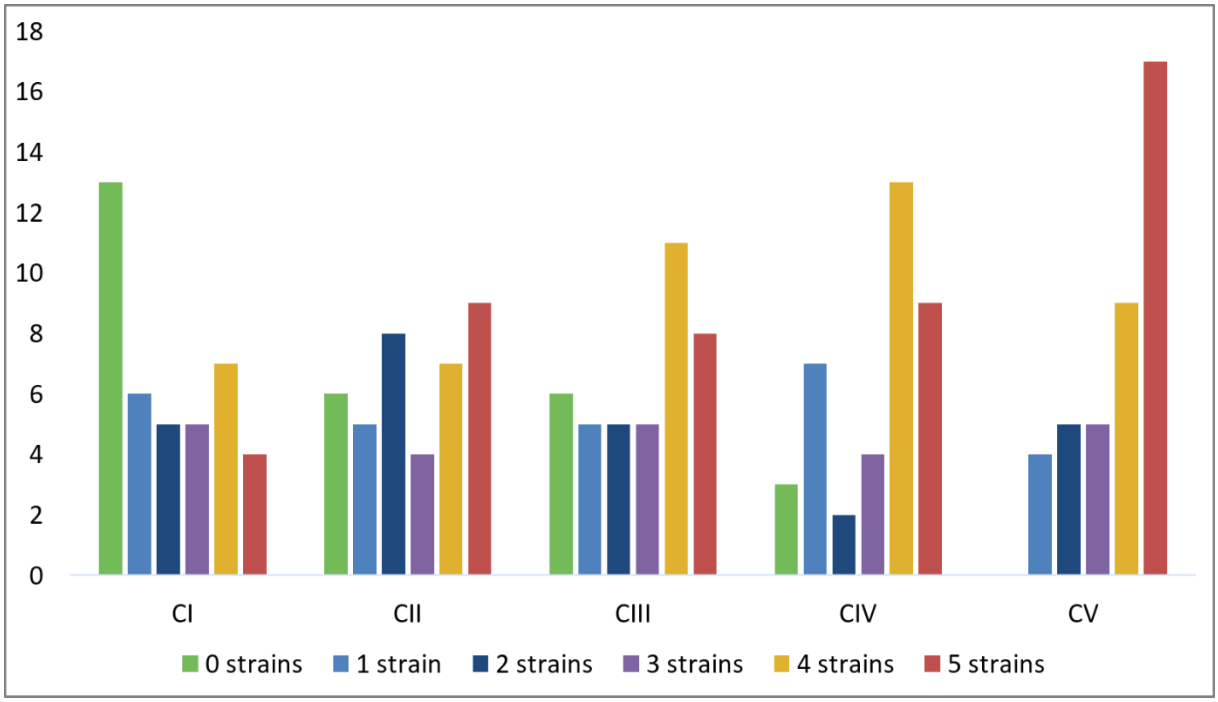

Figure 3. Frequency of progenies with favorable allelos (anthracnose severity score 1.0 – 3.0) to none, one, two, three, four and five *C. lindemuthianum* strains in the five recurrent selection cycles aiming anthracnose resistance

The reaction of the progenies was more similar among the strains of race 73, 81, 89 and race 65 (Lv238) (Figure 4). The race 65 (Lv134) were the most virulent to the progenies. The reaction of the progenies for this strain were less coincident compared with the other strains, especially in the first cycle of recurrent selection (Figure 4). In addition, although Lv134 and Lv238 belong to race 65, the progenies have a reaction different to them (Figure 5).

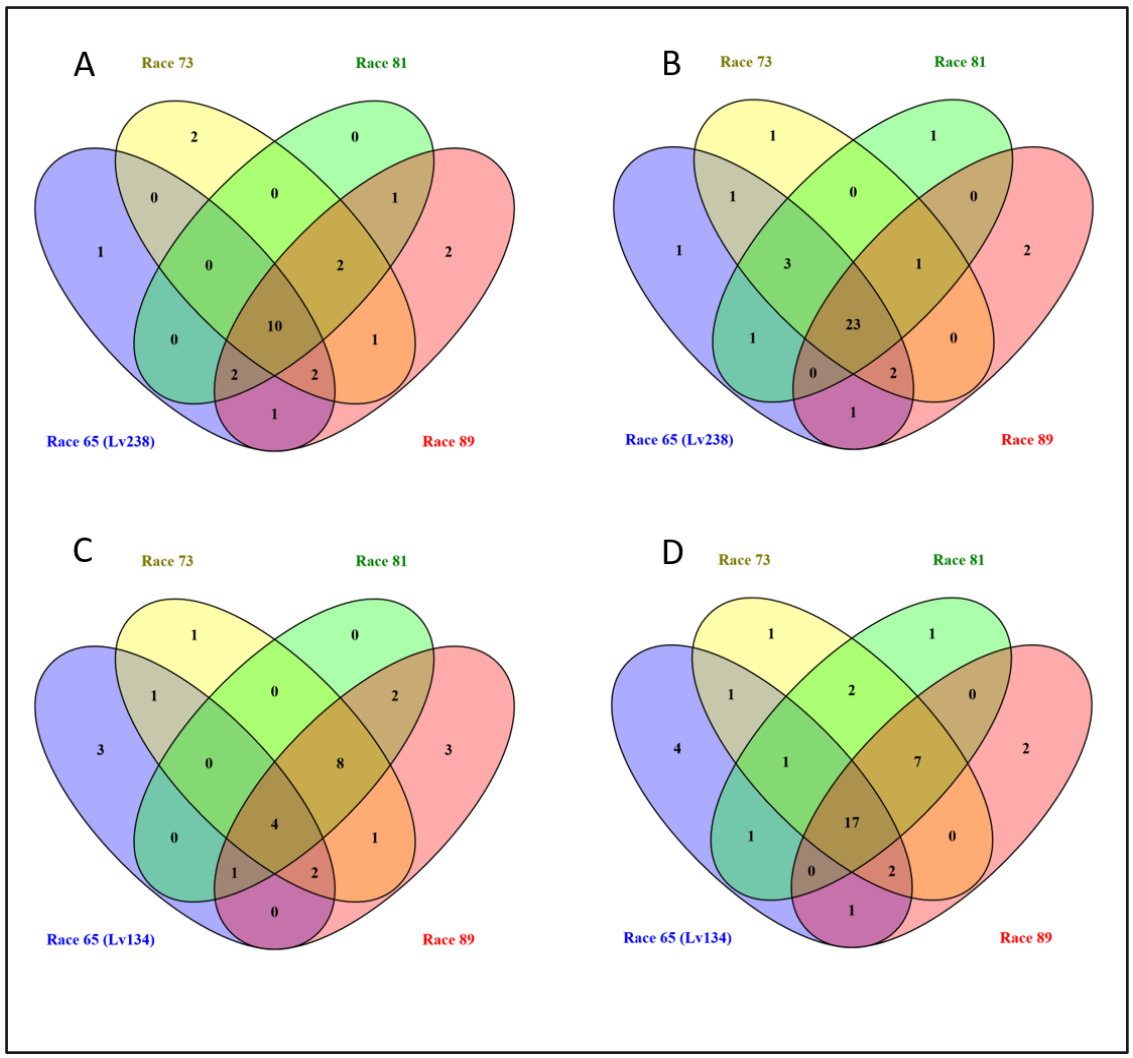

Figure 4. Coincident level of resistant progenies (score 1-3) regarding Cycle I (A) and Cycle V(B) among the strains of races 73, 81, 89 and Lv238 (race 65) and regarding cycle I (C) and cycle V (D) among strains of races 73, 81, 89 and Lv134 (race 65)

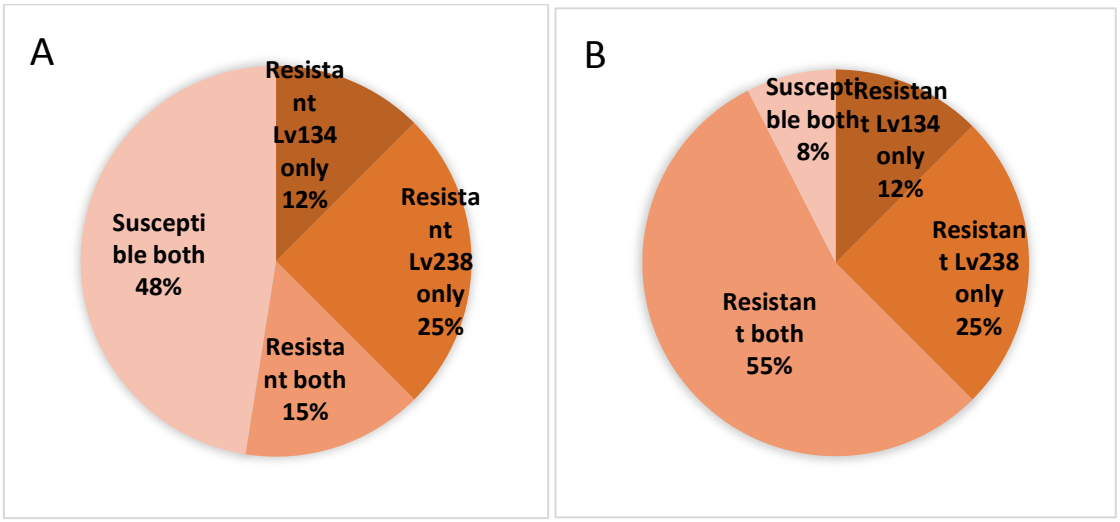

Figure 5. Reaction of the progenies to the strains Lv134 and Lv238 of race 65 of *C. lindemuth*ianum in the first (A) and fifth cycle (B) of recurrent selection

Inoculation using mixture of different *C. lindemuthianum* strains

Resistant progenies to the equitable mixture of eight different *C. lindemuthianum* strains were obtained since the first recurrent selection cycle (Figure 1). Considering three phenotypic classes according to the disease severity score: 1-3: Resistant; 3.1-6.0: Moderately resistant; 6.1-9.0: Susceptible, the mixture presented similar virulence in the progenies compared with the strain of higher virulence inoculated individually. For instance, if a progeny was susceptible to at least one strain, this progeny was also susceptible to the mixture of eight different strains. Nevertheless, the virulence of the mixture was lower in 13% of the progenies, compared with the strains of higher virulence inoculated independently (Figure 6). For the common bean cultivars Cometa and Madrepérola the inoculation with the mixture of different *C. lindemuthianum* strains also resulted in milder symptoms compared with the inoculation with some more virulent strains inoculated individually (Table 2). Conversely, 3% of the progenies were susceptible to the mixture and moderately resistant for each one of the five strains inoculated individually.

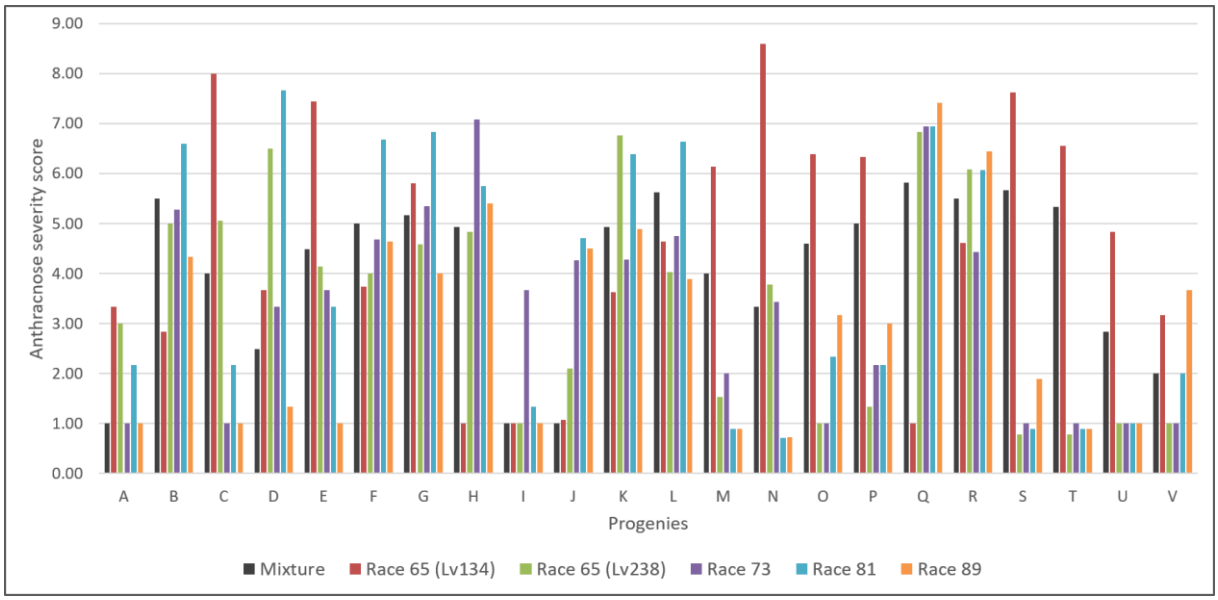

Figure 6. Comparison among the virulence of the mixture of eight different *C. lindemuthianum* strains and five strains inoculated individually in 22 $S_{0:2}$ progenies from different cycles.

DISCUSSION

The use of genetic resistance is certainly the main component of the integrated common bean anthracnose management. The current common bean lines are more resistant to anthracnose compared with the lineages of the past. However, it is still challenging to obtain common bean cultivars with durable resistance to *C. lindemuthianum* since this pathogen presents great genetic variability. This variability is generated through sexual (meiosis) and different asexual (hyphae and conidial anastomoses) recombination mechanisms (Castro-Prado et al., 2007; Pinto et al., 2012), which can also explain the chromosomal polymorphism that has been observed within this species (Gonçalves, 2016). Furthermore, especially in Brazil, where common bean is cultivated all year long in different regions, under different environmental conditions and production systems, the plasticity of *C. lindemuthianum* genome contributes to the rapid evolution and adaptation of the pathogen to these different conditions, which increases the genetic variability of the species. There is variability even within *C. lindemuthianum* races (Rodríguez-Suárez et al., 2005; Davide and Souza, 2009; Costa et al., 2017). For this reason, obtaining resistant cultivars to the main races of *C. lindemuthianum* that occur in the regions where common bean is cultivated is an ongoing process. Therefore, the breeding strategies aimed at durable resistance to this fungal disease should be dynamic and consider the great evolutionary potential of this pathogen to overcome resistance.

In the present study, a recurrent selection program as alternative to obtain durable resistance to different *C. lindemuthianum* strains, belonging to the main races that occur in Brazil, was initiated. It is the first time that this method has been used aiming anthracnose resistance in common bean. Five cycles of selection, recombination and evaluation were conducted. As the reaction of the common bean to *C. lindemuthianum* is a trait of high heritability, the selection of resistant plants, by artificial inoculations, was carried out exclusively in greenhouse using S_0 population. This strategy has been successfully used to obtain resistant cultivars to *Pseudocercopora griseola*, another important fungal disease that affect the crop (Librelon, 2006; Pádua, 2017). Despite the predominance of vertical resistance in the pathosystem *P. vulgaris*-*C. lindemuthianum*, considering the several major genes already identified, conferring resistance to specific races (Ferreira et al., 2013; Oblessuc et al., 2014; Castro et al., 2017; Valentini et al., 2017) or specific strains of a same race (Costa et al., 2017) and the minor genes that have also been reported in some studies (Oblessuc et al., 2014; Perseguini et al., 2016; Zuiderveen et al., 2016), the interaction of *P. vulgaris* - *C.*

lindimuthianum displays a polygenic inheritance. Therefore, the recurrent selection is a good alternative to accumulate these anthracnose resistance genes. After five selective cycles, considerable genetic progress was achieved for resistance to five *C. lindemuthianum* strains and also for a mixture of eight different *C. lindemuthianum* strains (Figure 2).

In general, the coefficients of determination (R^2) of the linear regression equation, for the most strains, were very high, close to 1 (Figure 2), which indicate that the data had a good fit in the linear regression model. However, the mean of anthracnose severity score of the $S_{0:2}$ progenies, inoculated with race 65 (Lv134) did not decrease linearly over the selective cycles and, therefore, the R^2 was lower for this strain (42.98%). There was even an increase in the mean of anthracnose severity scores of the $S_{0:2}$ progenies from CII to CIII when inoculated with this strain (Table 3) and, consequently, a decrease in the number of $S_{0:2}$ progenies resistant to the five strains from CII to CIII (Figure 2). One possible explanation is that the selected plants and recombination occurred in that cycle may not have favored the resistance to this strain. The genetic gain was also not linear when the progenies were inoculated with a mixture of eight different *C. lindemuthianum* strains. In this case, the mean of disease severity score of the progenies from CII to CIV were practically constant, since, probably, several resistance alleles were required to get resistance to all *C. lindemuthianum* strains of the mixture.

In the very first cycle, four progenies were resistant (disease severity score ≤ 3) to the five strains assessed. However, in the first selection cycle, 13 out of 40 $S_{0:2}$ progenies (32.5%) were susceptible to all five strains of *C. lindemuthianum* (Figure 3). This number decreased over the selective cycles and, conversely, the number of progenies, resistant to the five strains used increased from the first to the last cycle, which demonstrate the efficacy of the method (Figure 3). Some $S_{0:2}$ progenies were resistant to none or just few *C. lindemuthianum* strains even in the last cycles (Figure 5). However, throughout the five cycles of selection and recombination, the plants were evaluated and selected for resistance to three more *C. lindemuthianum* strains, Cl1610. Cl 1614, Cl1532 (Table 2) for which the $S_{0:2}$ progenies were not assessed individually. Therefore, those $S_{0:2}$ progenies may also be resistant to some of those three other *C. lindemuthianum* strains.

The coincident level for the resistant progenies among the strains of *C. lindemuthianum* were also analyzed (Figure 4). Ten out of 40 progenies were resistant to the strains of races 73, 81, 89 and 65 (Lv238) in the first cycle. This number has reached 23 in the last cycle. Considering the other strain of race 65, Lv134 and races of 73, 81 and 89, the coincidence level for resistant to these four strains were 4 and 17 progenies, respectively.

Indeed, the race 65 (Lv134) were the most virulent strains for the progenies (Figure 1). Among the 47 progenies that were resistant to any four of the five strains evaluated only eight were resistant to race 65 (Lv134) (Figure 3). Furthermore, the progenies presented different reaction to strains Lv134 e Lv238, even in the last cycle (Figure 5), which highlights the variability within strains of this race that have been reported (Costa et al., 2017; Ishikawa et al., 2011; Davide; Souza, 2009).

Evaluations by artificial inoculation of different *C. lindemuthianum* strains can be laborious, time consuming and requires large amount of seeds. An alternative would be the use of mixtures of different races of this pathogen. The feasibility of this alternative has been studied and demonstrated to be efficient to select resistant cultivars to three different races using a single inoculation (Falleiros et al., 2018). For the large majority of the progenies evaluated in the present study, the mixture presented similar virulence compared with the strains of higher virulence inoculated individually. Therefore, in a recurrent selection program, in which the objective is to accumulate favorable alleles gradually, without exhausting the genetic variability of the population, the use of a mixture of several *C. lindemuthianum* strains may be not the most appropriate strategy. If a genotype presents resistance-race/strain-specific and it is susceptible to at least one strains of a mixture of different strains, this genotype should be also susceptible to the mixture. So, if only an inoculation with the mixture of strains is used to select resistant genotypes, for the recombination in each recurrent selection cycle, a large number of promising genotypes, resistant to several *C. lindemuthianum* strains could be discarded. This is a very important point to be considered in *C. lindemuthianum* - *P. vulgaris* pathosystem, since several major resistance genes to specific race/strains are involved in the resistance (Ferreira et al., 2013; Murube et al., 2019).

In the present study, for 25 out of 198 progenies evaluated (13%), the mixture was less virulent compared with some of the five strains inoculated individually (Figure 6). The same thing was observed when the common bean cultivars, Cometa and Madrepérola, were inoculated with the mixture of different strains (Table 2). As the mixture was composed by eight different *C. lindemuthianum* strains, the competition among the strains of the mixture, may have disadvantaged the strain(s) of higher virulence. Another explanation is the possible synergistic interaction among the strains present in the mixture. This effect has been reported among different microbial pathogens, including fungi (Lamichhane and Venturi, 2015). The synergistic effect among the *C. lindemuthianum* strains of a mixture can also contribute for the higher virulence of the mixture in common bean compared with the inoculation of the

strains separately. This fact has already been verified in mixtures of different *C. lindemuthianum* strains (Falleiros et al., 2018; Chilipa et al., 2016) as well as in mixture of different *P. griseola* strains (Pereira et al., 2010). In the present study, six out of 198 (3%) progenies were higher virulent to the mixture compared with the five strains individually assessed. Nevertheless, this may also have happened due to the other three race 65 strains of *C. lindemuthianum* present in the mixture, Cl1532, Cl1610 and Cl1614 (Table 2) that were not assessed separately.

In some studies of recurrent selection aiming *P. griseola* resistance, it has been observed that the reaction of the progenies to the pathogen may vary considering the artificial inoculation in greenhouse and the natural occurrence of the pathogen in common bean field (Librelon, 2016; Pádua, 2017). In this photosystem there is a predominance of quantitative resistance and occurs the so-called resistance of adult plants, which is not expressed in stage V2, in which the reaction to the disease is commonly evaluated in greenhouse (Costa et al., 2006; Pereira et al., 2017). The difference of the reaction of the plants evaluated in greenhouse and field also might occur, especially, because in field conditions other virulent strains of the pathogen can be present. However, different of *P.griseola*, in which its spores spread through the wind, *C. lindemuthianum* spores does not spread so much by the action of the wind. Thus, the evaluation by natural occurrence of the pathogen is more difficult and should be done only in some region of historical and high occurrence of the disease.

As this pathogen have great evolutionary potential, the breeders should obtain new *C. lindemuthianum* strains, periodically, in the regions where common bean is cultivated, for the use in artificial inoculations in greenhouse to select resistant plants. However, the evaluation of the common bean for a large number of *C. lindemuthianum* strains individually, that represent the population of the pathogen in the fields, is unfeasible. In this case, instead of using a mixture with several *C. lindemuthianum* strains, an alternative would be the use of several mixtures with few *C. lindemuthianum* strains. The breeders may also choose to evaluate the progeny for the reaction to a few more virulent strains at a first time and then, to evaluate only the cultivars resistant to these first strains for the reaction to other strains.

In all selective cycles conducted, only common bean plants with few or no symptoms of anthracnose were selected. As a large number of plants with no symptoms were selected, the selection probably most favored the qualitative resistance genes, which is conferred by major genes and provides complete race-specific-resistance (Vanderplank, 1963). When a genotype presents qualitative resistance, it is not meaning that the quantitative resistance is not present, it also can be there but its expression is inhibited due the presence of qualitative

resistance (Parlevit,1981). The quantitative resistance, which has also been identified in pathosystem *P. vulgaris-C. lindemuthianum* (Oblessuc et al., 2014; González et al., 2015) is conferred by many genes of small effect, which are effective for a wide spectrum of pathogenic races and, therefore, can be more stable and durable. In this type of resistance, intermediate degrees of resistance occur and there are reactions since maximum susceptibility up to maximum resistance. For this reason, to ensure the selection of genotypes for both types of resistance, qualitative and quantitative, besides plants with no symptoms, plants with intermediate disease severity scores, around 3-4 should be selected. The combination of the two types of resistance can provide more effective and durable anthracnose resistance.

Many of resistant genes against different *C. lindemuthianum* strains have been mapped in different linkage groups of common bean (Richards et al., 2014; Meziadi et al., 2016). The common bean genome sequencing (Schmutz et al., 2014) has revealed most of cloned resistance genes, which encodes intracellular proteins containing a nucleotide-binding site and a C-terminal leucine rich-repeat domain (NB-LRR proteins) (Nelson et al., 2018), are frequently organized in large clusters located in the subtelomeric regions of the chromosomes. This suggests that these regions in common bean are favorable "niches" for resistance gene proliferation. Indeed, many resistant genes to different pathogens have been identified in these regions. In the short arm of Pv04, for instance, where there is one of the largest clusters of resistance genes in common bean, many different race-specific genes to *C. lindemuthianum* have been identified (Murube et al., 2019). In this context, recurrent selection is an interesting alternative. Since this method involves a selection and recombination step of the most resistant genotypes in each cycle, the favorable combinations can be constantly explored through genetic recombination, especially within those clusters. In this way, the host can respond dynamically to the high genetic variability of the pathogen.

It is important to highlight that the bulk method within progenies is widely used in recurrent selection programs for conducting segregating populations (Moreira et al., 2010). The selection is made only among progenies and, therefore, at the end of the process, usually in $F_{2:6}$ or $S_{0:6}$, the progenies are composed by a mixture of pure lines, which gives them, greater stability, as well as in a multiline formed by a mixture of different lines. Recently, the use of multilines has been efficient to decrease anthracnose severity (Carvalho, 2018; Botelho et al., 2009. Carvalho (2018) observed that the common bean cultivar MAV-336, which came from a recurrent selection program aiming at resistance to angular spot in common bean, presented similar or greater stability of resistance compared with multilines composed by different common bean lines. This most probably happened because the cultivar MAV-336 is

a mixture of pure lines, with several fixed resistant alleles.

Although pyramiding of resistance genes has been suggested for increase the durability of resistance (Brown, 2015; Consortium, 2016), the gene pyramid, in a single line of common bean, favor the directional selection in *C. lindemuthianum* population. As this pathogen has several recombination mechanisms, new virulence alleles can be created in response to selection imposed by the resistance genes pyramid, leading to a rapid resistance breakdown (Mcdonald and Linde, 2002). On the other hand, the common bean cultivars that come from recurrent selection aiming anthracnose resistance, besides being a mixture of pure lines and presenting all the advantages of a multiline, they can also present resistance genes pyramid in each pure line, since there is a recombination step in each recurrent selection cycle. Therefore, due to the stabilizing selection in the pathogen population, it is expected that the cultivars obtained by this method will present more durable resistance. Interestingly, in recurrent selection for anthracnose resistance is possible to select for other important traits simultaneously, for example, grain type and grain yield, in order to maintain the homogeneity for agronomic traits and to avoid the effect of intergenotypic competition. Furthermore, different of a multiline composed by a mixture of different common bean lines, it is not necessary to mix the common bean lines at each crop season since the cultivar is already a mixture of pure lines.

Since great variability among the progenies was detected even after the fifth cycle, the recurrent selection for resistance to *C. lindemuthianum* should be continued. It important emphasize that in this study was carried out three selective cycles per year, which contradict the argument that recurrent selection is a slow process. Moreover, the process is dynamic, new sources of genetic resistance can be recombined together with the selected resistance plants in any cycle.

Conclusion

Recurrent selection with the use of artificial inoculation of different *C. lindemuthianum* strains was an efficient method to obtain resistant progenies to different races of the pathogen.

References

Arantes, L.D.O., A.D.F.B. Abreu, and M.A.P. Ramalho. 2010. Eight cycles of recurrent selection for resistance to angular leaf spot in common bean. Crop Breed. Appl. Biotechnol. 10(2010): 232–237.

Botelho, F.B.S., M.A.P. Ramalho, A.D.F.B. Abreu, and H.J.A. Rosa. 2011. Multiline as a Strategy to Reduce Damage Caused by *Colletotrichum lindemuthianum* in Common Bean. J. Phytopathol. 159(3): 175–180.

Broughton, W., G. Hernandez, M. Blair, S. Beebe, P. Gepts, et al. 2003. Beans (Phaseolus spp.) - model food legumes. Plant Soil 252: 55–128.

Brown, J.K.M. 2015. Durable Resistance of Crops to Disease: A Darwinian Perspective. Annu. Rev. Phytopathol. 53(1): 513–539.

Carvalho, S.G.M. Misturas de feijão carioca como estratégia na obtenção de resistência durável a diferentes raças de *Colletotrichum lindemuthianum.* 2018. 58p. Dissertação (Mestrado em Genética e Melhoramento de Plantas) – Universidade Federal de Lavras, Lavras.

Castro-Prado, M.A.A., C.B. Querol, J.R. Sant'Anna, C.T. Miyamoto, C.C.S. Franco, et al. 2007.
Vegetative compatibility and parasexual segregation in *Colletotrichum lindemuthianum*, a fungal pathogen of the common bean. Genet. Mol. Res. 6(3): 634–642.

Castro, S.A. de L., M.C. Gonçalves-Vidigal, T.A.S. Gilio, G.F. Lacanallo, G. Valentini, et al. 2017.
Genetics and mapping of a new anthracnose resistance locus in Andean common bean Paloma. BMC Genomics 18(1): 1–12.

Consortium, R.E.X. 2016. Combining Selective Pressures to Enhance the Durability of Disease Resistance Genes. Front. Plant Sci. 7(December): 1–8.

Chilipa L.N.K, L Tembo. 2016. Multiple race inoculation as an option in breeding for resistance to *Colletotrichum lindemuthianum* in com- mon beans. Journal ofAgriculture and Crops 2:45–50

COSTA, I.F.D. et al. 2006. Resistance of six soybean cultivars to Colletotrichum truncatum (Schwein) in two phenologic stages. *Ciência Rural*, Santa Maria, v. 36, n. 6, p. 1684-1688.

Costa, L.C., R.S. Nalin, M.A.P. Ramalho, and E.A. De Souza. 2017. Are duplicated genes responsible for anthracnose resistance in common bean? PLoS One 12(3): 1–15.

Davide, L.M.C., and E.A. De Souza. 2009. Pathogenic variability within race 65 of *Colletotrichum lindemuthianum* and its implications for common bean breeding. Crop Breed. Appl. Biotechnol. 9: 23–30.

Falleiros, M.O., S.F. Mota, A.N. Ferreira, and E.A. de Souza. 2018. Mixture of

Colletotrichum lindemuthianum races in anthracnose resistance screening and its implication for common bean breeding. Trop. Plant Pathol. 43(3): 271–277.

Ferreira, J.J., A. Campa, and J.D. Kelly. 2013. Organization of Genes Conferring Resistance to Anthracnose in Common Bean. In: Varshney, R.K. and Tuberosa, R., editors, Translational Genomics for Crop Breeding. 1st ed. p. 151–176

Garzón, L.N., G.A. Ligarreto, and M.W. Blair. 2008. Molecular marker-assisted backcrossing of anthracnose resistance into Andean Climbing beans (*Phaseolus vulgaris* L.). Crop Sci. 48(2): 562–570.
Gonçalves, P.R.C. 2016. Cariotipagem de Linhagens de *Colletotrichum lindemuthianum* E *Glomerella* spp. Isoladas de Lesões De Antracnose no Feijoeiro. Univ. Fed. Lavras.

González, A.M., F.J. Yuste-Lisbona, a P. Rodiño, A.M. De Ron, C. Capel, et al. 2015. Uncovering the genetic architecture of *Colletotrichum lindemuthianum* resistance through QTL mapping and epistatic interaction analysis in common bean. Front. Plant Sci. 6: 13.

Ishikawa, F.H., M.A.P. Ramalho, and E.A. Souza. 2011. Common bean lines as potential diffetential cultivars for race 65 of *Colletotrichum lindemuthianum*. J. Plant Pathol. 93(2): 461–464.

Knapp SJ, Stroup WW and Ross WM (1985) Exact confidence intervals for heritability on a progeny mean basis. Crop Science 25: 192-194.

Lamichhane, J.R., and V. Venturi. 2015. Synergisms between microbial pathogens in plant disease complexes: a growing trend. Front. Plant Sci. 06: 1–12.

Leite, M.E., J.A. Dias, D.A. de Souza, F.C. Alves, L.R. Pinheiro, et al. 2016. Increasing the resistance of common bean to *White mold* through recurrent selection. Sci. Agric. 73(1): 71–78.

LIBRELON, S. S. 2016. Seleção recorrente visando resistência à mancha angular no feijoeiro com inoculação artificial precoce. 70p. Tese (Doutorado em Genética e Melhoramento de Plantas). Universidade Federal de Lavras, Lavras.

Marcondes, E.H.K., H.S. Pereira, and J.B. Santos. 2010. Seleção de linhagens de feijoeiro com tipo de grão carioca e com os alelos Co-4 e Co-5 de resistência à antracnose. Ciência e agrotecnologia 34(4): 975–982.

Mcdonald, B.A., and C. Linde. 2002. The population genetics of plant pathogens and breeding strategies for durable resistance. Euphytica 124: 163–180.

Meziadi, C., M.M.S. Richard, A. Derquennes, V. Thareau, S. Blanchet, et al. 2016. Development of molecular markers linked to disease resistance genes in common bean based on whole genome sequence. Plant Sci. 242: 351–357.

Miklas, P.N., J.D. Kelly, S.E. Beebe, and M.W. Blair. 2006. Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. Euphytica 147(1–2): 105–131.

MOREIRA, S.O. et al. 2010. Breeding methods and history of bean cultivars released in CBAB - Crop Breeding and Applied Biotechnology. *Crop Breeding and Applied Biotechnology***,** v.10, p. 345-350.

Mundt, C.C. 2014. Durable resistance: A key to sustainable management of pathogens and pests. Infect. Genet. Evol. 27: 446–455.

Murube, E., A. Campa, and J. Jose. 2019. Integrating genetic and physical positions of the anthracnose resistance genes described in bean chromosomes Pv01 and Pv04. : 1–17.

Nelson, R., T. Wiesner-Hanks, R. Wisser, and P. Balint-Kurti. 2018. Navigating complexity to breed disease-resistant crops. Nat. Rev. Genet. 19(1): 21–33.

Oblessuc, P.R., R.M. Baroni, G. da Silva Pereira, A.F. Chiorato, S.A.M. Carbonell, et al. 2014. Quantitative analysis of race-specific resistance to *Colletotrichum lindemuthianum* in common bean. Mol. Breed. 34(3): 1313–1329.

Padder, B.A., P.N. Sharma, H.E. Awale, and J.D. Kelly. 2017. *Colletotrichum lindemuthianum*, the Causal Agent of Bean Anthracnose. J. Plant Pathol. 99(2): 317–330.

Parlevliet, J. E. 1981. Disease resistance in plants and its consequences for plant breeding. In: FREY, K. J. (Ed.). Plant breeding II. Iowa: The Iowa State University.

Pastor-Corrales, M.A. 1991. Estandarizacion de variedades diferenciales y de designacion de razas de *Colletotrichum lindemuthianum*. Phytopathology 81: 694.

Pádua, P.F. 2017 Viabilidade da condução de três ciclos de seleção recorrente visando à resistência a *Pseudocercospora griseola* no feijoeiro. 70p. Tese (Mestrado em Genética e Melhoramento de Plantas). Universidade Federal de Lavras, Lavras .

Ramalho M.A.P, J.B Santos and M.J.O. Zimmermann.1993 Genética quantitativa em plantas autógamas: aplicações ao melhoramento do feijoeiro. Editora UFG, Goiânia, 271p

Pereira, R., F.H. Ishikawa, J.M.A. Pinto, and E.A. de Souza. 2010. Occurence of anthracnose in common bean cultivars collected in the state of Minas Gerais, Brazil. Annu. Rep. Bean Improv. Coop. 53: 224–225.

Perseguini, J.M.K.C., P.R. Oblessuc, J.R.B.F. Rosa, K.A. Gomes, A.F. Chiorato, et al. 2016. Genome-Wide Association Studies of Anthracnose and Angular Leaf Spot Resistance in Common Bean (*Phaseolus vulgaris* L.). PLoS One 11(3): 1–19.

Pinto, J.M.A., R. Pereira, S.F. Mota, F.H. Ishikawa, and E.A. Souza. 2012. Investigating phenotypic variability in *Colletotrichum lindemuthianum* populations. Phytopathology 102(5): 490–497.

Ramalho, M.A.P. et al. 2005. Progresso genético do quinto ao sétimo ciclo de seleção recorrente no melhoramento genético do feijoeiro. In: CONGRESSO NACIONAL DE FEIJÃO, 8., Goiânia. Anais... Goiânia: Embrapa.

Resende, M.D.V., and J.B. Duarte. 2007. Precisão e controle de qualidade em experimentos

de avaliação de cultivares. Pesqui. Agropecuária Trop. 37(3): 182–194.

Rezende, B.A., A. F.B. Abreu, M.A.P. Ramalho, and E.A. Souza. 2014. Severity evaluation methods in common bean recurrent selection programme for resistance to angular leaf spot. J. Phytopathol. 162(10): 643–649.

Ribeiro, T., J.A. de F. Esteves, D.A. Silva, J.G.R. Gonçalves, S.A.M. Carbonell, et al. 2016. Classification of *Colletotrichum lindemuthianum* races in differential cultivars of common bean. Acta Sci. Agron. 38(2): 179–184.

Richards, R.A., G.J. Rebetzke, A.G. Condon, L. Zhang, and D.C. Liu. 2014. Recurrent selection for wider seedling leaves increases early biomass and leaf area in wheat (Triticum aestivum L.). J. Exp. Bot. 66(5): 1215–1226. doi: 10.1093/jxb/eru468.

Rodríguez-Suárez, C., A. Pañeda, A. Campa, J.J. Ferreira, and R. Giraldez. 2005. Anthracnose resistance spectra of breeding lines derived from the dry bean landrace Andecha. Annu. Rep. Bean Improv. Coop. 48: 48–49.

De Ron, A.M., A.M. González, L. Godoy, and A.P. Rodiño. 2016. History of the common bean crop : its evolution beyond its areas of origin and domestication. Arbor Ciencia, Pensam. y Cult. 192(779): 1–11.

Schmutz, J., P.E. McClean, S. Mamidi, G.A. Wu, S.B. Cannon, et al. 2014. A reference genome for common bean and genome-wide analysis of dual domestications. Nat. Genet. 46(7): 707–713.

Schoonhoven, A. van, and M.A. Pastor-Corrales. 1987. Standard system for the evaluation of bean germplasm. CIAT - Centro Internacional de Agricultura Tropical.

Silva, K.J.D. e, E. a. de Souza, and F.H. Ishikawa. 2007. Characterization of *Colletotrichum lindemuthianum* Isolates from the State of Minas Gerais, Brazil. J. Phytopathol. 155(4): 241–247.

Singh, S.P., and H.F. Schwartz. 2010. Breeding common bean for resistance to diseases: A review. Crop Sci. 50(6): 2199–2223.

Tryphone, G., L. Chilagane, D. Protas, P. Kusolwa, and S. Nchimbi- Msolla. 2013. Marker assisted selec- tion for common bean diseases improvements in Tanzania: prospects and future needs. Plant breeding from laboratories to fields. p. 121–147

Valentini, G., M.C. Gonçalves-Vidigal, O.P. Hurtado-Gonzales, S.A. de Lima Castro, P.B. Cregan, et al. 2017. High-resolution mapping reveals linkage between genes in common bean cultivar Ouro Negro conferring resistance to the rust, anthracnose, and angular leaf spot diseases. Theor. Appl. Genet. 130(8): 1705–1722. d

Vanderplank, J.E. 1963. Plant diseases: Epidemics and control. Academic Press, New York.
Zuiderveen, G.H., B. a Padder, K. Kamfwa, Q. Song, and J.D. Kelly. 2016. Genome-Wide Association Study of Anthracnose Resistance in Andean Beans (*Phaseolus vulgaris*). PLoS One 11(6): 1–17.

Supplementary material

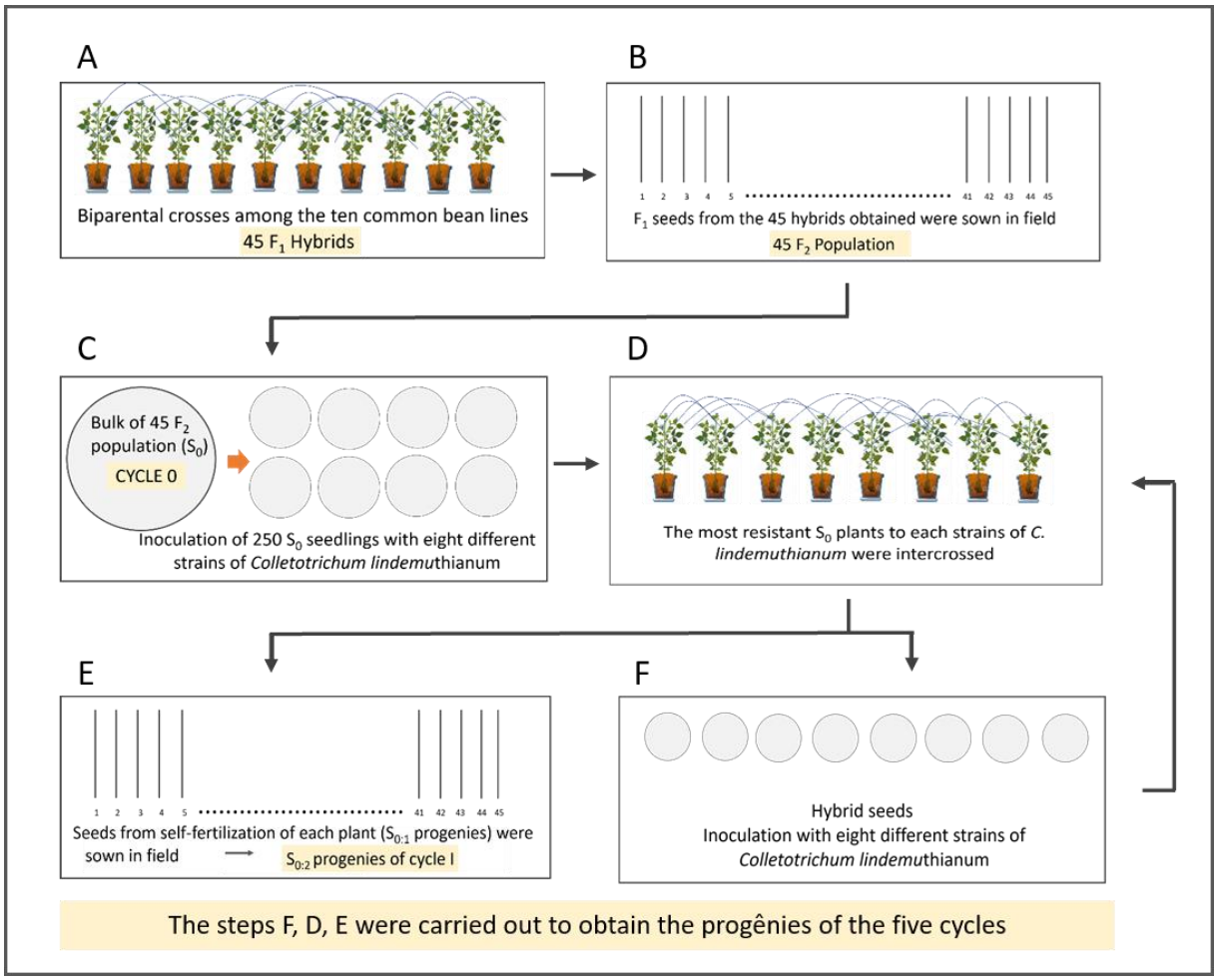

Figure S1. Flowchart of the methodology used to obtain the base population and progenies to cycle I to V of recurrent selection program.

Table S1. Summary of variance analyzes for anthracnose severity scores of $S_{0:2}$ progenies from five recurrent selection cycles aiming *C. lindemuthianum* resistance

SV	DF	Race 65 (Lv134)	Race 65 (Lv238)	Race 81
		MS	MS	MS
Progenies	197	15.23**	8.28**	8.46**
Among progenies of CI	39	13.7**	8.71**	8.68**
Among progenies of CII	39	13.75**	4.29**	5.61**
Among progenies of CIII	39	13.05**	8.92**	8.56**
Among progenies of CIV	37	13.46**	8.44**	10.31**
Among progenies of CV	39	20.22**	8.56**	7.87**
Among cycles	4	33.33**	32.4**	21.98**
Blocks	2	11.41**	45.4**	0.82
Residuals	394	0.82	0.8	0.87
SV	DF	Race 73	Race 89	Mixture[1]
		MS	MS	MS
Progenies	197	10.9**	8.661**	12.35**
Among progenies of CI	39	14.78**	10.9371**	19**
Among progenies of CII	39	5.22**	5.0245**	11.66**
Among progenies of CIII	39	11.35**	7.6198**	6.66**
Among progenies of CIV	37	10.89**	11.4377**	9.16**
Among progenies of CV	39	10.36**	8.4001**	10.26**
Among cycles	4	29.08**	8.9353**	59.28**
Blocks	2	12.83**	10.663**	0.23
Residuals	394	1.52	0.772	0.3888

[1] Inoculation performed with an equitable mixture of eight different *C. lindemuthianum* strains (Lv134, Lv238, Cl1610, Cl1614, and Cl1532 of race 65 and one strain of 73, 81 and 89).
"**" Significant at 1% probability level

Table S2. Estimates of genetic and phenotypic parameters from the six experiments for evaluation of $S_{0:2}$ progenies of the five recurrent selection cycles against different strains of *C. lindemuthianum*.

Strains	Cycles	Variance		Heritability					
		$\hat{\sigma}_G^2$,	$\hat{\sigma}_F^2$	h^2	LL	UL	Mean	Min	Max
Race 65 (Lv134)	Progenies	4.80	5.08	94.63	93.18	95.81	3.77	1.00	8.87
	CI	4.08	4.35	93.73	90.40	96.26	4.36	1.00	8.40
	CII	4.29	4.57	94.03	90.86	96.44	3.73	1.00	8.63
	CIII	6.47	6.74	95.95	93.81	97.59	4.28	1.00	8.87
	CIV	4.21	4.49	93.92	90.61	96.43	3.81	1.00	8.16
	CV	4.31	4.58	94.05	90.89	96.44	2.66	1.00	8.33
Race 65 (Lv238)	Progenies	2.49	2.76	90.34	87.74	92.47	2.65	1.00	8.08
	CI	2.71	2.97	91.04	86.28	94.66	3.45	1.00	7.01
	CII	2.64	2.90	90.82	85.94	94.52	2.91	1.00	8.08
	CIII	2.59	2.85	90.66	85.70	94.43	2.59	1.00	7.38
	CIV	2.55	2.81	90.53	85.37	94.44	2.40	1.00	6.83
	CV	1.16	1.43	81.34	71.43	94.52	1.87	1.00	4.50
Race 73	Progenies	3.13	3.63	86.09	82.34	89.15	2.83	1.00	9.00
	CI	3.28	3.78	86.65	79.56	92.04	3.67	1.00	7.69
	CII	4.42	4.93	89.75	84.31	93.89	2.89	1.00	9.00
	CIII	2.95	3.45	85.38	77.61	91.28	2.79	1.00	8.22
	CIV	3.13	3.63	86.09	78.51	91.83	2.74	1.00	7.66
	CV	1.23	1.74	70.97	55.55	93.89	2.09	1.00	6.00
Race 81	Progenies	2.53	2.82	89.76	86.99	92.01	2.67	1.00	7.50
	CI	2.57	2.85	89.88	84.49	93.96	3.36	1.00	7.50
	CII	2.60	2.89	90.01	84.70	94.04	2.84	1.00	6.90
	CIII	2.33	2.62	88.98	83.12	93.43	2.61	1.00	7.41
	CIV	3.15	3.44	91.59	87.00	95.06	2.51	1.00	7.17
	CV	1.58	1.87	84.54	76.32	94.04	2.01	1.00	5.99
Race 89	Progenies	2.63	2.89	91.09	88.68	93.05	2.74	1.00	7.85
	CI	2.283	2.54	89.87	84.49	93.96	3.37	1.00	7.81
	CII	3.388	3.646	92.94	89.19	95.79	2.89	1.00	7.85
	CIII	2.543	2.8	90.81	85.93	94.52	2.64	1.00	7.69
	CIV	3.555	3.813	93.25	89.57	96.03	2.64	1.00	7.41
	CV	1.418	1.675	84.64	76.47	95.79	2.14	1.00	4.67
Mixture	Progenies	3.99	4.12	96.85	96.00	97.54	4.20	1.00	9.00
	CI	2.09	2.22	94.16	91.06	96.52	4.80	1.33	8.44
	CII	6.20	6.33	97.95	96.87	98.78	4.44	1.00	9.00
	CIII	3.29	3.42	96.21	94.20	97.74	4.43	1.00	7.78
	CIV	2.92	3.05	95.76	93.45	97.50	4.41	1.00	7.77
	CV	3.76	3.89	96.67	94.90	98.78	2.94	1.00	6.48

$\hat{\sigma}_G^2$: Genetic variance; $\hat{\sigma}_F^2$: Phenotypic variance; h^2: Broad-sense heritability with its respective lower (LL) and upper (UL) limits; Minimum (Min) and maximum (Max) value of the anthracnose severity scores of $S_{0:2}$ progenies

Table S3. Summary of joint variance analyzes of the six experiments for anthracnose severity scores of $S_{0:2}$ progenies from five recurrent selection cycles aiming anthracnose resistance

SV	DF	MS
Blocks	2	200.52896**
Progenies	197	46.73726**
C. lindemuthianum strains	5	303.19217**
Progenies x Strains	985	6.76024**
Residual	2375	3.21878

"**" Significant at 1% probability level

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