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MELHORAMENTO GENÉTICO DO FEIJÃO-COMUM ASSISTIDO POR MARCADORES MOLECULARES: IDENTIFICAÇÃO, CARACTERIZAÇÃO, MAPEAMENTO E PIRAMIDAÇÃO DE ALELOS DE RESISTÊNCIA A DOENÇAS

LUCAS MATIAS GOMES MESSIAS

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Goiânia - GO
Brasil

Outubro - 2022

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Tese apresentada ao Programa de Pós-Graduação em Genética e Melhoramento de Plantas, da Faculdade de Agronomia, da Universidade Federal de Goiás (UFG), como requisito para obtenção do título de Doutor em Genética e Melhoramento de Plantas.
Área de concentração: Genética e Melhoramento de Plantas
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DEDICO

Aos meus avós maternos, José Inácio Filho e Adelícia Inácio Damasceno (in memoriam) e aos meus avós paternos, Antônio Gomes da Abadia e Maria Benedita Gomes (in memoriam).

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"A coragem está um passo à frente do medo"

Coleman Young

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SUMÁRIO

RESUMO GERAL 10

GENERAL ABSTRACT 11

1 INTRODUÇÃO GERAL 12

2 REFERÊNCIAS 22

CAPÍTULO 1 27

3 PIRAMIDAÇÃO DE ALELOS DE RESISTÊNCIA À DOENÇAS E ESCURECIMENTO LENTO EM FEIJÃO CARIOCA 27

4 INTRODUÇÃO 30

5 MATERIAL E MÉTODOS 33

5.1 Material genético e alelos-alvo 33

5.2 Sistema de cruzamentos e avanço de gerações 34

5.3 Seleção assistida por marcadores moleculares - SAM 36

5.4 Análise da segregação dos alelos-alvo na geração F_2 39

5.5 Cor do grão das progênies $F_{2:3}$ após armazenamento 39

6 RESULTADOS 40

6.1 Híbridos simples 40

6.2 Híbrido duplo e múltiplo 42

6.3 Geração F_2: seleção precoce de alelos-alvo 42

7 DISCUSSÃO 49

7.1 Seleção precoce dos alelos-alvo 49

7.2 Cor dos grãos após armazenamento - CGA 51

7.3 Valor dos alelos-alvo no melhoramento 51

8 CONCLUSÕES 57

9 REFERÊNCIAS 58

10 APÊNDICES 65

CAPÍTULO 2 77

11 CHARACTERIZATION AND GENETIC MAPPING OF A NEW ANDEAN ANTHRACNOSE RESISTANCE GENE PRESENT IN THE COMMON BEAN CULTIVAR BRSMG REALCE 77

ABSTRACT 78

12 INTRODUCTION 79

13 MATERIALS AND METHODS 80

13.1 Genetic material and crosses 80

13.2 Phenotyping of F_2 populations 80

13.3 Genotyping with SNP markers and SilicoDArT 81

13.4 Genetic mapping with SNP markers 81

13.5 QTL analysis and physical mapping 82

13.6 Gene annotation 82

13.7 Selection efficiency 82

14 RESULTS 82

14.1 Reaction of parents to selected *C. lindemuthianum* pathotypes 82

14.2 Inheritance studies and allelism tests 83

14.3 Genetic map 84

14.4 Major locus associated with anthracnose resistance 85

14.5 Increasing of mapping resolution in *Co-Realce* genomic region 85

15 DISCUSSION 88

16 CONCLUSIONS 93

17 REFERENCES 94

18 SUPPLEMENTARY FILES 102

CAPÍTULO 3 123

19 MOLECULAR CHARACTERIZATION OF PARENTAL LINES AND VALIDATION OF SNP MARKERS FOR ANTHRACNOSE AND ANGULAR LEAF SPOT IN COMMON BEAN[*] 123

20 CONCLUSÃO GERAL 138

RESUMO GERAL

GOMES-MESSIAS, L. M. **Melhoramento genético do feijão-comum assistido por marcadores moleculares: identificação, caracterização, mapeamento e piramidação de alelos de resistência a doenças**. 2022. 138 f. Tese (Doutorado em Genética e Melhoramento de Plantas) – Escola de Agronomia, Universidade Federal de Goiás, Goiânia, 2022.[1]

A produtividade é a principal característica a ser melhorada nas novas cultivares, sendo limitada pela ocorrência de doenças. Existem mais de 40 doenças que causam prejuízos à produção e à qualidade dos grãos de feijão em todo o mundo, comprometendo a segurança alimentar das pessoas em vulnerabilidade social. Além da produtividade, a qualidade nutricional e menores alterações pós-colheita (escurecimento lento) são de grande relevância para a aceitação do produto no mercado. À luz disso, objetivou-se com esse estudo aplicar modernas ferramentas moleculares para assistir os programas de melhoramento de feijão-comum na seleção precoce e eficiente de genótipos superiores. Aqui, apresento uma abordagem desde os estudos básicos da herança da resistência, teste de alelismo e mapeamento de genes até a validação e aplicação de marcadores moleculares no programa nacional de melhoramento de feijão-comum coordenado pela Embrapa Arroz e Feijão. Os principais achados desse trabalho foram: **1)** A seleção assistida com marcadores codominantes permite a identificação precoce de progênies combinando os alelos-alvo em homozigose. Os marcadores P8282v3-817, ANAAJK6, ANCFFDU e PvbHLHp12804 possuem segregação mendeliana 1:2:1. Além da identificação precoce dos alelos-alvo, é possível obter, nas fases iniciais, progênies com atributos que atendem o padrão comercial de grão carioca. **2)** A resistência à antracnose presente na cultivar andina BRSMG Realce é controlada por um único loco de efeito maior (R^2=54,6%) no cromossomo 4, sendo diferente dos genes R já mapeados nesse mesmo cromossomo, *Co-3*, *Co-15* e *Co-16*. A região genômica do *Co-Realce* inclui genes candidatos previamente descritos como associados aos mecanismos da interação patógeno-hospedeiro. Os snp12782 (1,182,123 pb), snp3308 (505,696 pb) e snp1327 (477,285 pb) são indicados para monitorar a introgressão do alelo *Co-Realce*, com 99,0% de eficiência de seleção. **3)** Sete marcadores oriundos do projeto de Genotipagem de Alto Rendimento (High-Throughput Genotyping - HTPG) apresentaram potencial para serem incorporados à rotina da seleção assistida em programas de melhoramento do feijão-comum (snpPV0025-*Phg-2*; snpPV0027-*Phg-5*; snpPV0046-*Co-u*; snpPV0068-*Co-4*^{2}; snpPV0070-*Co-4*^{2}; snpP8282v3-817-*Co-4*^{2}; snpPV0079-*Phg-5*). Os marcadores snpPV0025 e snpPV0079 são indicados para monitorar a introgressão dos alelos *Phg-5* e *Phg-5*, respectivamente. O sistema de genotipagem baseado em ensaios de hidrólise do tipo TaqMan® para os marcadores snpPV0070, snpP8282v3-817 e snpPV0025 foram específicos para os alelos-alvo *Co-4*^{2}, *Co-4*^{2} e *Phg-2*, respectivamente. Os marcadores snpPV0070 e snpP8282v3-817 possuem 99,0% de eficiência na seleção de genótipos superiores com o alelo *Co-4*^{2}, que confere resistência à antracnose. À vista do exposto, a seleção assistida por marcadores moleculares através de ensaios não destrutivos reduz o tempo e os custos com a seleção de alelos-alvo nas gerações iniciais. A validação de marcadores moleculares previamente identificados como ligados aos alelos-alvo é essencial para a seleção eficiente de genótipos com combinações alélicas superiores. Propõe-se que o *Co-Realce* seja nomeado oficialmente de acordo com as normas estabelecidas pelo Comitê de Genética da BIC (Bean Improvement Cooperative).

Palavras-chave: *Phaseolus vulgaris* L., genética molecular, seleção precoce

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GENERAL ABSTRACT

GOMES-MESSIAS, L. M. **Common bean breeding assisted by molecular markers: identification, characterization, mapping and pyramiding of disease resistance alleles**. 2022. 138 f. Thesis (Doctor of Science in Genetics and Plant Breeding) – Escola de Agronomia, Universidade Federal de Goiás, Goiânia, 2022.[1]

Yield is the main trait to be improved in new cultivars but can be limited by the occurrence of diseases. More than 40 diseases can damage the production and quality of common beans grains worldwide, compromising the food security of people in social vulnerability. In addition to productivity, nutritional quality and minor post-harvest changes (slow darkening) are of great importance for the acceptance of the product in the market. In view of this, the central aim of this study was to apply modern molecular tools to assist common bean breeding programs in the early and efficient selection of superior genotypes. Here, I present an approach from the basic studies of the inheritance of resistance, allelism testing and gene mapping to the validation and application of molecular markers in the common bean breeding program of Embrapa Rice and Beans. The main findings of this work were: **1)** Assisted selection with codominant markers allows for early identification of progenies matching the target alleles in homozygosity. The markers P8282v3-817, ANAAJK6, ANCFFDU and PvbHLHp12804 have 1:2:1 Mendelian segregation. In addition to the early identification of target alleles, it is possible to obtain progenies with commercial grain quality. **2)** The resistance to anthracnose present in the Andean cultivar BRSMG Realce is controlled by a single dominant locus 'major locus' (R^2=54.6%) on chromosome 4, which is different from the R genes already mapped on this same chromosome, *Co-3*, *Co-15* and *Co-16*. The genomic region of *Co-Realce* includes candidate genes previously described as associated with the pathogen-host interaction. The snp12782 (1,182,123 bp), snp3308 (505,696 bp) and snp1327 (477,285 bp) are indicated to monitor the introgression of the Co-Realce allele, with 99.0% selection efficiency. **3)** Seven markers provided by the High-Throughput Genotyping project showed potential for routine use in the marker assisted-selection in common bean breeding programs (snpPV0025-*Phg-2*; snpPV0027-*Phg-5*; snpPV0046-*Co-u*; snpPV0068 -*Co-4*^{2}; snpPV0070-*Co-4*^{2}; snpP8282v3-817-*Co-4*^{2}; snpPV0079-*Phg-5*). The markers snpPV0025 and snpPV0079 are indicated to monitor the introgression of the *Phg-2* and *Phg-5* alleles, respectively. The genotyping system based on TaqMan®-type hydrolysis assays for the markers snpPV0070, snpP8282v3-817 and snpPV0025 were specific for the target alleles *Co-4*^{2}, *Co-4*^{2} and *Phg-2*, respectively. Markers snpPV0070 and snpP8282v3-817 have high selection efficiency (99.0%) to identify superior genotypes with the *Co-4*^{2} allele, which confers resistance to anthracnose. The key messages of this study are: marker-assisted selection through non-destructive assays reduces the time and costs of selecting target alleles in early generations, validation of molecular markers previously identified as linked to target alleles is essential to improve the selection efficiency of genotype with superior allelic combinations, and that the *Co-Realce* be considered by Genetics Committee as a unique gene and named officially following the norms established by the BIC (Bean Improvement Cooperative).

Keywords: *Phaseolus vulgaris* L., molecular genetics, molecular breeding, early selection.

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1 INTRODUÇÃO GERAL

O feijão-comum (*Phaseolus vulgaris* L.) é uma espécie diploide, com 11 pares de cromossomos (Altrock et al., 2011), e está organizada em dois pools gênicos (Mesoamericano e Andino) que divergiram a partir de uma população ancestral comum há cerca de 165 mil anos (Geps, 1998; Schmutz et al., 2014; Valdisser et al., 2017). É uma importante fonte de proteínas, carboidratos, fibras, vitaminas e minerais, principalmente para pessoas de classes sociais menos favorecidas (Ferreira et al., 2018). Seu alto valor nutricional e a alta taxa de consumo faz do feijão uma importante fonte de alimento e renda para milhões de pessoas nos países em desenvolvimento da África, Ásia e América Latina (Valentini et al., 2017). Estima-se que 50% da produção de feijão-comum ocorre em países em que há predomínio de indivíduos de baixa renda, tornando-a fundamental para a segurança alimentar (Porch et al., 2013).

No Brasil, as condições favoráveis e diversificadas de clima, solo, relevo e luminosidade favorecem as práticas agrícolas e garantem safras produtivas. Exemplo disso, do volume total de feijão-comum produzido nas Américas, o Brasil foi responsável por cerca de 37% da produção, contribuindo com 2,4 milhões de toneladas em uma área de aproximadamente 1,6 milhão de ha, com rendimento médio de 1498 kg ha^{-1} em 2020 (Embrapa Arroz e Feijão, 2022). O fato de o Brasil também figurar como um dos maiores consumidores, limita o volume de produto excedentes exportável e gera pequena demanda de importação para suprir o mercado interno (Conab, 2022).

A produção de feijão-comum é realizada por pequenos, médios e grandes produtores, em diversas regiões do país, utilizando diferentes níveis tecnológicos (Brusamarello et al., 2017). Seu cultivo ocorre durante todo o ano, em três safras distintas, "águas", "seca" e "irrigada", em que o início da semeadura de cada safra é dependente da região produtora (Brusamarello et al., 2017). A primeira safra, "safra das águas", com semeadura ocorrendo entre os meses de agosto a novembro, contribuiu com 39,4% da produção nacional em 2020 (Embrapa Arroz e Feijão, 2022). A segunda safra, "safra da seca", com semeadura de dezembro a abril, respondeu por 36,5% da produção nacional em 2020 (Embrapa Arroz e

Feijão, 2022). A terceira safra, ocorre em grande parte sob irrigação, uma vez que o calendário coincide com períodos secos das principais regiões produtoras, com a semeadura entre os meses de abril e julho (Brusamarello et a., 2017). Essa safra concentra-se no Oeste da Bahia, em Mato Grosso, na região do Distrito Federal, incluindo municípios goianos e mineiros vizinhos, como a região de Cristalina - GO e Unaí - MG (Ana, 2022). A safra sob irrigação possui elevados rendimentos, podendo ser até duas vezes superior ao cultivo sem irrigação (Ana, 2022), sendo responsável por 24% da produção brasileira em 2020 (Embrapa Arroz e Feijão, 2022).

Os avanços em soluções tecnológicas para que a cadeia produtiva do feijão-comum produza cada vez mais com responsabilidade socioambiental estão estreitamente ligadas com as pesquisas desenvolvidas pelo setor público, incluindo universidades, institutos e a Embrapa. O lançamento de cultivares resilientes às condições edafoclimáticas e os avanços no manejo da cultura permitiram que houvesse, ao longo do tempo (1985 – 2020), uma correlação inversamente proporcional entre produtividade e área plantada (**Figura 1A e 1B**). Em uma análise de regressão linear simples realizada com dados do período entre 1985 e 2020 (Embrapa Arroz e Feijão, 2022), a produtividade apresentou incremento anual de +34,6 kg enquanto a área cultivada reduziu cerca de -93491 hectares, fenômeno que pode ser observado pelas linhas de tendência de cada macrorregião produtora (**Figuras 1A e 1B**). Embora tenha ocorrido redução da área cultivada, observa-se na **Figura 1B** que após o ano de 2010 houve uma retomada no crescimento da "safra irrigada" no Centro-Oeste, muito em função do aumento das áreas de cultivo sob pivôs centrais no estado de Mato Grosso (**Figura 1C**).

Considerando um recorte dos últimos dez anos, a produtividade média nacional foi de 1439 kg ha^{-1}, valor inferior às médias das principais regiões produtoras, onde foram obtidas produtividades de 2114,7 kg ha^{-1} (Centro-Oeste), 1720,3 kg ha^{-1} (Sudeste) e 1533,1 kg ha^{-1} (Sul) (**Figura 2A**). A macrorregião Centro-Oeste, composta pelo Distrito Federal, Goiás, Mato Grosso e Mato Grosso do Sul, se destaca em termos de produtividade (**Figura 2A**). Essa região possui média superior as demais macrorregiões produtoras devido à alta tecnologia aplicada à produção de feijão-comum, principalmente, no Distrito Federal, Goiás e Mato Grosso (**Figura 2C**). Esse destaque em termos de produtividade é explicado pela adoção do sistema de cultivo em pivô central (3° Safra – Irrigado), em que se observa produtividade superior a 3 toneladas na região produtora do Distrito Federal (**Figura 2C**).

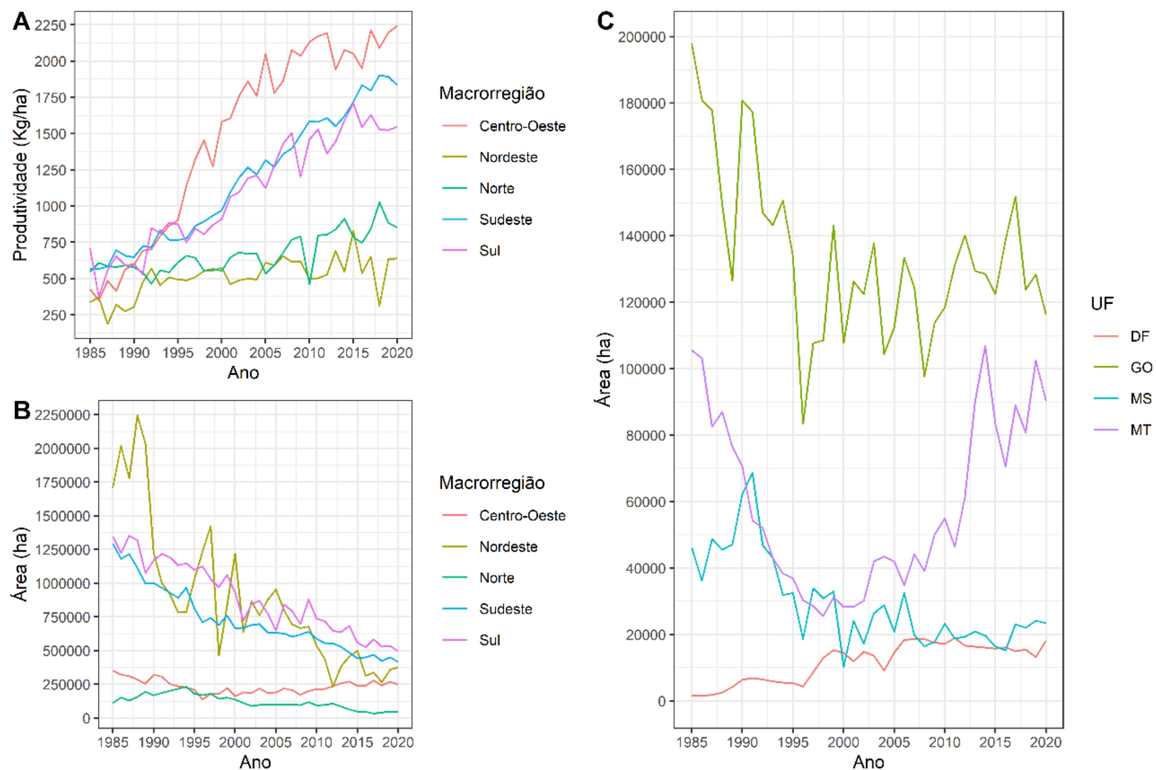


Figura 1. A – Comportamento temporal da produtividade de feijão-comum das cincos macrorregiões produtoras a partir dos dados de cultivo do período entre 1985 e 2020, considerando a média anual das três safras (“Águas”, “Seca” e “Irrigado”). **B** – Comportamento temporal da área cultivada de feijão-comum das cincos macrorregiões produtoras a partir dos dados de cultivo do período entre 1985 e 2020, considerando a média anual das três safras (“Águas”, “Seca” e “Irrigado”). **C** – Comportamento temporal da área cultivada de feijão-comum nos estados de GO, MT, MS e o Distrito Federal (macrorregião Centro-Oeste) no período entre 1985 e 2020, considerando a média anual das três safras (“Águas”, “Seca” e “Irrigado”).

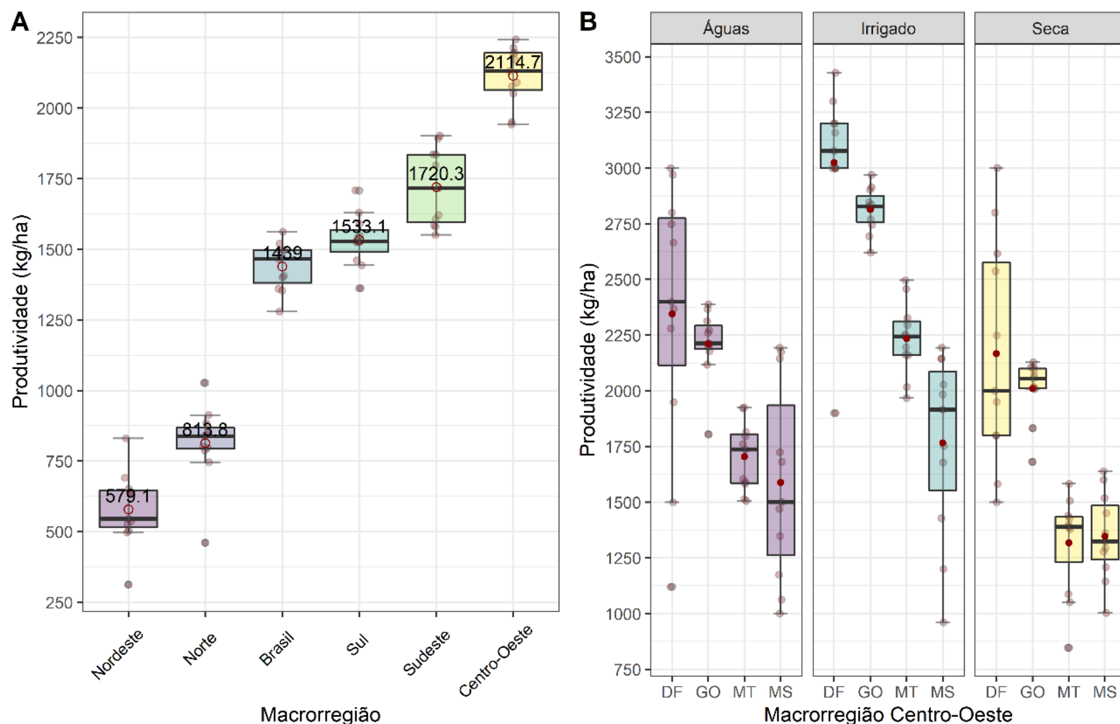


Figura 2. A – Produtividade de feijão-comum das cincos macrorregiões produtoras e do Brasil a partir de um recorte dos últimos dez anos (2010 a 2020) de cultivo, considerando a média anual das três safras ("Águas", "Seca" e "Irrigado"). **B** - Produtividade de feijão-comum na primeira ("Águas"), segunda ("Seca") e terceira ("Irrigado") safra por estado da macrorregião Centro-Oeste, a partir de um recorte dos últimos dez anos (2010 a 2020) de cultivo. Fonte dos dados: Adaptado do Levantamento Sistemático da Produção Agrícola - IBGE (1985-2020) e elaborado na Embrapa Arroz e Feijão por Osmira Fátima da Silva (SILVA, O. F. da), em agosto de 2021.

Esses dados revelam a grande heterogeneidade ambiental que existe entre e dentro das regiões produtoras de feijão-comum, sendo o posicionamento técnico uma prática indispensável para que as cultivares disponíveis no mercado expressem o máximo do seu potencial produtivo (Pereira et al., 2018). Ao mesmo tempo, evidencia a existência de oportunidades para os programas de melhoramento desenvolverem cultivares mais adaptadas às condições de déficit hídrico, como no Norte e Nordeste (**Figura 2A**).

Pelo fato de o feijão-comum ser cultivado em todo Brasil, as demandas e os problemas relacionados à produção em cada região são diferentes, necessitando de ações de pesquisa especificas (Faria et al., 2013; Faria et al., 2014; Barili et al., 2016a, 2016b). Apesar de características como produtividade, precocidade, arquitetura de planta, tamanho e coloração dos grãos serem de grande importância para todas as regiões (Faria et al., 2013; Faria et al., 2014; Barili et al., 2016a, 2016b), permanecem ainda muitos desafios

relacionados a fatores bióticos e abióticos que tendem a limitar o potencial produtivo, proporcionando uma redução na produção (Valdisser et al., 2020; Barili et al., 2016a, 2016b). Entre os fatores que limitam a produtividade, causam instabilidade e risco de implantação da cultura, encontram-se às doenças (Valentini et al., 2017; Meziadi et al., 2016), sendo relatadas na literatura mais de 45, de maior ou menor importância (Singh & Schwartz, 2010; Ragagnin et al., 2009). No tocante às doenças fúngicas, destacam-se a antracnose (*Colletotrichum lindemuthianum*), a mancha angular (*Phaeoisariopsis griseola* (Sacc.) Ferraris), a murcha de *fusarium* (*Fusarium oxysporum* f. sp. *phaseoli*), podridão-radicular-seca (F. *solani* f. sp. *phaseoli*), mofo branco (*Sclerotinia sclerotiorum*) e podridão-radicular de Rhizoctonia (*Rhizoctonia solani*) (Singh & Schwartz, 2010). A capacidade destrutiva destas doenças passa pela interação entre o patógeno, o hospedeiro e o ambiente, em que dependendo do nível de suscetibilidade das cultivares, das condições ambientais favoráveis e da presença do inóculo inicial, leva a perdas na produção e na qualidade dos grãos de algo entre 70 e 100% (Maina et al., 2017; Souza et al., 2014; Ragagnin et al., 2009).

A maioria dos patógenos têm grande variabilidade patogênica e, normalmente, conseguem suplantar a resistência obtida via melhoramento genético (Basavaraja et al., 2020). Por isso, estratégias que possibilitem a obtenção de uma resistência mais estável têm sido adotadas, como a piramidação de alelos de resistência (Terán et al., 2013), retrocruzamentos (Almeida et al., 2021) e a seleção recorrente (Arantes et al., 2010; Pádua et al., 2021). Continuamente, busca-se por genes/alelos mediante a identificação e mapeamento de novas fontes de resistência, procurando desenvolver genótipos com base genética mais ampla que proporcione maior estabilidade, permitindo maximizar os ganhos com a seleção (Assefa et al., 2019).

Os marcadores moleculares, quando validados, prestam auxílio no monitoramento da introgressão de alelos-alvo de resistência através de ensaios não destrutivos, promovendo a redução de tempo e de custos durante o processo de avaliação e seleção (Miklas et al., 2006; Assefa et al., 2019). Sanglard et al. (2005) utilizaram o método de retrocruzamentos assistidos por marcadores moleculares para monitorar a introgressão dos alelos de resistência à antracnose (*Co-3*^{4}), mancha angular (*Phg-3*) e ferrugem (*Ur-14*) da cultivar 'Ouro Negro' para a cultivar 'Pérola'. Para tanto, além da avaliação fenotípica da reação de resistência, os autores utilizaram os marcadores OPX-11550, OPF-101050, SCARBA-08560 e SCARF-101050 (ligados aos genes *Co-3*^{4} e *Ur-14*), OPAA-19400, OPM-02425 e OPBA-16669

(ligados ao alelo *Phg-3*) para monitorar a transferência destes alelos. Ao final de três ciclos de retrocruzamentos ($BC_3F_{2:5}$), os autores concluíram que a associação do método de melhoramento convencional com o uso de marcadores moleculares permitiu a rápida e simultânea transferência dos três alelos-alvo de resistência para cultivar 'Pérola'.

Em um estudo conduzido por Ragagnin et al. (2009) foram introgredidos, por retrocruzamentos assistidos por marcadores moleculares, alelos de resistência à antracnose, mancha angular e ferrugem utilizando como parental recorrente a cultivar 'Rudá'. Ao todo, um conjunto de nove marcadores foram usados, sendo eles: OPH13490 e SCARH13490 ligados ao alelo *Phg-1* que confere resistência ao patótipo 63-23 de *P. griseola*; OC8900 (*Co-4*), OB31800 (*Co-4*), OAZ4560 (*Co-6*), OX11550 (*Ur-14*/*Co-3*^{4}), SCARF101050 (*Ur-14*/*Co-3*^{4}), SCARY20830 (*Co-4*) e SCARZ20940 (*Co-6*), ligados aos alelos que conferem resistência aos patótipos 65 e 89 de antracnose e patótipos 32, 45, 46, 47, 49, 50, 52, 54, 58 e 59 de *U. appendiculatus*. Ao final do estudo, os autores obtiveram quatro linhagens $F_{4:7}$ com resistência aos três patógenos. A partir deste estudo, Costa et al. (2010) adotaram a mesma estratégia para realizarem a transferência dos alelos *Co-4*, *Co-6* e *Co-3*^{4} (SCARY20830 e SCARAZ20845), *Ur-14* (SACR101050) e *Phg-1* (SACR13490) a partir do cruzamento inicial entre a cultivar 'Rudá' (parental doador) e a cultivar 'Diamante Negro' (parental recorrente). Como resultado deste trabalho, quatro linhagens de grãos do grupo comercial preto (DNR7, DNR8, DNR9 e DNR10) foram indicadas para serem utilizadas pelos programas brasileiros de melhoramento de feijão-comum, pois cada uma possuem os alelos *Co-4*, *Co-3*^{4}, *Ur-14* e *Phg-1*, que conferem resistência aos principais patótipos de antracnose (raças 65, 89, 73 e 81), ferrugem (patótipos 21-3 e 29-15) e mancha angular (patótipos 63-21 e 63-23) que predominam nos campos de produção no Brasil. Souza et al. (2014) realizaram a piramidação dos alelos *Ur-5*, *Ur-11* e *Ur-14* de resistência à *U. appendiculatus* na cultivar Rudá (grão carioca) com o auxílio dos marcadores moleculares SI19, SAE19 e OPX11, respectivamente. Com esta estratégia, os autores selecionaram 16 linhagens $F_{4:7}$, das quais nove (TL-006, TL-009, TL-015, TL-016, TL-026, TL-032, TL-034, TL-035 e TL-037) foram altamente resistentes (HR) aos três patótipos testados no estudo (21-3, 29-15 e 53-3), indicando que os alelos *Ur-5*, *Ur-11* e *Ur-14* em homozigose.

O melhoramento assistido por marcadores moleculares tem contribuído para o progresso genético quanto à resistência aos principais patógenos que acometem a cultura do feijão-comum (Basavaraja et al., 2020). No entanto, devido à grande variabilidade genética

observada nas populações dos patógenos, os esforços na busca por novas fontes de resistência, no mapeamento de genes de resistência e no desenvolvimento e validação de marcadores moleculares ligados aos principais genes de resistência devem ser feitos de forma contínua e ininterrupta (Gomes-Messias et al., 2022; Vieira et al., 2018). Neste caminho, estudos mais recentes têm focado no mapeamento de alta resolução reduzindo a distância entre marcadores e alelos de interesse, seguido pela validação desses marcadores em genótipos diversos, consequentemente, aumentando a eficiência dos programas de piramidação alélica (Vieira et al., 2018; Miller et al., 2018; Valentini et al., 2017; Perseguini et al., 2016; Fritsche-Neto et al., 2019; Gil et al., 2019, Nay et al., 2019).

Vieira et al. (2018) verificaram que dos quatro marcadores (SH18, SAS13, SAB03 e SAZ20) validados para a piramidação de alelos de resistência à antracnose, apenas o SH18 foi capaz de discriminar o alelo *Co-4*^{2} dos alelos *Co-4* e *Co-4*^{3}, sendo este útil para a seleção assistida. Em outro estudo, Miller et al. (2018) verificaram que o alelo *Phg-2*, que confere resistência ao patótipo 63-39 de *P. griseola*, está a uma distância de 3 cM do marcador STS g796. Mais recentemente, Nay et al. (2019) identificaram SNPs-alvos em blocos haplotípicos posicionados nessa região do marcador STS g796 e que poderão ser utilizados para a SAM. Adicionalmente, Valentini et al. (2017) revelaram que os alelos *Co-3*^{4} e *Phg-3* co-segregam e estão ligados a uma distância de 0.0 cM do marcador g2303. Além disso, com base no mapa altamente saturado produzido nesse estudo, o gene *Ur-14* foi posicionado a 2.2 cM do loco *Co-3*^{4}/*Phg-3* no final do cromossomo Pv04. Com estes resultados, o marcador g2303 é indicado para programas de piramidação alélica, visto que este permite a seleção rápida e simultânea dos alelos *Co-3*^{4}, *Phg-3* e *Ur-14*, que conferem resistência ao patótipo 73 de *C. lindemuthianum*, aos patótipos 31-23, 31-31, 31-35, 53-47, 63-39 e 63-63 de *P. griseola* e patótipos 41, 47, 53 e 58 de *U. appendiculatus*, respetivamente.

Perseguini et al. (2016) realizaram estudo de associação genômica ampla (GWAS) para resistência à antracnose e mancha angular utilizando 24 genótipos de origem Andina e 156 genótipos Mesoamericanos. Os autores encontraram 28 marcadores SSR e 38 SNP significativamente associados com a resistência aos dois patógenos, sendo que dois SSR (SSR-IAC167 e PvM95, localizados no cromossomo 3) e um SNP (scaffold00021_89379) foram associados com a resistência de ambas as doenças, indicando que possa existir efeito pleiotrópico. Além disso, dois marcadores presentes no cromossomo 1 (scaffold00024_916410 e PvM97) foram mapeados na posição correspondente ao alelo *Co-*

1, que confere resistência aos patótipos 73 e 65 de antracnose. Enquanto outros dois marcadores SNP (scaffold0060_115096 e scaffold00060_401853) foram localizados na mesma região do cromossomo 4 que corresponde aos alelos *Co-3*^{4}, *Phg-3* e *Ur-14*, sendo estes dois marcadores de grande importância para os programas de piramidação alélica. Os marcadores PvM124 e scaffold00045_345513 mapeados no Pv03 foram associados especificamente a resistência à antracnose, enquanto o marcador BMc225 (Pv04) foi associado a resistência ao patótipo 0-39 de *P. griseola*.

Fritsche-Neto et al. (2019) realizaram a genotipagem (5,398 SNP) de 60 linhagens de feijão-comum que constituem o germoplasma elite desenvolvido pela Embrapa ao longo de 22 anos, sendo as mesmas avaliadas sob condição de campo quanto a reação à antracnose e à mancha angular. A partir da análise de associação (GWAS) foi possível identificar dois SNP significativamente (BARCPV_1.0_Chr02_23542475_A_G e BARCPV_1.0_Chr02_23644618_G_A) associados a reação de resistência à antracnose (explicando 25% da variação fenotípica) e o SNP BARCPV_1.0_Chr10_20935383_C_T respondendo por 19% da reação de resistência à mancha angular. Este estudo revelou novas regiões genômicas relevantes que respondem pela reação de resistência a estes dois patógenos, sendo os marcadores SNP úteis para os programas de seleção assistida por marcadores moleculares.

Gil et al. (2019) realizaram o mapeamento de alta resolução (fine-map) da região genômica do loco *Phg-2* visando a identificação de marcadores SNP para serem utilizados em programas de melhoramento assistido por marcadores moleculares. Para isso, foram feitos cruzamentos entre linhagens de feijão-comum de origem Mesoamericana (*Phg-2*) e a cultivar comercial CAL 96 de origem Andina com o objetivo de transferir o alelo *Phg-2* para este pool gênico. Em uma das etapas do estudo de mapeamento, os autores avaliaram 97 linhagens F_4 quanto a reação de resistência à mancha angular após a inoculação com uma mistura de isolados de origem Andina e Mesoamericana. Foi constatado que as linhagens com o alelo ALS_Chr08_62193174 (SNP) apresentaram os maiores níveis de reação de resistência à antracnose tanto na folha quanto nas vagens. Segundo os autores, este marcador SNP não é específico para o alelo *Phg-2* no pool gênico Mesoamericano, podendo ser utilizado como marcador desse loco em background Andino. Além disso, os autores concluem que entre os cinco alelos descritos (*Phg-1* ao *Ph-5*), o *Phg-2* é o que promove maior nível resistência a diferentes patótipos de *P. griseola*.

Um forte aliado ao desenvolvimento crescente das ferramentas para a seleção assistida tem sido o avanço na genômica do feijão-comum. O conhecimento da estrutura, funcionalidade e organização genômica do feijão tem gerado considerável progresso nos programas de melhoramento genético, desde o desenvolvimento de inúmeros recursos genômicos, até a manipulação de genes, permitindo estudos mais específicos (Valdisser et al., 2017). O feijão-comum possui o tamanho do genoma estimado em ~587 milhões de pares de base (Mpb), distribuídos em 11 cromossomos e, atualmente, são muitos os recursos biotecnológicos disponíveis, tanto para o germoplasma de origem Andina (Schmutz et al., 2014), quanto para o de Mesoamericana (Vlasova et al. 2016). A disponibilidade de dois genomas de referência tem facilitado o desenvolvimento de abordagens de re-sequenciamento, pois as informações geradas são facilmente alinhadas e comparadas, revelando diferenças ao longo de todo genoma (Valdisser et al., 2017; Wu et al., 2019). Além de favorecerem enormemente a condução de estudos por busca de genes candidatos e anotação de sequências.

Para o feijão-comum, conjuntos de alguns milhares de SNP amplamente distribuídos no genoma encontram-se desenvolvidos (Song et al. 2015; Cichy et al. 2015; Müller et al. 2015; Valdisser et al. 2016; 2017) e vêm sendo utilizado em pesquisas (Valdisser et al., 2020; Vidigal Filho et al., 2020; Costa et al., 2021). O acesso crescente à abordagem de whole-genome sequencing (WGS), ou sequenciamento total do genoma, tem possibilitado avaliar variações a nível de bases em todo genoma (Schmutz et al. 2014; Vlasova et al., 2016). Recentemente, 40 linhagens/cultivares do programa de melhoramento de feijão do Brasil (incluindo importantes fontes de resistência para diversas doenças) tiveram seus genomas integralmente sequenciados e alinhados (cobertura de 10X), possibilitando uma ampla varredura genômica e a identificação de mais de ~10 milhões de SNPs/Indels/CNV (dados ainda não publicados). A capacitação para lidar com esse grande volume de dados genômicos, aliados aos inúmeros recursos de bioinformática em constante desenvolvimento, têm possibilitado acelerar/aumentar a compreensão dos padrões de herança para as mais variadas características que poderão ser convertidas em valiosas ferramentas para manipulação dos genótipos em regiões, por exemplo, de cluster de genes de resistência (Meziadi et al., 2014).

Os objetivos deste estudo foram: 1) Piramidar alelos-alvo de resistência à antracnose, mancha angular, murcha de *fusarium* e escurecimento lento dos grãos, usando ferramentas modernas de biotecnologia. 2) Usar marcadores codominantes (SNP) para mapear a região genômica associada com a resistência a antracnose em uma população F_2 derivada do cruzamento entre a cultivar do pool gênico Andino, BRSMG Realce (Resistente), com uma cultivar do pool gênico Mesoamericano, BRS FC104 (Suscetível). Identificar marcadores que co-segregam com o alelo de resistência para uso na SAM do programa de melhoramento de feijão-comum da Embrapa. 3) avaliar e validar marcadores SNP previamente identificados como ligados a alelos de resistência à antracnose (*Co-4*^{2} e *Co-u*) e mancha angular (*Phg-1*, *Phg-2* e *Phg-5*), usando um painel diverso de genótipos de feijão-comum contendo importantes fontes de resistência e uma população segregante (BRS Cometa x SEL 1308), para serem incorporados à rotina da seleção assistida por marcadores moleculares (SAM) do programa de melhoramento de feijão-comum.

2 REFERÊNCIAS

ALMEIDA, C. P.; PAULINO, J. F. C.; SANTOS, I. L.; BAJAY, M. M.; GONÇALVES, J. G. R.; CARVALHO, C R. L.; CARBONELL, S. A. M.; CHIORATO, A. F.; BENCHIMOL-REIS. Marker-assisted backcrossing for disease resistance and agronomic traits in carioca beans. **agronomy**, v. 61, n. 4, 2510-2521, 2021. Doi: 10.1002/csc2.20528.

ALTROCK, S.; FONSÊCA, A.; PEDROSA-HARAND, A. Chromosome identification in the Andean common bean accession G19833 (*Phaseolus vulgaris* L., Fabaceae). **Genetics and Molecular Biology**, v. 34, n. 3, p. 459-463, 2011. Doi: 10.1590/S1415-47572011005000029.

ANA. Agência Nacional de Águas e Saneamento Básico. **Atlas de irrigação: uso da água na agricultura irrigada**. Brasília: Agência Nacional de Águas e Saneamento Básico. 2022. Disponível em: <https://portal1.snirh.gov.br/ana/apps/storymaps/stories/a874e62f27544c6a986da1702a911c6b>. Acesso em: 01 de agosto de 2022.

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**, v. 10, p. 232-237, 2010. Doi: 10.1590/S1984-70332010000300008.

ASSEFA, T.; MAHAMA, A.; BROWN, A. V.; CANNON, E. K. S.; RUBYOGO, J. C.; RAO, I. M.; BLAIR, M. W.; CANNON, S. B. A review of breeding objectives, genomic resources, and marker-assisted methods in common bean (*Phaseolus vulgaris* L.). **Molecular Breeding**, v. 39, 20, 2019. Doi 10.1007/s11032-018-0920-0.

BARILI, L. D.; VALE, N. M.; CARNEIRO, J. E. S.; FONSECA E SILVA, F.; SILVA, F. L. Five decades of black common bean genetic breeding in Brazil. **Pesquisa Agropecuária Tropical**, v. 46, n. 3, p. 259-266, 2016b. Doi: 10.1590/1983-40632016v4641371.

BARILI, L. D.; VALE, N. M.; MOURA, L. M.; PAULA, R. G.; SILVA, F. F.; CARNEIRO, J. E. S. Genetic progress resulting from forty-three years of breeding of the carioca common bean in Brazil**. Genetics and Molecular Research**, v. 15, n. 3, gmr.15038523, 2016a. Doi: http://dx.doi.org/10.4238/gmr.15038523.

BASAVARAJA, T.; PRATAP, A.; DUBEY, V.; GURUMURTHY, S.; GUPTA, S.; SINGH, N. P. Molecular and conventional breeding strategies for improving biotic stress resistance in common bean. In: GOSAL, S. S.; WANI, S. H. (eds.) **Accelerated Plant Breeding**. v. 3. Switzerland: Springer Nature, 2020. ch. 13, p. 389-421. Doi: 10.1007/978-3-030-47306-8_13.

BRUSAMARELLO, A. P.; OLIVEIRA, P. H.; SEBIM, D. E.; BARETTA, D. R. Performance of bean (*Phaseolus vulgaris* L.) genotypes in the second-season under high and low technology management in Parana, Brazil. **Acta Agronómica**, v. 66, n. 3, p. 436-441, 2017. Doi: 10.15446/acag.v66n3.55555.

CICHY, K. A.; PORCH, T. G.; BEAVER, J. S.; CREGAN, P.; FOURIE, D.; GLAHN, R. P.; GRUSAK, M. A.; KAMFWA, K.; KATUURAMU, D. N.; MCCLEAN, P.; MNDOLWA, E.; NCHIMBI-MSOLLA, S.; PASTOR-CORRALES, M. A.; MIKLAS, P. N.

A Phaseolus vulgaris diversity panel for Andean bean improvement. Crop Science, v. 55, p. 2149-2160, 2015. Doi 10.2135/cropsci2014.09.0653.

CONAB. Companhia Nacional de Abastecimento. **Boletim da safra de grãos: 10º Levantamento - Safra 2021/22**. Brasília: Companhia Nacional de Abastecimento. 2022. Disponível em: <https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos>. Acesso em: 01 de agosto de 2022.

COSTA, L. C.; NALIN, R. S.; DIAS, M. A.; FERREIRA, M. E.; SONG, Q.; PASTOR-CORRALES, M. A. Different loci control resistance to different isolates of the same race of *Colletotrichum lindemuthianum* in common bean. **Theoretical and Applied Genetics**, v. 134, p. 543–556, 2021. Doi: 10.1007/s00122-020-03713-x.

COSTA, M. R.; TANURE, J. P. M.; ARRUDA, K. M. A.; CARNEIRO, J. E. S.; MOREIRA, M. A.; BARROS, E. G. Development and characterization of common black bean lines resistant to anthracnose, rust and angular leaf spot in Brazil. **Euphytica**, 176:149-156, 2010.

EMBRAPA ARROZ E FEIJÃO. **Dados conjunturais da produção de feijão comum (Phaseolus vulgaris L.) e caupi (Vigna unguiculata (L.) Walp) no Brasil (1985 a 2020): área, produção e rendimento**. Santo Antônio de Goiás: Embrapa Arroz e Feijão. 2022. Disponível em: <http://www.cnpaf.embrapa.br/socioeconomia/index.htm>. Acesso em: 01 de agosto de 2022.

FARIA, L. C.; MELO, P. G. S.; PEREIRA, H. S.; DEL PELOSO, M. J.; BRÁS, A. J. B. P.; MOREIRA, J. A. A.; CARVALHO, H. W. L.; MELO, L. C. Genetic progress during 22 years of improvement of carioca-type common bean in Brazil. **Field Crops Research**, v. 142, p. 68-74, 2013. Doi.org/10.1016/j.fcr.2012.11.016.

FARIA, L. C.; MELO, P. G. S.; PEREIRA, H. S.; WENDLAND, A.; BORGES, S. F.; PEREIRA-FILHO, I. A.; DIAZ, J. L. C.; CALGARO, M.; MELO, L. C. Genetic progress during 22 years of black bean improvement. **Euphytica**, v. 199, p. 261-272, 2014. Doi 10.1007/s10681-014-1135-z.

FERREIRA, C. M.; FIGUEIREDO, R. S.; LUZ, T. C. L. A. Arroz e Feijão: Intervenção multi-institucional em prol do Brasil. **Revista Política Agrícola**, v. 27, n. 1, p. 96-112. 2018.

FRITSCHE-NETO, R.; SOUZA, T. L. P. O.; PEREIRA, H. S.; FARIA, L. C.; MELO, L. C.; NOVAES, E.; BRUM, I. J. B.; JANNINK, J. L. Association mapping in common bean revealed regions associated with anthracnose and angular leaf spot resistance. **Scientia Agricola**, v. 76, p. 321-327, 2019. Doi: 10.1590/1678-992X-2017-0306.

GEPTS, P. 1998. Origin and evolution of common bean: past events and recent trends. **HortScience**, v. 33, p. 1124-1130. Doi: 10.21273/HORTSCI.33.7.1124.

GIL, J.; SOLARTE, D.; LOBATON, J. D.; MAYOR, V.; BARRERA, S.; JARA, C.; BEEBE, S.; RAATZ, B. Fine-mapping of angular leaf spot resistance gene *Phg-2* in common bean and development of molecular breeding tools. **Theoretical and Applied Genetics**, 4: 1-14, 2019. Doi: 10.1007/s00122-019-03334-z.

GOMES-MESSIAS, L. M.; VIANELLO, R. P.; MONTEIRO-JÚNIOR, J. P.; RODRIGUES, L. A.; MOTA, A. P. S.; PEREIRA, H. S.; MELO, L. C.; RAATZ, B.;

SOUZA, T. L. P. O. Molecular characterization of parental lines and validation of SNP markers for anthracnose and angular leaf spot in common bean. **Euphytica**, v. 218, 49, 2022. Doi 10.1007/s10681-022-03002-2.

MAINA, P. K.; WACHIRA, P. M.; OKOTH, S. A.; KIMENJU, J. W. Cultural, morphological and pathogenic variability among *Fusarium oxysporum* f. sp. *phaseoli* causing wilt in french bean (*Phaseolus vulgaris* L.). **Journal of Advances in Microbiology**, v. 2, n. 4, p. 1-9, 2017. Doi 10.9734/JAMB/2017/32684.

MEZIADI, C.; RICHARD, M. M. S.; DERQUENNES, A.; THAREAU, V.; BLANCHET, S.; GRATIAS, A.; PFLIEGER, S.; GEFFROY, V. Development of molecular markers linked to disease resistance genes in common bean based on whole genome sequence. **Plant Science**, v. 242, p. 351-357, 2016. doi: 10.1016/j.plantsci.2015.09.006.

MIKLAS, P. N.; KELLY, J. D.; BEEBE, S. E.; BLAIR, M. W. Common bean breeding for resistance against biotic and abiotic stresses: from classical to mas breeding. **Euphytica**, 147:105-131, 2006. Doi: 10.1007/s10681-006-4600-5.

MILLER, T.; GEPTS, P.; KIMMO, S.; ARUNGA, E.; CHILAGANE, L. A.; NCHIMBI-MSOLLA, S.; NAMUSOKE, A.; NAMAYANJA, A.; TEDLA, Y. R. Alternative markers linked to the *Phg-2* angular leaf spot resistance locus in common bean using the *Phaseolus* genes marker database. **African Journal of Biotechnology**, v. 17, p. 818-828, 2018. Doi: 10.5897/AJB2018.16493.

MÜLLER, B. S. F.; PAPPAS, J. R.; G. J.; VALDISSER, P. A. M. R.; COELHO, G. R. C.; MENEZES, I. P. P.; ABREU, A. G.; BORBA, T. C. O.; SAKAMOTO, T.; BRONDANI, C.; BARROS, E. G.; VIANELLO, R. P. An operational SNP panel integrated to SSR marker for the assessment of genetic diversity and population structure of the common bean. **Plant Molecular Biology Reporter**, v. 33, p. 1697-1711, 2015. Doi: 10.1007/s11105-015-0866-x.

NAY, M. M.; MUKANKUSI, C. M.; STUDER, B.; RAATZ, B. Haplotypes at the Phg-2 locus are determining pathotype-specificity of angular leaf spot resistance in common bean. **Frontiers in Plant Science**, v. 10, 1126, 2019. Doi 10.3389/fpls.2019.01126.

PÁDUA, P. F.; PEREIRA, R.; ABREU, A. F. B.; RAMALHO, M. A. P.; SOUZA, E. A. Efficiency of a recurrent selection method to achieve resistance of common beans to *Pseudocercospora griseola* in a short period. **Ciência e Agrotecnologia**, v. 45, e013421, 2021. Doi: 10.1590/1413-7054202145013421.

PEREIRA, H. S.; FARIA, L. C.; WENDLAND A.; COSTA J. G. C.; SOUZA, T. L. P. O.; MELO, L. C. Genotype by environment interaction for disease resistance and other important agronomic traits supporting the indication of common bean cultivars. **Euphytica**, v. 214, 12, 2018. Doi.org/10.1007/s10681-017-2093-z.

PERSEGUINI, J. M. K. C.; OBLESSUC, P. R.; ROSA, J. R. B. F.; GOMES, K. A.; CHIORATO, A. F.; CARBONELL, S. A. M.; GARCIA, A. A. F.; VIANELLO, R. P.; BENCHIMOL-REIS, L. L. Genome-wide association studies of anthracnose and angular leaf spot resistance in common bean (*Phaseolus vulgaris* L.). **PLoS ONE**, v. 11, e0150506, 2016. Doi: 10.1371/journal.pone.0150506.

PORCH, T. G.; BEAVER, J. S.; DEBOUCK, D. G.; JACKSON, S. A.; KELLY, J. D.; DEMPEWOLF, H. Use of wild relatives and closely related species to adapt common bean to climate change. **agronomy**, v. 3, p. 433-461, 2013. Doi:10.3390/agronomy3020433.

RAGAGNIN, V. A.; SOUZA, T. L. P. O.; SANGLARD, D. A.; ARRUDA, K. M. A.; COSTA, M. R.; ALZATE-MARIN, A. L.; CARNEIRO, J. E. S.; MOREIRA, M. A.; DE BARROS, E. G. Development and agronomic performance of common bean lines simultaneously resistant to anthracnose, angular leaf spot and rust. **Plant Breeding**, v. 128, p. 156-163, 2009. Doi: 10.1111/j.1439-0523.2008.01549.x.

SANGLARD, D. A.; SOUZA, T. L. P. O.; SOUSA, C. S.; RAGAGNIN, V. A.; BARROS, E. G.; MOREIRA, M. A. Molecular marker assisted backcrossing for developing lines with cv. Pérola genetic background, resistant to rust, anthracnose and angular leaf spot. **Annual report of the Bean Improvement Cooperative**, n. 48, p. 84-85, 2005.

SCHMUTZ, J.; MCCLEAN, P. E.; MAMIDI, S.; WU, G. A.; CANNON, S. B.; GRIMWOOD, J. et al. A reference genome for common bean and genome-wide analysis of dual domestications. **Nature Genetics**, v. 46, p. 707-713, 2014. Doi 10.1038/ng.3008.

SINGH, S. P.; SCHWARTZ, H. F. Breeding Common Bean for Resistance to Diseases: A Review. **Crop Science**, v.v50, p. 2199–2223, 2010. Doi: 10.2135/cropsci2009.03.0163.

SONG, Q.; JIA, G.; HYTEN, D. L.; JENKINS, J.; HWANG, E.; SCHROEDER, S. G.; OSORNO, J. M.; SCHMUTZ, J.; JACKSON, S. A.; MCCLEAN, P. E.; CREGAN, P. B. SNP assay development for linkage map construction, anchoring whole genome sequence and other genetic and genomic applications in common bean. **G3**, v. 5, p. 2285-2290, 2015. Doi: 10.1534/g3.115.020594.

SOUZA, T. L. P. O.; RAGAGNIN, V. A.; DESSAUNE, S. N.; SANGLARD, D. A.; CARNEIRO, J. E. S.; MOREIRA, M. A.; BARROS, E. G. DNA marker-assisted selection to pyramid rust resistance genes in ‘‘carioca’’ seeded common bean lines. **Euphytica**, 199: 303-316, 2014. Doi: 10.1007/s10681-014-1126-0.

TERÁN, H.; JARA, C.; MAHUKU, G.; BEEBE, S.; SINGH, S. P. Simultaneous selection for resistance to five bacterial, fungal, and viral diseases in three Andean x Middle American inter-gene pool common bean populations. **Euphytica**, v. 189, p. 283-292, 2013. Doi: 10.1007/s10681-012-0803-0.

VALDISSER, P. A. M. R.; MÜLLER, B. S. F.; DE ALMEIDA FILHO, J. E.; MORAIS JÚNIOR, O. P.; GUIMARÃES, C. M.; BORBA, T. C. O.; DE SOUZA, I. P.; ZUCCHI, M. I.; NEVES. L. G.; COELHO, A. S. G.; BRONDANI, C.; VIANELLO, R. P. Genome-wide association studies detect multiple QTLs for productivity in mesoamerican diversity panel of common bean under drought stress. **Frontiers in Plant Science**, v. 11, 574674, 2020. doi: 10.3389/fpls.2020.574674.

VALDISSER, P. A. M. R.; PAPPAS JR, G. J.; MENEZES, I. P. P.; MÜLLER, B. S. F.; PEREIRA, W. J.; NARCISO, M. G.; BRONDANI, C.; SOUZA, T. L. P. O.; BORBA, T. C. O.; VIANELLO, R. P. SNP discovery in common bean by restriction-associated DNA (RAD) sequencing for genetic diversity and population structure analysis. **Molecular Genetics and Genomics**, v. 291, p. 1277-1291, 2016. Doi: 10.1007/s00438-016-1182-3.

VALDISSER, P. A. M. R.; PEREIRA, W. J.; ALMEIDA FILHO, J. E.; MÜLLER, B. S. F.; COELHO, G. R. C.; DE MENEZES, I. P. P.; VIANNA, J. P. G.; ZUCCHI, M. I.; LANNA, A. C.; COELHO, A. S. G.; OLIVEIRA, J. P.; MORAES, A. C.; BRONDANI, C.; VIANELLO, R. P. In-depth genome characterization of a Brazilian common bean core collection using DArTseq high-density SNP genotyping. **BMC Genomics**, v. 18, 423, 2017. Doi: 10.1186/s12864-017-3805-4.

VALENTINI, G.; GONÇALVES-VIDIGAL, M. C.; HURTADO-GONZALES, O. P.; CASTRO, S. A. L.; CREGAN, P. B.; SONG, Q.; PASTOR-CORRALES, M. A. High-resolution mapping reveals linkage between genes in common bean cultivar Ouro Negro conferring resistance to the rust, anthracnose, and angular leaf spot diseases. **Theorical and Applied Genetics**, v. 130, p. 1705-1722, 2017. Doi: 10.1007/s00122-017-2920-6.

VIDIGAL FILHO, P. S.; GONÇALVES-VIDIGAL, M. C.; BISNETA, M. V.; SOUZA, V. B.; GILIO, T. A. S.; CALVI, A. A.; LIMA, L. R. L.; PASTOR-CORRALES, M. A.; MELOTTO, M. Genome-wide association study of resistance to anthracnose and angular leaf spot in Brazilian Mesoamerican and Andean common bean cultivars. **Crop Science**, v. 60, p. 2931–2950, 2020. Doi: 10.1002/csc2.20308.

VIEIRA, A. F.; ALMEIDA, L. C. S.; RODRIGUES, L. A.; COSTA, J. G. C.; MELO, L. C.; PEREIRA, H. S.; SANGLARD, D. A.; SOUZA, T. L. P. O. Selection of resistance sources to common bean anthracnose by field phenotyping and DNA marker-assisted screening. **Genetics and Molecular Research**, v. 17, n. 3, gmr18066, 2018. Doi: 10.4238/gmr18066.

VLASOVA, A.; CAPELLA-GUTIÉRREZ, S.; RENDÓN-ANAYA, M.; HERNÁNDEZ-OÑATE, M.; MINOCHE, A. E.; ERB, I. 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**, v. 17, 32, 2016. Doi 10.1186/s13059-016-0883-6.

WU, J.; WANG, L.; FU, J.; JIBAO, C.; WEI, S.; ZHANG, S. et al. Resequencing of 683 common bean genotypes identifies yield component trait associations across a north–south cline. **Nature Genetics**, v. 52, p. 118-125, 2020. Doi 10.1038/s41588-019-0546-0.

CAPÍTULO 1

3 PIRAMIDAÇÃO DE ALELOS DE RESISTÊNCIA À DOENÇAS E ESCURECIMENTO LENTO EM FEIJÃO CARIOCA

RESUMO

As doenças causam prejuízos à produção e à qualidade dos grãos do feijão-comum em todo o mundo, comprometendo a segurança alimentar das pessoas em vulnerabilidade social. O objetivo desse estudo foi selecionar precocemente progênies de feijão carioca que combinem os alelos-alvo de resistência à antracnose (*Co-Realce* e *Co-4*^{2}), à mancha angular (*Phg-2*), à murcha de *fusarium* (*Fop*) e do escurecimento lento dos grãos (*sd*), usando modernas ferramentas biotecnológicas. Seis genitores compuseram o bloco de cruzamentos, Progênie-218311376 (BRSMG Realce x BRS Cometa, *Co-Realce*), K10 (*Co-4*^{2}), CNFC16902 (*sd*), DM103 (*Phg-2*), BRS Sublime (*Phg-Sublime*) e CNFC15826 (*sd* + *Fop*). Foram obtidas 387 plantas F_2 a partir da autofecundação do híbrido múltiplo envolvendo todos os genitores supracitados. As plantas da geração F_2 foram genotipadas com os marcadores P8282v3-817 (A/G - *Co-4*^{2}, o alelo-alvo está sublinhado), g796 (209/233 – *Phg-2*), SE04 (640 – *Phg-2*), ANYMUJW (G/T – *Phg-2*), ANAAJK6 (G/C - *Fop*) e PvbHLHp12804 (A/G - *sd*). Foram selecionadas onze plantas combinando os alelos *Co-4*^{2} (AA) e *Fop* (CC) e outras onze com os alelos *Fop* (CC) e *sd* (AA), todas em homozigose para os alelos-alvo. Os marcadores SNP P8282v3-817, ANAAJK6 e PvbHLHp12804 são úteis para a seleção precoce de plantas portadoras dos alelos *Co-4*^{2}, *Fop* e *sd*. Além de selecionar progênies $F_{2:3}$ com os alelos-alvo, foi possível identificar 28,8% delas com grão carioca dentro dos padrões comerciais. Os marcadores g796, SE04 e ANYMUJW não foram específicos para o alelo de resistência à mancha angular presente no genitor DM103, inviabilizando a seleção de plantas contendo, simultaneamente, os alelos *Co-4*^{2}, *Phg-2*, *Fop* e *sd*. Visando combinar todos os alelos-alvo, as onze progênies $F_{2:3}$ com os alelos *Co-4*^{2} e *Fop* serão cruzadas com outras onze progênies $F_{2:3}$ que carregam os alelos *Fop* e *sd*. Além disso, a linhagem-elite MAIII-16.159 do programa de seleção recorrente da Universidade Federal de Lavras é indicada como fonte de resistência à mancha angular nos próximos blocos de cruzamentos. A eficiência da seleção precoce dos alelos-alvo via marcadores moleculares deve ser confirmada através da inoculação em condição controlada ou em avaliação de campo.

Palavras-chave: *Phaseolus vulgaris* L., genética molecular, seleção precoce, melhoramento genético.

PYRAMIDING OF RESISTANCE ALLELES TO DISEASES AND SLOW DARKENING IN CARIOCA BEANS ASSISTED BY MOLECULAR MARKERS

ABSTRACT

The diseases cause damage to the production and quality of common beans grains worldwide, compromising the food security of low-income social classes. The objective was to select early carioca bean progenies that combine the target alleles of resistance to anthracnose (*Co-Realce* and $Co\text{-}4^2$), angular leaf spot (*Phg-2*), fusarium wilt (*Fop*), and slow darkening (*sd*), using modern biotechnological tools. Six parents composed the crossing block, Progeny-218311376 (BRSMG Realce x BRS Cometa, *Co-Realce*), K10 ($Co\text{-}4^2$), CNFC16902 (*sd*), DM103 (*Phg-2*), BRS Sublime (*Phg-Sublime*) and CNFC15826 (*sd* + *Fop*). 387 F_2 plants were obtained from the self-fertilization of the multiple-hybrid involving all the parents mentioned above. The plants of the F_2 generation were genotyped with the markers P8282v3-817 (A/G - $Co\text{-}4^2$, the target allele is underlined), g796 (209/233 - *Phg-2*), SE04 (640 - *Phg-2*), ANYMUJW (G/T - *Phg-2*), ANAAJK6 (G/C - *Fop*) and PvbHLHp12804 (A/G - *sd*). Eleven plants were selected combining the $Co\text{-}4^2$ (AA) and *Fop* (CC) alleles and another eleven plants with the *Fop* (CC) and *sd* (AA) alleles, all in homozygosity. The SNP markers P8282v3-817, ANAAJK6 and PvbHLHp12804 are useful for the early selection of progenies carrying the $Co\text{-}4^2$, *Fop*, and *sd* alleles. In addition to the target alleles, it was possible to identify 28,8% of them with commercial carioca grain. The markers g796, SE04, and ANYMUJW were not specific for the angular leaf spot resistance allele present in the DM103 parent, making it impossible to select plants containing, simultaneously, the $Co\text{-}4^2$, *Phg-2*, *Fop* and sd alleles. In order to combine all the target alleles, the eleven $F_{2:3}$ progenies with the $Co\text{-}4^2$ and *Fop* alleles will be crossed with another eleven $F_{2:3}$ progenies that carry the Fop and sd alleles. Furthermore, the elite-line MAIII-16.159 from the recurrent selection program of the Federal University of Lavras should be used as an angular leaf spot-resistant parent in the next crossing blocks. The efficiency of early selection of target alleles via molecular markers must be confirmed through inoculation under controlled conditions or in-field evaluation.

Keywords: *Phaseolus vulgaris* L., molecular genetics, molecular breeding, early selection.

4 INTRODUÇÃO

O feijão-comum (*Phaseolus vulgaris* L.) é cultivado em mais 120 países (Long et al., 2020), sendo fonte de macro e micronutrientes essenciais à dieta humana (Kotue et al., 2018). Com alto valor nutricional, o feijão é o alimento mais consumido nos países em desenvolvimento, com as maiores médias de consumo per capita podendo chegar a 40 - 60 kg ano^{-1} em Ruanda, Quênia e Uganda (Mukankusi et al., 2019). O Brasil figura entre os principais produtores de feijão-comum do mundo, com consumo per capita de 15 kg ano^{-1} (Ferreira et al., 2018). A importância socioeconômica do feijão-comum torna-o uma cultura-chave para a segurança alimentar (Nadeen et al., 2021), sendo necessário um incremento de 30% em sua produção até 2050 (Porch et al., 2013).

A estabilidade da produção de feijão-comum é constantemente desafiada pela ocorrência de doenças nas regiões produtoras (Brown & Hovmoller, 2002; Bebber et al., 2014). Estima-se que cerca de 45 doenças, de maior ou menor importância, causam perdas significativas na produção e na qualidade dos grãos de feijão (Singh & Schwartz, 2010). No tocante as doenças fúngicas, a antracnose, a mancha angular e a murcha de fusarium apresentam ampla variabilidade patogênica e podem causar perdas na produção e na qualidade dos grãos em algo entre 70 e 100% (Ragagnin et al., 2009; Souza et al., 2014; Maina et al., 2017).

Para antracnose, já foram relatados cerca de 182 patótipos em todo o mundo (Padder et al., 2017), em que 71 foram descritos no Brasil (Pereira et al., 2018). A resistência à antracnose é conferida, principalmente, por genes de efeito principal (genes R) e independentes, identificados pelo símbolo *Co* (Kelly & Young, 1996; Kelly & Vallejo, 2004; Gonçalves-Vidigal et al., 2013). Atualmente, 14 genes R foram mapeados em diferentes cromossomos de P. vulgaris (Paulino et al., 2022) e que seguem a nomenclatura '*Co*': *Co-1* ao *Co-6*, co-8 (recessivo) e *Co-11* ao *Co-17* (http://www.bic.uprm.edu). A mancha angular também possui variabilidade considerável, sendo identificados 26 patótipos em dez estados brasileiros, em que os patótipos 63-31, 63-23, 63-55, 63-39, 63-47 e 63-63 foram os mais frequentes (Nietsche et al., 2002; Pereira et al., 2015). A resistência à mancha angular é governada por cinco genes R, em que três foram identificados em fontes do pool gênico Andino (*Phg-1*, *Phg-4* e *Phg-5*) e dois foram mapeados em genótipos do pool gênico Mesoamericano (*Phg-2* e *Phg-3*) (Souza et al., 2016; Nay et al., 2019). No que tange à murcha de fusarium, foram identificados 27 patótipos entre os isolados coletados no Brasil

(Henrique et al., 2014), indicando que a variabilidade na população de *F. oxysporum* f. sp. *phaseoli* é superior a previamente relatada na literatura (Woo et al., 1996). Estudos indicam que o controle genético da resistência à murcha de *fusarium* pode ser monogênico (Fall et al., 2001; Batista et al., 2017), oligogênico (até três genes) (Batista et al., 2017) ou mesmo poligênico (Cross et al., 2000; Paulino et al., 2021).

Para reduzir os danos causados por essas doenças, as principais estratégias de manejo são baseadas na aplicação de produtos químicos, rotação de culturas e uso de cultivares resistentes (Souza et al., 2014; Batista et al., 2016). O uso da resistência genética é considerado o método mais promissor, ambientalmente sustentável e economicamente mais rentável, além de fácil adoção pelo produtor (Assefa et al., 2019). Adicionalmente, garante a maior estabilidade do desempenho agronômico das cultivares, reduz o custo de produção e minimiza os riscos de contaminação do ambiente por moléculas químicas aplicadas no controle das doenças (Miklas et al., 2006; Souza et al., 2013). Com isso, a obtenção de cultivares com resistência efetiva contra patótipos de um patógeno ou combinando resistência à dois ou mais patógenos tem sido o grande desafio dos programas de melhoramento (Valentini et al., 2017).

A piramidação de alelos de resistência oriundos de fontes de resistência de pool gênicos distintos pode ser uma estratégia útil ao melhoramento para o desenvolvimento de cultivares com resistência ampla e duradoura às principais doenças que acometem o feijão-comum (Meziadi et al., 2016). Essa estratégia é embasada nas evidências da coevolução entre patógeno e hospedeiro (*P. vulgaris*), onde isolados de origem Andina são mais patogênicos, principalmente, aos genótipos de feijão oriundos deste mesmo pool gênico (Miklas et al., 2006). Por sua vez, os isolados de origem Mesoamericana, além de serem patogênicos aos genótipos Mesoamericanos, também podem infectar feijões de origem Andina (Kelly & Miklas, 1998; Miklas et al., 2006; Terán et al., 2013). Esta particularidade em termos evolutivos dentro e entre pools gênicos influencia diretamente nos métodos de melhoramento que serão adotados pelos programas de melhoramento, sendo o seu conhecimento muito importante no desenvolvimento de cultivares resistentes. Além disso, o entendimento acerca da diversidade populacional do patógeno bem como os patótipos que ocorrem em regiões específicas são cruciais para o sucesso do programa de piramidação alélica (Cruz et al., 2018).

O emprego da piramidação encontra limitações quando são utilizados métodos de melhoramento convencionais, visto que o efeito individual de um gene de resistência nem sempre é facilmente identificado ou mensurado quando se têm outros genes de resistência presentes no mesmo genótipo (Ragagnin et al., 2009). No entanto, com a validação de marcadores moleculares ligados aos alelos-alvo, tornou-se viável a introgressão de dois ou mais alelos de resistência ao mesmo tempo (Kelly & Miklas, 1998). Além do mais, a adoção da seleção assistida promove uma redução de tempo e de custos durante o processo de avaliação e seleção (Miklas et al., 2006; Kelly & Miklas, 1998).

Por intermédio do melhoramento genético novas cultivares são lançadas a cada ano com algum nível de resistência aos principais patógenos de relevância para a cultura do feijão-comum, como a BRS FC402, resistente à antracnose e moderadamente resistente à murcha de *fusarium* (Melo et al., 2017), a BRS FC406, resistente à mancha angular e antracnose (Pereira et al., 2021*a*) e a BRS Notável, moderadamente resistente à antracnose e murcha de *fusarium* (Pereira et al., 2012). Até o presente momento, não foram desenvolvidas cultivares que combinem resistência à antracnose, mancha angular e murcha de *fusarium*, havendo então, a necessidade de buscarmos por soluções tecnológicas nesse sentido. Neste contexto, o objetivo deste estudo foi desenvolver linhagens-elite de feijão carioca contendo alelos de resistência à antracnose, mancha angular e murcha de *fusarium*, além de possuírem escurecimento lento dos grãos, usando ferramentas modernas de biotecnologia.

5 MATERIAL E MÉTODOS

5.1 Material genético e alelos-alvo

Para este estudo foram selecionados seis genitores-elite, que além da resistência genética, também possuíam atributos relacionados à qualidade comercial dos grãos (**Tabela 1.1**).

Tabela 1.1. Genitores utilizados nos blocos de cruzamentos visando a piramidação de alelos-alvo de resistência à antracnose, mancha angular, murcha de fusarium e escurecimento lento dos grãos.

Genitor	Critério de seleção	Alelo-alvo	Fenótipo	Pool gênico	Referência
K10	ANT	*Co-4*^{2}	R	M	Vieira et al. (2018)
Progênie-218311376	ANT	*Co-Realce*	R	A	Gomes et al. (2019)
DM103	MA	*Phg-2*	R	M	Sanglard (2010)
BRS Sublime	MA	*Phg-Sublime*	R	M	Wendland et al. (2018)
CNFC 15826	FU + EL	*Fop + sd*	R + EL	M	Rodrigues et al. (2019a, 2019b)
CNFC 16902	EL	*sd*	EL	M	Pereira et al. (2021b)

M: Mesoamericano; A: Andino; ANT: Resistência à antracnose; MA: Resistência à mancha angular; FU: Resistência à murcha de *fusarium*; EL: Escurecimento lento; R: Resistente; MR: Moderadamente resistente.

O genitor K10 é de origem Mesoamericana e carrega os alelos *Co-3*^{4}, *Co-4*^{2}, *Co-5* e *Co-6*, que conferem às plantas de feijão-comum o fenótipo de reação de resistência à antracnose (Vieira et al., 2018). A Progênie-218311376, obtida do cruzamento entre BRSMG Realce e BRS Cometa, possui o alelo *Co-Realce* e pertence ao background genético carioca (Gomes et al., 2019). A linhagem DM103, de origem Mesoamericana, foi selecionada por Sanglard (2010) por carregar o alelo *Phg-2* (Mesoamericano) de resistência à mancha angular, com nota média de 3,0. BRS Sublime é uma cultivar de grão carioca, resistente à mancha angular (alelo *Phg-Sublime*) e com aptidão para a colheita mecanizada, além de apresentar grão carioca com alta qualidade nutricional e potencial produtivo estimado em 4667 kg ha^{-1} (Wendland et al., 2018). As linhagens CNFC 15826 e CNFC 16902 carregam o alelo *sd* em homozigose, que confere o fenótipo de escurecimento lento em grãos de feijão carioca (Rodrigues et al., 2019a; Pereira et al., 2021b). Além do escurecimento lento dos grãos, a linhagem CNFC 15826 é resistente à murcha de fusarium, sendo fonte do alelo *Fop* (Rodrigues et al., 2019b).

5.2 Sistema de cruzamentos e avanço de gerações

O planejamento dos cruzamentos foi realizado de modo que os genitores a serem cruzados apresentassem coincidência de florescimento. Para tanto, a semeadura foi realizada em duas épocas e com intervalos de aproximadamente uma semana entre si.

Neste estudo foram realizados três cruzamentos simples, um duplo e um múltiplo, visando a piramidação dos alelos-alvo (**Figura 1.1**). As hibridações artificiais foram conduzidas em casa de vegetação climatizada, durante o período de temperatura mais amena (das 8 horas às 11 horas). A emasculação, na fase de botão floral (antes da liberação do pólen), foi realizada com o auxílio de uma pinça e a exposição do estigma foi promovida retorcendo as duas pétalas maiores (estandarte) no sentido horário. A confirmação dos cruzamentos e o monitoramento da introgressão dos alelos-alvo foram feitos via Seleção Assistida por Marcadores Moleculares (SAM) (**Tabela 1.2; Apêndice 1.A**).

O número de plantas necessárias para identificar pelo menos uma com o genótipo de interesse na geração F_t foi obtido conforme descrito por Ramalho et al. (2012), com a seguinte expressão:

$$n = \frac{log\ (1 - P)}{log\ (1 - p)}$$

Em que n é o tamanho mínimo da geração F_t para que se tenha pelo menos uma planta com os alelos *Co-Realce*, *Co-4*^{2}, *Phg-2*, *Fop* e *sd*; P é a probabilidade de ocorrência do genótipo desejado (P = 0,95) e p a frequência esperada na geração t de obter pelo menos uma planta com todos os alelos-alvo (k) em homozigose ou em heterozigose, que pode ser obtida pelas equações $p = \left(\frac{(2^{t-1} - 1)}{2^t}\right)^k$ e $p = \left(\frac{(2^{t-1} + 1)}{2^t}\right)^k$, sendo a primeira para genótipos homozigotos e a segunda para genótipos heterozigotos.

Inicialmente, foram obtidos dois híbridos simples: $F_{1(AB)}$ [Prog. 218311376♀ X K10♂] e $F_{1(CD)}$ [CNFC 16902♀ X DM 103♂] (**Figura 1.1**). O híbrido duplo $F_{1(AB)(CD)}$ (**Figura 1**) foi obtido intercruzando as plantas oriundas os híbridos simples $F_{1(AB)}$ e $F_{1(CD)}$. Simultaneamente a obtenção do híbrido duplo, obteve-se o híbrido simples $F_{1(EF)}$ [BRS Sublime♀ x CNFC 15826♂] (**Figura 1.1**). O híbrido múltiplo foi obtido cruzando as plantas do híbrido duplo $F_{1(AB)(CD)}$ com as plantas do híbrido simples $F_{1(EF)}$ (**Figura 1.1**). As sementes híbridas do cruzamento múltiplo foram semeadas para o avanço de geração

$F_1 \longrightarrow F_2$. Após um período de aproximadamente três meses, as plantas contendo os alelos-alvo tiveram suas sementes F_2 colhidas individualmente. Com objetivo de se obter maior número de sementes, avançou-se mais uma geração, $F_{2:3} \longrightarrow F_{2:4}$ (**Figura 1.1**). Todas as etapas de cruzamentos e avanço de gerações foram conduzidas em casa de vegetação na Embrapa Arroz e Feijão usando vasos de 10 litros preenchidos com solo. A adubação de semeadura foi realizada com fosfato monoamônico (MAP), com 48% de P_2O_5. A adubação de cobertura foi realizada com uréia (44% de N) em dois momentos, o primeiro aos 15 dias após a semeadura e o segundo no início da floração. Em cada etapa do avanço de geração, realizou-se o tratamento das sementes com fungicida Vitavax®-Thiram na dose de 250 mL $100kg^{-1}$ de semente para o controle da podridão radicular e do tombamento, ambos causados pelo fungo Rhizoctonia solani. Quando necessário, o controle da mosca minadora (Liriomyza huidobrensis) foi realizada com o inseticida Benevia® (FMC) na dose de 250 mL ha^{-1}.

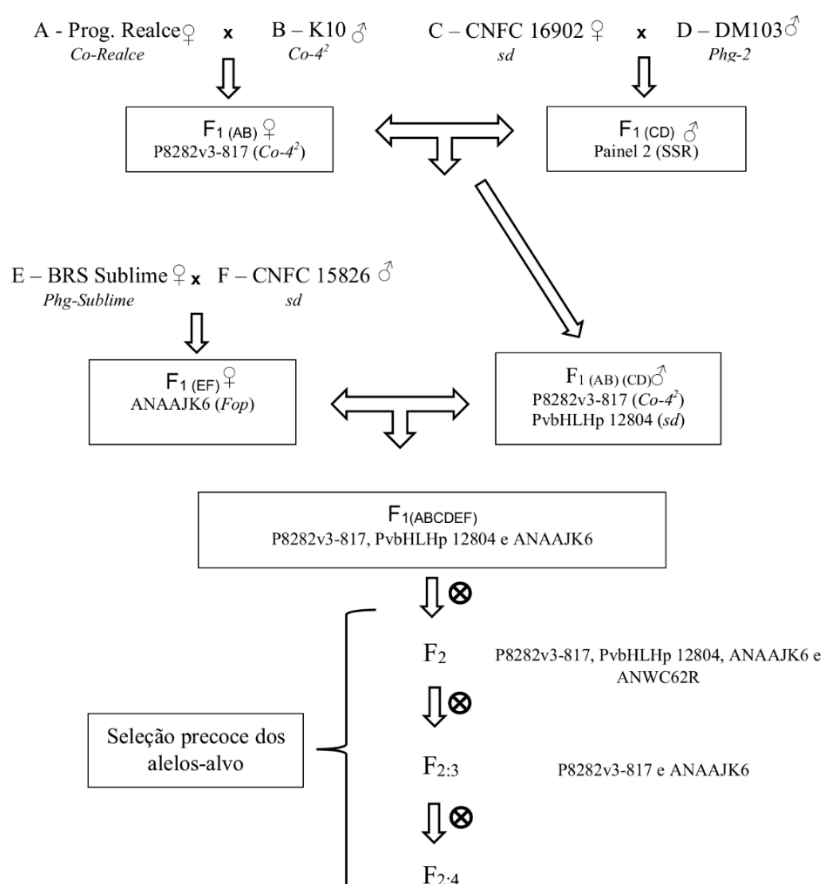


Figura 1.1. Esquema ilustrativo dos cruzamentos visando a piramidação dos alelos-alvo *Co-Realce*, *Co-4²*, *Phg-2*, *Fop* e *sd*. Genitores: **A** - Progênie 218311376 (Prog. Realce, *Co-Realce*); **B** - K10 (*Co-4²*); **C** - CNFC 16902 (*sd*); **D** - DM103 (*Phg-2*); **E** – BRS Sublime (*Phg-Sublime* + *Car-11*) e **F** – CNFC 15826 (*sd* + *Fop*).

5.3 Seleção assistida por marcadores moleculares - SAM

Todas as etapas de genotipagem foram realizadas no laboratório de Biotecnologia da Embrapa Arroz e Feijão. A extração do DNA genômico foi realizada a cada geração de cruzamentos com o objetivo de monitorar a introgressão dos alelos-alvo bem como eliminar plantas oriundas de autofecundação. Foram coletados, em microtubos devidamente identificados, dois discos foliares de aproximadamente 5 mm de diâmetro de cada planta (estádio V3) oriundas dos cruzamentos exemplificados na **Figura 1.1**. As amostras de tecido foliar foram armazenadas em freezer com temperatura ajustada para -20°C. A extração foi realizada com base no protocolo CTAB, adaptado de Brondani et al. (1998) para isolamento de DNA genômico de *Phaseolus vulgaris* L.

A concentração do DNA extraído foi estimada por espectrometria em NanoDrop 2000 (Thermo Scientific®, Waltham/EUA) e a integridade foi verificada por meio de eletroforese em gel de agarose 1%, corado com SYBR Green (Life Technologies®, São Paulo, Brasil). O resultado foi fotodocumentado por fotografia sob luz UV, utilizando-se o equipamento transiluminador (Geldoc – Bio-Rad) e programa computacional QuantityOne. As amostras foram diluídas em água ultrapura e foram padronizadas em 100 ng μL^{-1} para serem utilizadas nas reações de PCR (Polymerase Chain Reaction).

A introgressão dos alelos-alvo e a natureza híbrida das plantas foram monitoradas utilizando-se os marcadores descritos nas **Tabela 1.2 e Apêndice 1.A**.

Cada reação de PCR com o marcador g796 foi realizada com um volume final de 5,0 µL, sendo 0,5 µL de H_2O RNAse-free, 2,5 µL de Master Mix (ThermoFisher) [2X], 0,5 µL de Q-Solution (Qiagen), 0,5 µL do F-g796/R-g796 [10µM] e 1 µL de DNA [100 µg/uL]. As reações de PCR para o marcador SE04 foram conduzidas com volume final de 10 µL, contendo 0,5 µL de H_2O RNAse-free, 2,5 µL de Master Mix (ThermoFisher) [2X], 0,5 µL de Q-Solution (Qiagen), 0,25 µL do F-SE04 [10µM], 0,25 µL do R-SE04 [10µM] e 1 µL de DNA [100 µg/uL]. Todas as reações de amplificação foram performadas em termociclador Veriti (Applied Biosystems®, USA) e os programas de amplificação específicos para cada marcador molecular estão apresentados no **Apêndice 1.B**. Os fragmentos específicos para os marcadores g796 (209/233 pb) e SE04 (640 pb) foram obtidos via eletroforese em gel de agarose 2,5% com marcador de peso molecular conhecido. As reações de PCR loco-específicas para os marcadores em ensaio de hidrólise do tipo TaqMan® (ThermoFisher) (Shen et al., 2009; Appliedbiosystems, 2021) foram constituídas de 100 ng μL^{-1} de DNA,

2,5 µL de TaqMan™ GTXpress™ Master Mix (Applied Biosystems™), 0,125 µL do ensaio TaqMan (Thermo Fisher ScientificTM) e 1,375 µL de H_20 RNAse-free, totalizando 5 µL. As condições de amplificação, anelamento e extensão usando o equipamento QuantStudio 7 Flex (Applied Biosystems) podem ser observadas no **Apêndice 1.B**. Ao final, a análise dos alelos foi realizada utilizando o programa Genotyping Analysis Module, V.3.7.

Na ausência de marcadores polimórficos entre os genitores para os alelos-alvo, a natureza híbrida das plantas foi determinada com marcadores SSR (Morais et al., 2016) (**Apêndice 1.A**). A amplificação dos locos SSR foi conduzida com o kit Qiagen® PCR Multiplex (Qiagen), conforme descrito pelo fabricante. O volume final de cada reação foi de 5 µL, contendo 100 ng μL^{-1} de DNA e as concentrações de pares de primers individuais variando de 0,06 µM a 1,2 µM, dependendo da intensidade do produto amplificado (Valdisser et al., 2013). As reações de PCR foram conduzidas em termociclador ABI 9700 (Applied Biosystems), com uma etapa inicial de desnaturação do DNA e ativação da enzima HotStar Taq DNA Polimerase à 95 ºC por 15 minutos, seguida por 40 ciclos de desnaturação à 94 ºC por 30 segundos, anelamento à 56 ºC por 1 minuto e 30 segundos e extensão à 72 ºC por 1 minuto e 30 segundos. Ao final dos ciclos, foi realizada uma etapa de extensão à 72 ºC por 10 minutos (Valdisser et al., 2013).

Após a reação de PCR, os produtos derivados da amplificação foram diluídos na proporção de 10 vezes com água Milli-Q estéril para reduzir o nível de intensidade da fluorescência e proporcionar melhor padrão de detecção dos fragmentos (Valdisser et al., 2013). Após esta etapa, foi preparado o mix contendo uma alíquota de 0,5 µL do produto da PCR diluído, 9,4 µL de formamida (Hi-Di®, Applied Biosystems) e 0,08 µL do marcador padrão (GeneScan™ 500 Rox™ Size Standard). Os fragmentos amplificados foram separados via eletroforese capilar conduzida em analisador automático de DNA (ABI 3100 Genetic Analyzer, Applied Biosystems). Os dados de saída foram coletados utilizando o programa Data Colection versão 2.0 (Applied Biosystems) e analisados usando programa GeneMapper versão 4.1 (Applied Biosystems) para a chamada dos alelos em pares de base (Valdisser et al., 2013).

Tabela 1.2. Descrição dos marcadores moleculares que foram utilizados para monitorar a introgressão dos alelos-alvo no programa de piramidação alélica.

Marcador	Tipo	Position (pb)	Cromossomo	Polimorfismo	Alelo	Fonte	Trait*	Referência
g796	STS	61514592	Pv08	209/233 pb	*Phg-2*	México 54	Mancha angular	*Miller* et al. (201*8*)
SE04	SCAR	-	Pv08	640 pb	*Phg-2*	MAR-2	Mancha angular	Sanglard *(2010)*
ANYMVJW	SNP	62193174	Pv08	T/G	*Phg-2*	México 54	Mancha angular	Lobaton et al. (2018)
P8282v3-817	SNP	2278285	Pv08	G/A	*Co-4*^{2}	SEL1308	Antracnose	Gomes-Messias et al. (2022)
ANAAJK6	SNP	26864618	Pv02	G/C	*Fop*	CNFP10794	Murcha de fusarium	Torres (2020)
ANCFFDU	SNP	26953858	Pv02	T/A	*Fop*	CNFP10794	Murcha de fusarium	Torres (2020)
PvbHLHp12804	SNP	28765330	Pv07	G/A	*sd*	BRSMG Madrepérola	Escurecimento lento	Alvares et al. (2019)
ANWC62R	SNP	6128743	Pv08	T/A	*Car-11*	BRS Sublime	Carlavírus	Silva (2021)
snp12782	SNP	1182123	Pv04	C/T	*Co-Realce*	BRSMG Realce	Antracnose	Capítulo 2
snp3308	SNP	505696	Pv04	C/T	*Co-Realce*	BRSMG Realce	Antracnose	Capítulo 2
snp1327	SNP	477285	Pv04	T/C	*Co-Realce*	BRSMG Realce	Antracnose	Capítulo 2

O alelo-alvo associado ao fenótipo de interesse está sublinhado; *Refere-se ao fenótipo de resistência à antracnose, mancha angular, murcha de *fusarium*, carlavírus e do escurecimento lento dos grãos.

5.4 Análise da segregação dos alelos-alvo na geração F_2

Realizou-se a análise de segregação dos alelos-alvo na população F_2 oriunda do híbrido múltiplo $F_{1(ABCDE)}$ com base na informação genotípica dos marcadores P8282v3-817 (G/A – *Co-4²*), ANAAJK6 (G/C - *Fop*) e PvbHLHp12804 (G/A - *sd*). A estatística qui-quadrado ($\chi 2$) foi estimada para testar a aderência das frequências observadas às frequências esperadas, ao nível de 5% de probabilidade e considerando a segregação 1:2:1 (marcadores codominantes).

5.5 Cor do grão das progênies $F_{2:3}$ após armazenamento

Avaliou-se a cor dos grãos após um período de armazenamento (CGA) de 147 dias em câmara fria, visando identificar aquelas com padrão de cor de grão carioca. A escala de notas variou de 1 a 5: nota 1 - grãos com a cor do tegumento muito clara; 2 - grãos com a cor do tegumento intermediária tendendo para clara; 3 - grãos com a cor do tegumento intermediária sem tendência; 4 - grãos com a cor do tegumento intermediária tendendo para escura e 5 - grãos com a cor do tegumento escura (Silva et al., 2008).

6 RESULTADOS

6.1 Híbridos simples

O cruzamento $F_{1(AB)}$ [Prog-218311376♀ X K10♂] gerou 33 plantas, as quais foram genotipadas com o SNP P8282v3-817, resultando em 32 plantas heterozigotas (A/G, o polimorfismo associado ao alelo-alvo está sublinhado) e uma oriunda de autofecundação (GG), sendo, portanto, excluída. Do híbrido $F_{1(CD)}$ [CNFC 16902♀ X DM 103♂] foram obtidas 35 plantas, e como os marcadores g796 (209/233), SE04 (640/-) e ANYMVJW (G/T), específicos para o alelo *Phg-2* (**Tabela 1.2**) foram monomórficos entre os genitores DM103 e CNFC 16902 ou não amplificaram o alelo-alvo no parental doador (**Figuras 1.2A e 1.2B; Tabela 1.3**), a natureza híbrida das plantas foi confirmada usando os marcadores microssatélites do painel 2 (**Apêndice 1.A**). Os marcadores microssatélites PV5 e PV35 foram polimórficos entre os genitores DM103 e CNFC 16902, amplificando em 24 das 35 plantas, sendo todas híbridas (**Apêndice 1.C**). As plantas híbridas dos cruzamentos $F_{1(AB)}$ e $F_{1(CD)}$ foram intercruzadas para a obtenção do híbrido duplo, tendo o $F_{1(AB)}$ como parental masculino (**Figura 1.1**). Um total de 33 plantas do híbrido simples $F_{1(EF)}$ [BRS Sublime♀ x CNFC 15826♂] foram genotipadas com o ensaio ANAAJK6 (G/C), sendo seis homozigotas (GG) para o alelo do parental feminino e, portanto, eliminadas do bloco de cruzamentos com o híbrido duplo por serem frutos de autofecundação. A taxa de sucesso do processo de hibridação variou de 81,8% para o $F_{1(EF)}$ a 100% para o híbrido $F_{1(CD)}$.

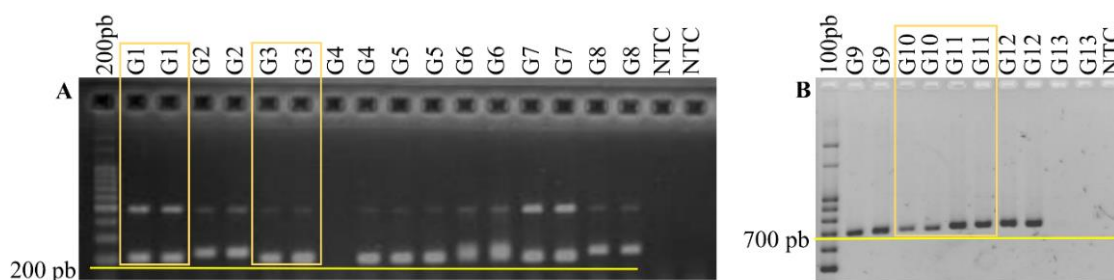


Figura 1.2. A - Gel de agarose à 2,5% do produto da PCR com o marcador codominante g796 (*Phg-2*): DM103 (G1), México54 (G2), CNFC16902 (G3), BRS Sublime (G4), DM103 x CNFC16902 (G5), México54 x CNFC16902 (G6), DM103 x BRS Sublime (G7) e México54 x BRS Sublime. Suscetível (209 pb); Resistente (233 pb). **B -** Gel de agarose à 2,5% do produto da PCR com o dominante marcador SE04 (*Phg-2*): MAR-2 (G9), DM103 (G10), CNFC16902 (G11), México54 (G12) e Rudá (G13). Suscetível (Ausência); Resistente (640 pb).

Tabela 1.3. Perfil molecular dos genitores utilizados neste estudo quanto à presença dos alelos-alvo em ensaios TaqMan.

Genótipo	P8282v3-817 (*Co-4*^{2})		ANYMVJW (*Phg-2*)		ANAAJK6 (*Fop*)		PvbHLHp12804 (*sd*)		ANWC62R (*Car-11*)	
	A/G	ANT	G/T	MA	G/C	FOP	A/G	EL	T/A	Carlavírus
BRS Sublime	GG	-	TT	R	GG	-	GG	ER	TT	2 (R)
CNFC 15826	GG	-	TT	-	CC	R	AA	2.0 (EL)	TT	-
CNFC 16902	GG	-	TT	-	-	-	AA	2.0 (EL)	TT	-
DM 103	GG	-	TT	MR	-	-	GG	-	AA	-
K10	AA	R	TT	-	-	-	-	-	AA	-
Prog-218311376	GG	R	TT	-	-	-	-	-	AA	-
BRS Horizonte	GG	-	-	S	GG	7.2 (S)	-	-	AA	-
BRSMG Madrepérola	GG	-	TT	-	-	-	AA	2.0 (EL)	-	
CNFCT 16207	-	-	-	-	-	-	-	-	AA	9 (S)
CNFP10794	GG	-	TT	-	CC	2.75 (R)	-	-	-	-
México 54	GG	-	GG	R	-	-	-	-	-	-
IPA 7419	GG	S	TT	-	-	-	-	-	-	-

O alelo-alvo associado ao fenótipo de interesse está sublinhado, ANT – Reação à antracnose; MA – Reação à mancha angular; FOP – Reação à murcha de *fusarium*; Carlavírus – Reação ao carlavírus; Fenótipo: R - Resistente; MR – Moderadamente Resistente; S – Suscetível; EL – Escurecimento Lento; ER – Escurecimento Regular

6.2 Híbrido duplo e múltiplo

Do cruzamento duplo, foram obtidas 151 plantas, das quais 77 e 75 plantas foram heterozigotas para os alelos $Co\text{-}4^2$ (A/G) e sd (A/G), respectivamente. Entre as plantas heterozigotas, obteve-se 33 combinando os alelos $Co\text{-}4^2$ e *sd*. As 27 plantas híbridas do cruzamento $F_{1(EF)}$ foram cruzadas com as plantas heterozigotas do híbrido duplo para obtenção do híbrido múltiplo $F_{1(ABCDEF)}$. A etapa de obtenção do híbrido múltiplo ocorreu em plena pandemia (Set/2020), prejudicando o número de cruzamentos realizados diariamente, o que resultou em apenas três hibridações efetivas (**Apêndice 1.D**). Foram obtidas sete plantas do híbrido $F_{1(ABCDEF)}$, as quais foram individualizadas, sendo codificadas como LM01, LM02, LM03, LM04, LM05, LM06 e LM07 (**Tabela 1.4**). A genotipagem dessas plantas revelaram diferentes combinações dos alelos-alvo $Co\text{-}4^2$, *Fop* e *sd* (**Tabela 1.4**). Apenas a planta LM04 foi homozigota para o alelo associado à suscetibilidade à antracnose (GG – $co\text{-}4^2/co\text{-}4^2$), homozigota para o alelo associado ao fenótipo de resistência à murcha de fusarium (CC – *Fop*/*Fop*) e para o alelo associado ao escurecimento lento dos grãos (AA – *sd*/*sd*) (**Tabela 1.4**). As demais plantas apresentaram pelo menos um dos alelos-alvo em heterozigose (**Tabela 1.4**). As plantas F_2 oriundas de LM04 não foram usadas na análise de segregação dos marcadores P8282v3-817 (G/A – $Co\text{-}4^2$), ANAAJK6 (G/C - *Fop*) e PvbHLHp12804 (G/A - *sd*). Para a análise de aderência das frequências observadas às esperadas, foram utilizadas apenas as plantas F_2 oriundas das plantas do híbrido $F_{1(ABCDEF)}$ que eram heterozigotas em relação aos alelos-alvo desse estudo (**Tabela 1.4**). As sete plantas F_1 supracitadas foram autofecundadas e geraram 394 sementes F_2 (**Apêndice 1.D**).

6.3 Geração F_2: seleção precoce de alelos-alvo

As plantas LM01 e LM07 produziram o maior (97) e o menor (14) número de sementes F_2, respectivamente (**Apêndice 1.D**). Ao todo, foram obtidas 387 plantas F_2. Nesta etapa, realizou-se a genotipagem com os marcadores em ensaios TaqMan P8282v3-817 (A/G), ANAAJK6 (G/C) e PvbHLHp12804 (A/G) (**Tabela 1.2**), o que resultou na seleção de onze plantas combinando os alelos $Co\text{-}4^2$ (AA) e *Fop* (CC) em homozigose e de outras 82 plantas combinando os alelos *Fop* (CC) e *sd* (AA) em homozigose. Das onze plantas F_2 homozigotas para os alelos $Co\text{-}4^2$ (AA) e *Fop* (CC), sete produziram sementes $F_{2:3}$ (**Tabela 1.5**). As sementes da planta P1551-LM01-p63 (AA/CC) (**Tabela 1.5**) foram subdivididas em pequenas e graúdas. Com essa subdivisão, foram gerados oito grupos de progênies $F_{2:3}$ oriundos das sete plantas F_2, G01 ao G08 (**Tabela 1.6**). Os maiores números de progênies

$F_{2:3}$ foram obtidos nos grupos G02 e G03, por outro lado, o menor número foi obtido no grupo G02, com um total de 59 progênies $F_{2:3}$ (**Tabela 1.6**). Entre as 82 plantas homozigotas para os alelos *Fop* (CC) e *sd* (AA), onze foram selecionadas por possuírem escurecimento lento (nota da CGA = 1), grãos-carioca com fundo creme claro, rajas marrons claras, halo claro e hilo branco, atributos que atendem o padrão comercial (**Apêndice 1.E**).

Tabela 1.4. Genotipagem das sete plantas do cruzamento múltiplo $F_{1(ABCDEF)}$ com os P8282v3-817 (G/A – *Co-4²*), ANAAJK6 (G/C - *Fop*) e PvbHLHp12804 (G/A - *sd*).

Planta F_1	P8282v3-817	Alelo-alvo	ANAAJK6	Alelo-alvo	PvbHLHp12804	Alelo-alvo
LM01	GA	*co-4²/Co-4²*	GC	*fop/Fop*	GG	*Sd/Sd*
LM02	GG	*co-4²/co-4²*	GC	*fop/Fop*	GA	*Sd/sd*
LM03	GA	*co-4²/Co-4²*	GC[1]	*fop/Fop*	GG	*Sd/Sd*
LM04	GG	*co-4²/co-4²*	CC[2]	*Fop/Fop*	AA	*sd/sd*
LM05	GG	*co-4²/co-4²*	GG[3]	*fop/fop*	GA	*Sd/sd*
LM06	GA	*co-4²/Co-4²*	GG	*fop/fop*	GA	*Sd/sd*
LM07	GG	*co-4²/co-4²*	CC	*Fop/Fop*	AA	*sd/sd*

O alelo-alvo associado ao fenótipo de interesse está sublinhado. [1]Planta heterozigota em relação ao alelo-alvo; [2]Planta homozigota em relação ao alelo-alvo; [3]Planta homozigota para o alelo alternativo ao alelo-alvo.

Com o objetivo de aumentar o número de sementes, avançou-se uma geração com as 59 progênies $F_{2:3}$, sendo obtidas 2964 sementes $F_{2:4}$ (**Tabela 1.6**). 43 progênies $F_{2:3}$ tem como genealogia a planta LM01 e 16 a planta LM03 (**Tabela 1.6**). Como era esperado considerando a genealogia das plantas F_2 selecionadas com os alelos *Co-4²* e *Fop* (**Tabela 1.5**), as 59 progênies $F_{2:3}$ foram homozigotas para os alelos *Co-4²* (AA) e *Fop* (CC). Os fenótipos das sementes das progênies $F_{2:3}$ podem ser observados nos **Apêndices 1.F (A) ao 1.F (G)** e na **Tabela 1.6**.

Tabela 1.5. Plantas F_2 com os alelos-alvo piramidados. Plantas codificadas com a genealogia LM01 e LM03 possuem os alelos-alvo *Co-4*^{2} (AA) e *Fop* (CC) piramidados. Plantas com a genealogia LM04 possuem os alelos-alvo *Fop* (CC) e *sd* (AA) piramidados.

	Planta F_2	Sementes $F_{2:3}$	Genótipo Molecular			Combinação Alélica
			P8282v3-817	ANAAJK6	PvbHLHp12804	
LM01	P1489-LM01-p1	0	AA	CC	GG	*Co-4*^{2} + *Fop*
	P1493-LM01-p5	32	AA	CC	GG	
	P1505-LM01-p17	1	AA	CC	GG	
	P1540-LM01-p52	0	AA	CC	GG	
	P1542-LM01-p54	0	AA	CC	GG	
	P1551-LM01-p63	22	AA	CC	GG	
	P1556-LM01-p68	0	AA	CC	GG	
	P1557-LM01-p69	16	AA	CC	GG	
	P1581-LM01-p93	5	AA	CC	GG	
LM03	P1635-LM03-p9	30	AA	CC	GG	*Co-4*^{2} + *Fop*
	P1678-LM03-p52	22	AA	CC	GG	
LM04	P1719_21-LM04-p23	50	GG	CC	AA	*Fop* + *sd*
	P1721_21-LM04-p25	19	GG	CC	AA	
	P1722_21-LM04-p26	47	GG	CC	AA	
	P1724_21-LM04-p28	21	GG	CC	AA	
	P1726_21-LM04-p30	17	GG	CC	AA	
	P1727_21-LM04-p31	35	GG	CC	AA	
	P1730_21-LM04-p34	44	GG	CC	AA	
	P1733_21-LM04-p37	26	GG	CC	AA	
	P1734_21-LM04-p38	19	GG	CC	AA	
	P1745_21-LM04-p49	46	GG	CC	AA	
	P1757_21-LM04-p61	25	GG	CC	AA	

O alelo-alvo associado ao fenótipo de interesse está sublinhado.

Tabela 1.6. Descrição das 59 progênies $F_{2:3}$ obtidas a partir das plantas F_2 homozigotas para os alelos-alvo *Co-4*^{2} (AA) e *Fop* (CC).

Planta F_2	N° plantas $F_{2:3}$	Progênie $F_{2:3}$	N° sementes $F_{2:4}$	Massa de 20 sementes	CGA	Combinação alélica
P1493-LM01-P5	G01 (9)	LM2906	81	5.35	4	AA+CC (*Co-4*^{2} + *Fop*)
P1493-LM01-P5		LM2907	40	5.43	3	
P1493-LM01-P5		LM2908	32	5.63	4	
P1493-LM01-P5		LM2909	52	4.78	2	
P1493-LM01-P5		LM2910	80	6.29	4	
P1493-LM01-P5		LM2911	31	5.22	2	
P1493-LM01-P5		LM2912	64	4.63	4	
P1493-LM01-P5		LM2913	25	4.97	4	
P1493-LM01-P5		LM2914	75	5.53	3	
P1505-LM01-P17	G02 (1)	LM2915	61	7.33	3	AA+CC (*Co-4*^{2} + *Fop*)
P1551-LM01-P63 (1)	G03 (11)	LM2916	59	6.13	5	AA+CC (*Co-4*^{2} + *Fop*)
P1551-LM01-P63 (1)		LM2917	36	5.81	2	
P1551-LM01-P63 (1)		LM2918	50	6.15	4	
P1551-LM01-P63 (1)		LM2919	21	6.14	4	
P1551-LM01-P63 (1)		LM2920	22	5.06	3	
P1551-LM01-P63 (1)		LM2921	18	-	2	
P1551-LM01-P63 (1)		LM2922	22	6.16	5	
P1551-LM01-P63 (1)		LM2923	35	6.15	4	
P1551-LM01-P63 (1)		LM2924	23	5.74	4	
P1551-LM01-P63 (1)		LM2925	57	6.98	2	
P1551-LM01-P63 (1)		LM2926	40	6.55	3	
P1551-LM01-P63 (2)	G04 (8)	LM2927	34	6.08	4	AA+CC (*Co-4*^{2} + *Fop*)
P1551-LM01-P63 (2)		LM2928	16	-	4	
P1551-LM01-P63 (2)		LM2929	40	6.11	2	
P1551-LM01-P63 (2)		LM2931	15	-	4	
P1551-LM01-P63 (2)		LM2932	37	6.38	4	
P1551-LM01-P63 (2)		LM2933	47	6.45	4	
P1551-LM01-P63 (2)		LM2934	29	7.21	4	
P1551-LM01-P63 (2)		LM2935	18	-	4	
P1557-LM01-P69	G05 (8)	LM2936	51	4.83	2	AA+CC (*Co-4*^{2} + *Fop*)
P1557-LM01-P69		LM2937	48	5.14	3	
P1557-LM01-P69		LM2938	128	5.22	4	
P1557-LM01-P69		LM2939	37	4.8	4	

Tabela 1.6. Continuação.

P1557-LM01-P69		LM2940	22	5.48	4	
P1557-LM01-P69		LM2941	56	5.56	2	
P1557-LM01-P69		LM2942	50	5.2	3	
P1557-LM01-P69		LM2943	52	6.25	3	
P1581-LM01-P93		LM2944	114	5.75	2	
P1581-LM01-P93		LM2945	76	5.32	3	
P1581-LM01-P93	G06 (6)	LM2946	6	-	3	AA+CC (*Co-4*^{2} + *Fop*)
P1581-LM01-P93		LM2947	69	4.24	4	
P1581-LM01-P93		LM2949	10	-	3	
P1581-LM01-P93		LM2950	57	4.6	4	
P1635-LM03-P9		LM2951	65	6.7	2	
P1635-LM03-P9		LM2952	51	6.33	4	
P1635-LM03-P9		LM2953	35	6.45	3	
P1635-LM03-P9		LM2954	22	6.02	2	
P1635-LM03-P9		LM2955	14	-	2	
P1635-LM03-P9	G07 (11)	LM2956	99	5.37	3	AA+CC (*Co-4*^{2} + *Fop*)
P1635-LM03-P9		LM2957	90	5.84	2	
P1635-LM03-P9		LM2958	26	6.67	3	
P1635-LM03-P9		LM2959	158	5.99	3	
P1635-LM03-P9		LM2960	35	6.24	2	
P1635-LM03-P9		LM2961	84	6.65	2	
P1678-LM03-P52		LM2962	128	5.92	2	
P1678-LM03-P52		LM2963	71	6.58	3	
P1678-LM03-P52	G08 (5)	LM2964	36	7.43	3	AA+CC (*Co-4*^{2} + *Fop*)
P1678-LM03-P52		LM2965	27	7.22	2	
P1678-LM03-P52		LM2966	87	5.74	3	

$G0_n$– Grupos de progênies $F_{2:3}$ oriundas de cada planta F_2 selecionada com alelos-alvo *Co-4*^{2} (AA) e *Fop* (CC) em homozigose; CGA – Cor dos grãos após armazenamento por um período de 147 dias em câmara fria.

Tabela 1.7. Genotipagem dos genitores usados neste estudo, das onze plantas F_2 selecionadas com os alelos-alvo *Co-4*^{2} e *Fop* em homozigose e das plantas individuais resistentes e suscetíveis selecionadas da população da cultivar IPR Celeiro quanto a presença do alelo *Car-11* (ANWC62R – T/A) de resistência ao Carlavírus.

	Genótipo	ANWC62R (*Car-11*)	Reação	Fenótipo
Genitores	BRS Sublime	TT	2	R
	IPR Celeiro	TT	1	R
	BRS Ametista	AA	-	R
	BRS Horizonte	AA	-	-
	CNFCT 16207	AA	9	S
	CNFC 15826	TT	-	-
	CNFC 16902	TT	-	-
	DM 103	AA	-	-
	K10	AA	-	-
	Prog-218311376	AA	-	-
Plantas F_2 com *Co-4*^{2} e *Fop*	P1489-LM01-p1	AA	-	-
	P1493-LM01-p5	AA	-	-
	P1505-LM01-p17	AA	-	-
	P1540-LM01-p52	AA	-	-
	P1542-LM01-p54	AA	-	-
	P1551-LM01-p63	AA	-	-
	P1556-LM01-p68	T/A	-	-
	P1557-LM01-p69	AA	-	-
	P1581-LM01-p93	TT	-	-
	P1489-LM01-p1	AA	-	-
	P1493-LM01-p5	AA	-	-
Planta individual - IPR Celeiro	9-12-1	TT	1	R
	9-12-1	TT	1	R
	10-12-1	TT	1	R
	10-12-3	TT	1	R
	13-19-2	AA	1	R
	13-19-4	AA	1	R
	13-19-5	AA	1	R
	13-23-1	AA	1	R
	13-23-3	AA	1	R
	IPRCeleiro1*	AA	9	S
	IPRCeleiro2*	AA	9	S

O alelo-alvo associado ao fenótipo de resistência ao Carlavírus está sublinhado, R – Resistente, S – Suscetível, *Planta suscetível selecionada dentro da população de plantas da cultivar IPR Celeiro.

Adicionalmente, o marcador SNP ANWC62R (T/A) foi utilizado para monitorar o alelo *Car-11* presente no genitor BRS Sublime e que confere resistência ao Carlavírus (Silva, 2021). Realizou-se a genotipagem dos seis genitores utilizados neste estudo, das onze plantas combinando os alelos *Co-4*^{2} e *Fop* e de outras onze plantas individuais contrastantes quanto a reação ao Carlavírus selecionadas dentro da população de IPR Celeiro (**Tabela 1.7**). Houve amplificação do alelo T nos genitores BRS Sublime, CNFC 15826, CNFC 16902 e nas plantas F_2 P1556-LM01-p68 e P1581-LM01-p93 (**Tabela 1.7**). Entre as plantas individuais selecionadas dentro da população da cultivar IPR Celeiro, houve amplificação do alelo T (*Car-11*) apenas nas plantas resistentes (**Tabela 1.7**), enquanto o alelo alternativo A amplificou tanto em plantas resistentes quanto nas suscetíveis (**Tabela 1.7**). As plantas individuais resistentes não apresentaram nenhum sinal de sintomas de Carlavírus, com nível de resistência superior ao observado em BRS Sublime (Comunicação pessoal, Dr. Josias C. de Farias). As progênies $F_{2:3}$ oriundas das duas plantas F_2 que apresentaram o alelo T deverão ser fenotipadas quanto a reação ao Carlavírus.

7 DISCUSSÃO

7.1 Seleção precoce dos alelos-alvo

O sucesso na identificação do genótipo desejado, combinando os alelos-alvo, depende da probabilidade de ocorrência simultânea (P = 0,95), da frequência esperada de dada combinação e do número de alelos-alvo (k) (Ramalho et al., 2012). Neste estudo, cujo objetivo central foi selecionar precocemente plantas que combinassem os alelos-alvo *Co-4*^{2}, *Co-Realce*, *Phg-2* e *Fop* em homozigose, o número mínimo de plantas estimado da geração F_2 foi de 765 plantas (**Figura 1.3**). A simulação de dois cenários (**Figura 1.3**), o primeiro com quatro (k=4) alelos-alvo em homozigose (*Co-4*^{2}, *Co-Realce*, *Phg-2*, *Fop*) e o segundo com cinco (k=5) alelos-alvo (*Co-4*^{2}, *Co-Realce*, *Phg-2*, *Fop* e *sd*), mostra que a partir da geração F_3 o número mínimo de plantas reduz consideravelmente, visto que a taxa de homozigose aumenta em 50% em relação à geração F_2 (Ramalho et al., 2012). Nesse estudo, a geração F_2 foi composta de 387 plantas, número inferior ao estimado para a identificação de pelo menos uma planta combinando os quatro alelos de resistência, além do escurecimento lento. No entanto, as 387 plantas F_2 foram oriundas de plantas que possuíam apenas dois alelos-alvo piramidados (**Tabela 1.4**), impossibilitando a seleção de plantas combinando todos os alelos-alvo desse estudo. Por exemplo, as onze plantas selecionadas com os alelos *Co-4*^{2} e *Fop* em homozigose (**Tabela 1.5**) vieram das plantas LM01 e LM03, ambas combinando esses dois alelos (**Tabela 1.4**). Da mesma forma, as plantas combinando os alelos *Fop* e *sd* (**Tabela 1.5**) foram obtidas da planta LM04, que possuía essa combinação em seu genoma (**Tabela 1.4**). Portanto, para os próximos ciclos de cruzamentos, quando houver a probabilidade de selecionar plantas combinando todos os alelos-alvo, recomenda-se a adoção do número mínimo de plantas estimado na simulação dos dois cenários da **Figura 1.3**.

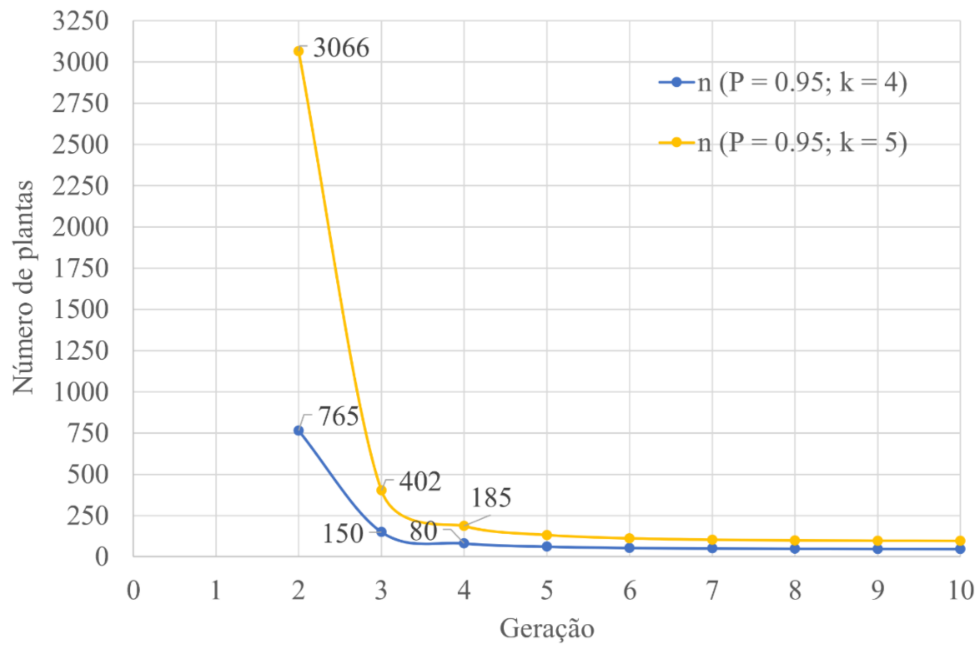


Figura 1.3. Simulação do número mínimo de plantas na geração t para identificar pelo menos um genótipo desejado. P é a probabilidade de ocorrência de determinada combinação e k é o número de alelos-alvo.

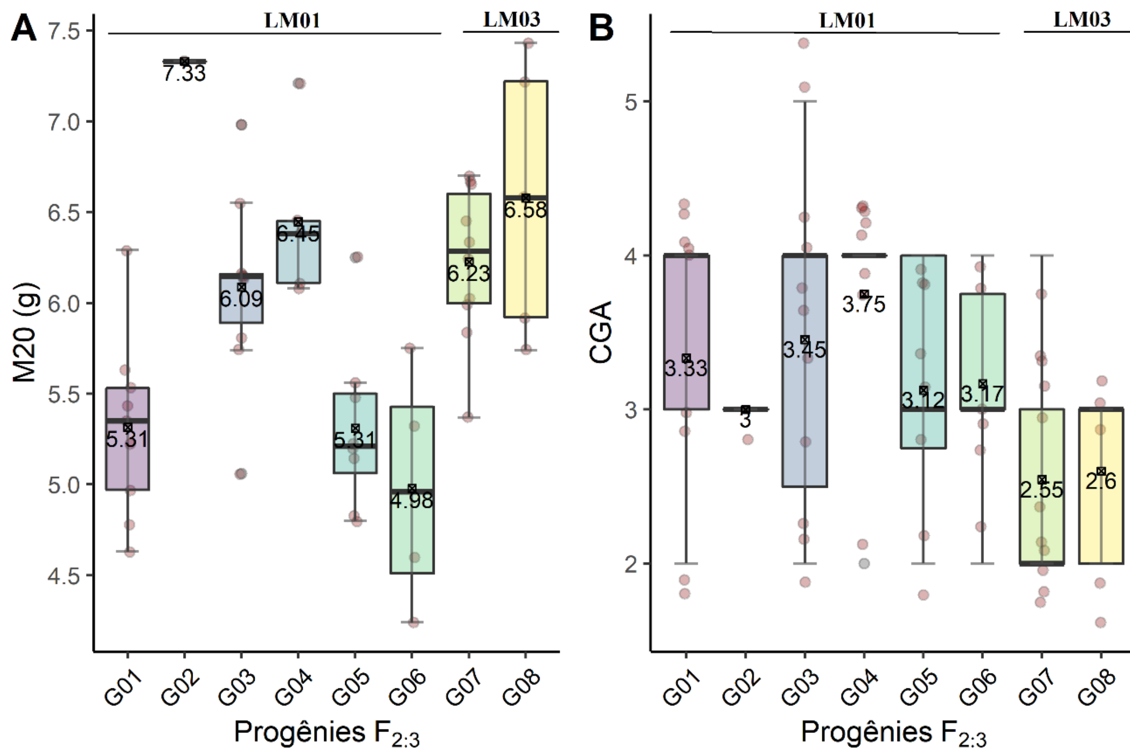


Figura 1.4. **A** – Variação dentro e entre grupos de progênies $F_{2:3}$ quanto a massa de 20 sementes (M20). **B** – Variação dentro e entre grupo de progênies $F_{2:3}$ em relação a nota de cor dos grãos após armazenamento (CGA).

7.2 Cor dos grãos após armazenamento - CGA

Existe variabilidade genética quanto cor do grão após o armazenamento por um período de 147 dias (**Figura 1.4B**). Observou-se que 28,8% das progênies $F_{2:3}$, combinando os alelos *Co-4*^{2} (AA) + *Fop* (CC), apresentaram nota de CGA igual a 2 (**Tabela 1.6**). As progênies dos grupos G07 e G08 apresentaram as menores médias para CGA (**Figura 1.4B**). Como comparativo, as cultivares BRS Sublime e BRS Estilo, com padrão de grão carioca que atende o mercado consumidor, possuem notas iguais a 3,0 e 3,1, respectivamente (Alvares et al., 2019). Portanto, para a próxima etapa de cruzamentos, recomenda-se que as progênies $F_{2:3}$ com nota 2 (**Tabela 1.6**), especialmente dos grupos G07 e G08, sejam priorizadas. Os grãos das onze plantas F_2 selecionadas com os alelos *Fop* (CC) e *sd* (AA) em homozigose apresentaram nota de CGA igual ao da cultivar BRSMG Madrepérola, padrão comercial para escurecimento lento dos grãos (Carneiro et al., 2012).

7.3 Valor dos alelos-alvo no melhoramento

O *Co-4*^{2} é um alelo do loco *Co-4* e foi mapeado próximo a região telomérica do cromossomo 8 a partir da fonte de resistência do pool gênico Mesoamericano SEL1308 (Oblessuc et al., 2015). Esse alelo confere resistência aos patótipos 65, 73 e 81 do fungo *C. lindemuthianum*, mais frequentes nos campos de produção do Brasil (Paulino et al., 2022). Entre os genitores selecionados para este estudo, o alelo *Co-4*^{2} está presente apenas no genitor K13 (AA) e foi monitorado via SAM usando o marcador SNP P8282v3-817 (**Tabela 1.3**). O SNP P8282v3-817 possui eficiência de seleção de 99,8% (Gomes-Messias et al., 2022). Nesse estudo, o marcador P8282v3-817 segregou conforme o esperado para marcadores codominantes, sendo os desvios entre as frequências observadas e esperadas devido ao acaso (**Tabela 1.8**). A eficiência de seleção deverá ser confirmada por meio da avaliação da reação aos patótipos mais comuns nos campos de produção brasileiros, 65, 73 e 81 (Paulino et al., 2022). O alelo-alvo *Co-Realce* confere resistência aos patótipos 65, 73, 81, 91, 475 e 1609, sendo mapeado no cromossomo 4 a partir da fonte de resistência do *pool* gênico Andino BRSMG Realce (Capítulo 2). A presença do alelo *Co-Realce* pode ser monitorada com os marcadores snp3308 (C/T), snp1327 (T/C) e snp12782 (C/T) (**Tabela 1.2**) no próximo bloco de cruzamentos e avanço de geração. A combinação de alelos oriundos de fontes de resistência à antracnose de pool gênicos distintos é uma importante estratégia para o desenvolvimento de cultivares com resistência ampla e duradoura (Paulino et al., 2022).

Tabela 1.8. Segregação genotípica dos marcadores P8282v3-817 (G/A – *Co-4*^{2}), ANAAJK6 (G/C - *Fop*) e PvbHLHp12804 (G/A - *sd*) na geração F_2.

SNP	Genótipo	Hipótese	Observado	Esperado	$\chi2$	p-valor
P8282v3-817	GG[1]		49	55		
	GA[2]	1:2:1	122	111	2,4	0,30
	AA[3]		50	55		
	Total	-	221	221		-
ANAAJK6	GG		40	48		
	GC	1:2:1	95	97	3,4	0,18
	CC		58	48		
	Total	-	193	193		-
PvbHLHp12804	GG		38	34		
	GA	1:2:1	72	69	2,1	0,35
	AA		27	34		
	Total	-	137	137		-

O alelo-alvo associado ao fenótipo de interesse está sublinhado. [1]Planta homozigota para o alelo alternativo ao alelo-alvo; [2]Planta heterozigota em relação ao alelo-alvo; [3]Planta homozigota em relação ao alelo-alvo.

O alelo *Phg-2* confere resistência aos patótipos 63-39, 63-19 e 63-63, sendo as duas últimas mais relevantes no Brasil (Bassi et al., 2017; Pereira et al., 2019). Esse alelo foi mapeado no cromossomo 8 a partir da fonte de resistência México 54 (Souza et al., 2016; Nay et al., 2019). Os marcadores g796 (209/233), SE04 (640/-) e ANYMVJW (G/T) estão em desequilíbrio de ligação com o alelo *Phg-2*, sendo úteis à seleção assistida (Sanglard, 2010; Miller et al., 2018; Lobaton et al., 2018). O marcador codominante g796 amplificou o tamanho de banda associada à suscetibilidade nos genitores DM103 e CNFC 16902 (**Figura 1.2A**). Embora esteja a uma distância de 3,0 cM do loco *Phg-2*, a não especificidade do marcador g796 pode ser devido a fonte de resistência usada nesse estudo, visto que o mesmo foi desenvolvido utilizando o genitor México 54 (Miller et al., 2018). O marcador dominante SE04 (presença e ausência), além de amplificar o tamanho de banda associada à resistência nos genótipos MAR-2, DM103 e México 54 (**Figura 1.2B**), também amplificou na linhagem CNFC 16902, que não possui em sua genealogia nenhum parental doador do alelo *Phg-2*. O SE04 foi usado pelo obtentor da linhagem DM103 para monitorar o alelo *Phg-2* oriundo do parental doador MAR-2 (Sanglard, 2010). O SNP ANYMVHW (G/T) foi mapeado no cromossomo 8 a partir da fonte de resistência México 54 (Lobaton et al., 2018). Esse marcador foi utilizado por Gomes-Messias et al. (2022), o qual foi específico para o alelo *Phg-2* nas linhagens México 54, PT-65 e MAIII-16.159, todas resistentes à mancha angular.

O ANYMVHW amplificou o alelo T associado à suscetibilidade nos genitores DM103 e CNFC 16902 (**Tabela 1.3**). Segundo Nay et al. (2019), o alelo de resistência à mancha angular presente na linhagem MAR-2, utilizada como parental doador no desenvolvimento da linhagem DM103, pode ser uma variação alélica na mesma região do *Phg-2*. Devido à falta de especificidade dos marcadores g796, SE04 e ANYMVHW em relação ao alelo de resistência à mancha angular do genitor DM103 (**Figuras 1.2A e 1.2B**; **Tabela 1.3**), não foi possível monitorar a introgressão do alelo *Phg-2*. Visando a continuidade do programa de piramidação alélica, recomenda-se que a linhagem MAIII-16.159, *background* carioca, seja utilizada como fonte do alelo *Phg-2* em cruzamentos futuros. Essa linhagem foi obtida pelo programa de seleção recorrente da Universidade Federal de Lavras, sendo resistente ao patótipo 63-63 de *P. griseola* e indicada para participar do novo grupo de cultivares diferenciadoras proposto para mancha angular (Pádua, 2022). Outro fato importante que pode ser aproveitado é a presença do alelo $Co\text{-}3^{4}$ no genitor K10 (Vieira et al., 2018). Esse alelo co-segrega com o alelo *Phg-3* de resistência à mancha angular (Gonçalves-Vidigal et al., 2013) e está fortemente ligado ao marcador g2303 (0,0 cM), permitindo a seleção da combinação alélica $Co\text{-}3^{4}$/*Phg-3* (Gonçalves-Vidigal et al., 2013). Portanto, recomenda-se que as progênies selecionadas sejam genotipadas com o marcador g2303 (Gonçalves-Vidigal et al., 2013).

Torres (2020) identificou dois SNP significativamente associados ao fenótipo de resistência à murcha de *fusarium* com avaliação a campo, os quais foram convertidos em ensaios TaqMan ANAAJK6 (G/C) e ANCFFDU (T/A). O ensaio ANAAJK6 amplificou o alelo C no genitor CNFC 15826 (**Tabela 1.3**) e, portanto, sendo útil para monitorar a introgressão do alelo *Fop*. Em análise realizada com quinze progênies oriundas do cruzamento CNFP10794 (*Fop*) x BRS Horizonte, aquelas homozigotas para o alelo de referência, CC, apresentaram o fenótipo resistente, com nota média menor que 3 (**Figura 1.5**). A média das progênies heterozigotas (GC) ou homozigotas para o alelo alternativo (GG) apresentaram média três vezes maior do que a observada nas progênies contendo o alelo de referência em homozigose, CC (**Figura 1.5**). Adicionalmente, o ensaio ANCFFDU foi 100% coincidente ao amplificar o alelo T nas mesmas progênies resistentes que apresentaram o alelo C do SNP ANAAJK6 (Comunicação pessoal). Portanto, recomenda-se a adoção do SNP ANCFFDU nos próximos ciclos de cruzamentos. Nesse estudo, o SNP ANAAJK6 segregou conforme esperado para marcadores codominantes, 1:2:1 (**Tabela 1.8**),

havendo a necessidade da avaliação das progênies $F_{2:3}$ selecionadas com o alelo CC – *Fop* (**Tabela 1.6**) quanto à reação à murcha de fusarium.

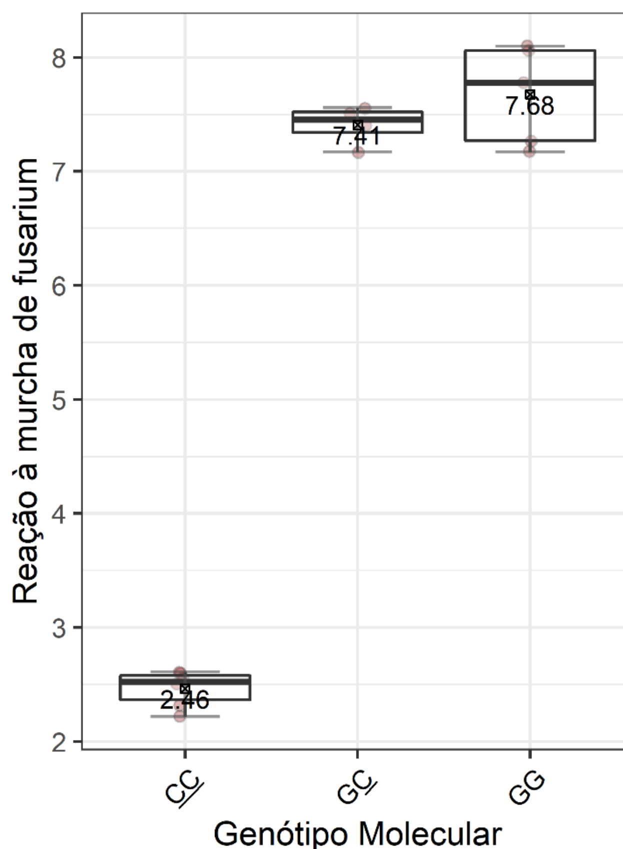


Figura 1.5. Diferença fenotípica da reação à murcha de fusarium entre progênies contendo os genótipos moleculares CC, GC e GG. Genotipagem realizada com o SNP ANAAJK6 (Torres, 2020). O alelo referência está sublinhado.

O escurecimento lento é controlado pela ação de dois genes, *J* e *Sd*, com interação do tipo epistasia recessiva (Elsadr et al., 2011). A presença do alelo dominante *J* é responsável pela ocorrência ou não do escurecimento, sendo que a presença da condição recessiva (*sdsd*) do gene *Sd* inibe a ação do gene *J* e resulta no escurecimento lento dos grãos (Elsadr et al., 2011). O loco *Sd* foi mapeado no cromossomo 7 e a introgressão do alelo *sd* pode ser monitorada com o marcador SNP PvbHLHp12804 (A/G), com eficiência de seleção superior a 90% (Rodrigues et al., 2019a; Alvares et al., 2019). As onze plantas F_2 selecionadas neste estudo combinando os alelos CC (*Fop*/*Fop*) e AA (*sd*/*sd*) apresentaram fenótipo de escurecimento lento dos grãos (**Figura 1.6**; **Apêndice 1.E**), com nota média igual a 1 aos 147 dias após a colheita. Os desvios entre as frequências observadas e esperadas do genótipo molecular do marcador PvbHLHp12804 foram devido ao acaso e, portanto, houve aderência à segregação mendeliana 1:2:1 (**Tabela 1.8**).

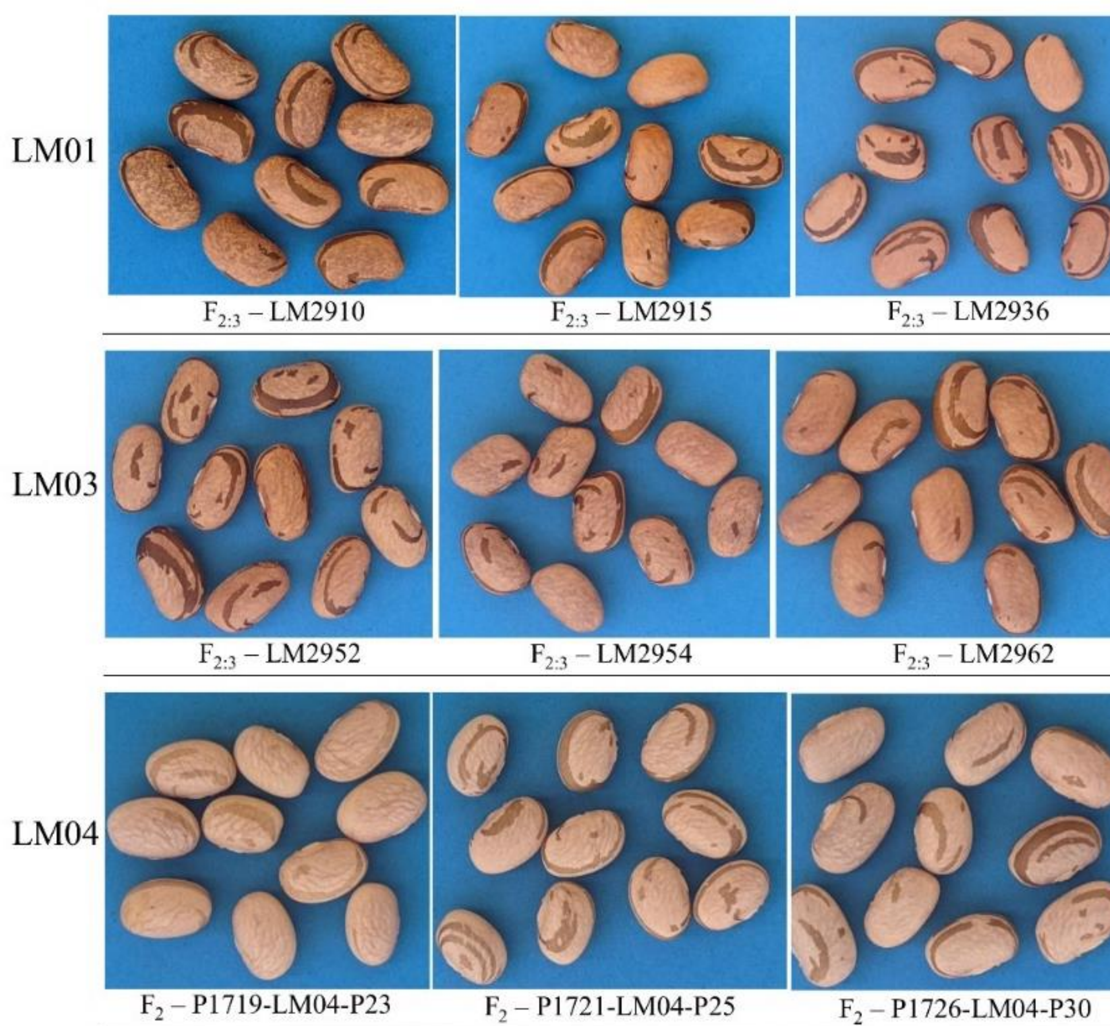


Figura 1.6. Comparação entre os fenótipos das sementes das progênies $F_{2:3}$ e das plantas F_2 selecionadas. LM01 e LM03 - Progênies com a combinação *Co-4*^{2} e *Fop*. LM04 – progênies selecionadas com os alelos *Fop* e *sd*.

A produtividade é a principal característica a ser melhorada nas novas cultivares, sendo limitada pela ocorrência de doenças. As doenças causam prejuízos à produção e à qualidade dos grãos em todo o mundo, comprometendo a segurança alimentar das classes sociais de baixa renda (Dormatey et al., 2020; Nadeem et al., 2021). Portanto, o acúmulo de alelos de resistência em um mesmo genótipo é uma importante estratégia para garantir maior estabilidade à produção de cada região e, consequentemente, assegurar o alimento para milhões de pessoas (Dormatey et al., 2020; Nadeem et al., 2021). Os avanços das tecnologias de genotipagem, o mapeamento de genes e a validação de marcadores ligados aos genes de interesse facilitaram a piramidação simultânea de dois ou mais alelos de resistência (Meziadi et al., 2016). Além da produtividade, para todos os tipos de grãos de feijão, a qualidade tecnológica, nutricional e menores alterações pós-colheita são de grande relevância para a

aceitação do produto no mercado (Siqueira et al., 2014; Dias et al., 2015). Dentre esses, características relacionadas à qualidade comercial dos grãos, como o escurecimento lento dos grãos armazenados, continuam mantendo sua relevância ao longo dos últimos anos frente exigência dos consumidores brasileiros (Silva et al., 2018; Duwadi et al., 2018). No Brasil, o feijão carioca com cores mais claras é o preferido pelos consumidores (Siqueira et al., 2014) e responde 70% do mercado consumidor. O escurecimento do tegumento limita o armazenamento dos grãos por longos períodos visando a comercialização em épocas de melhores preços, tornando-o um dos mais importantes atributos na comercialização de feijão tipo carioca.

Nesse estudo, utilizando-se de marcadores ligados aos alelos-alvo (**Tabela 1.2**), foi possível selecionar precocemente plantas combinando os alelos-alvo *Co-4*^{2} + *Fop* e *Fop* + *sd* (**Tabelas 1.4 e 1.6**). Devido ao padrão de cor dos grãos após armazenamento, recomenda-se que as progênies $F_{2:3}$, com nota de CGA igual a 2 (**Tabela 1.6; Figura 1.4B**), sejam utilizadas nos próximos cruzamentos. Como destaque, devido a uniformidade dos grãos e a presença de halo claro e hilo branco, as progênies $F_{2:3}$ LM2954, LM2955, LM2957, LM2960 e LM2962 devem ser priorizadas nos próximos cruzamentos (**Tabela 1.6**). As progênies $F_{2:3}$ dos grupos G2, G3, G4, G7 e G8 são indicadas para obtenção de linhagens com grãos mais pesados (**Figura 1.4A; Tabela 1.6**). Além disso, recomenda-se que a linhagem MAII-16.159 seja incluída como fonte de resistência à mancha angular nos blocos de cruzamentos futuros (Pádua, 2022). Atualmente, a cultivar BRS FC402 combina resistência à antracnose e à murcha de fusarium (Melo et al., 2017) e a cultivar BRS FC406 mostrou-se resistente à antracnose e à mancha angular (Pereira et al., 2021*a*). Portanto, faz-se necessária a continuidade do programa de piramidação alélica iniciado neste trabalho para o desenvolvimento de cultivares de feijão carioca combinando, simultaneamente, resistência efetiva contra à antracnose, à mancha angular e à murcha de fusarium, além de apresentar o fenótipo do escurecimento lento dos grãos.

Como perspectiva, espera-se que as progênies selecionadas neste estudo sejam fenotipadas quanto a reação à antracnose, mancha angular, murcha de fusarium e o escurecimento lento para estimar a eficiência da seleção precoce dos alelos-alvo via SAM.

8 CONCLUSÕES

A seleção assistida por marcadores moleculares através de ensaios não destrutivos reduz o tempo e os custos com a seleção de alelos-alvo nas gerações iniciais.

A seleção precoce assistida com os marcadores codominantes permite a identificação de progênies combinando os alelos-alvo em homozigose. Os marcadores P8282v3-817, ANAAJK6, ANCFFDU e PvbHLHp12804 possuem segregação mendeliana 1:2:1 na geração F_2.

Além da seleção precoce dos alelos-alvo, foi possível identificar progênies com grão carioca dentro dos padrões comerciais.

9 REFERÊNCIAS

ALVARES, R. C.; STONEHOUSE, R.; SOUZA, T. L. P. O.; MELO, P. G. S.; MIKLAS, P. N.; BETT, K. E.; MELO, L. C.; RODRIGUES, L. A.; SOUZA, L. L.; PEREIRA, H. S. Generation and validation of genetic markers for the selection of carioca dry bean genotypes with the slow-darkening seed coat trait. **Euphytica**, v. 215:141, 2019. Doi: 10.1007/s10681-019-2461-y.

APPLIED BIOSYSTEMS. **TaqMan® SNP Genotyping Assays**. USER GUIDE. Disponível em: < https://tools.thermofisher.com/content/sfs/manuals/TaqMan_SNP_Genotyping_Assays_man.pdf >. Acessado em: 18 set. 2022.

ASSEFA, T.; MAHAMA, A.; BROWN, A. V.; CANNON, E. K. S.; RUBYOGO, J. C.; RAO, I. M.; BLAIR, M. W.; CANNON, S. B. A review of breeding objectives, genomic resources, and marker-assisted methods in common bean (*Phaseolus vulgaris* L.). **Molecular Breeding**, v. 39:20, 2019. Doi: 10.1007/s11032-018-0920-0.

BASSI, D.; BRIÑEZ, B.; ROSA, J. S.; OBLESSUC, P. R.; ALMEIDA, C. P.; NUCCI, S. M.; SILVA, L. C. D.; CHIORATO, A. F.; VIANELLO, R. P.; CAMARGO, L. E. A.; BLAIR, M. W.; BENCHIMOL-REIS, L. L. Linkage and mapping of quantitative trait loci associated with angular leaf spot and powdery mildew resistance in common beans. **Genetics and Molecular Biology**, v. 40, n. 1, p. 109-122, 2017. Doi: 10.1590/1678-4685-GMB-2015-0314.

BATISTA, R. O.; OLIVEIRA, A. M. C.; SILVA, J. L. O.; NICOLI, A.; CARNEIRO, P. C. S.; CARNEIRO, J. E. S.; JÚNIOR, T. J. P.; QUEIROZ, M. V. Resistance to *Fusarium* wilt in common bean. **Crop Breeding and Applied Biotechnology**, v. 16, p. 226-233, 2016. Doi: 10.1590/1984-70332016v16n3a34

BATISTA, R. O.; SILVA, L. C.; MOURA, L. M.; SOUZA, M. H.; CARNEIRO, P. C. S.; CARVALHO FILHO, J. L. S.; CARNEIRO, J. E. S. Inheritance of resistance to *Fusarium* wilt in common bean. **Euphytica**, v. 213:133, 2017. Doi: 10.1007/s10681-017-1925-1.

BEBBER, D. P.; HOLMES, T.; GURR, S. J. The global spread of crop pests and pathogens. **Global Ecology and Biogeography**, v. 23, p. 1398-1407, 2014. Doi: 10.1111/geb.12214.

BRONDANI, R. P. V.; BRONDANI, C.; TARCHINI, R.; GRATTAPAGLIA, D. Development, characterization and mapping of microsatellite markers in *Eucalyptus grandis* and E. urophylla. **Theoretical and Applied Genetics**, v. 97, p. 816-827, 1998. Doi: 10.1007/s001220050961.

BROWN, J. K. M.; HOVMØLLER, M. S. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. **Science**, v. 297, p. 537-541, 2002.

CARNEIRO, J. E. S.; ABREU, A. F. B.; RAMALHO, M. A. P.; PAULA JÚNIOR, T. J.; PELOSO, M. J. Del; MELO, L. C.; PEREIRA, H. S.; PEREIRA FILHO, I. A.; MARTINS, M.; VIEIRA, R. F.; MARTINS, F. A. D.; COELHO, M. A. O.; CARNEIRO, P. C. S.; MOREIRA, J. A. A.; SANTOS, J. B.; FARIA, L. C.; COSTA, J. G. C.; TEIXEIRA, H. BRSMG Madrepérola: common bean cultivar with late-darkening carioca grain. **Crop Breeding Applied Biotechnology**, **v.** 12, p. 281-284, 2012. Doi: 10.1590/S1984-70332012000400008.

CROSS, H.; BRICK, M. A.; SCHWARTZ, H. F.; PANELLA, L. W.; BYRNE, P. F. Inheritance of resistance to *Fusarium* wilt in two common bean races. **Crop Science**, v. 40, p. 954-958, 2000. Doi: 10.2135/cropsci2000.404954x.

CRUZ, A. F.; SILVA, L. F.; SOUSA, T. V.; NICOLI, A.; JUNIOR, T. J. P.; CAIXETA, E. T.; ZAMBOLIM, L. Molecular diversity in *Fusarium oxysporum* isolates from common bean fields in Brazil. **European Journal of Plant Pathology**, v. 152, p. 343-354, 2018. Doi: 10.1007/s10658-018-1479-7.

DIAS, D. M.; MOREIRA, M. E. C.; GOMES, M. J. C.; TOLEDO, R. C. L.; NUTTI, M. R.; SANT´ANA, H. M. P.; MARTINO, H. S. D. Rice and Bean targets for biofortification combined with high carotenoid content crops regulate transcriptional mechanisms increasing iron bioavailability. **nutrients**, v. 7, p. 9683-9696, 2015. Doi: 10.3390/nu7115488.

DORMATEY, R.; SUN, C.; ALI, K.; COULTER, J. A.; BI, Z.; BAI, J. Gene pyramiding for sustainable crop improvement against biotic and abiotic stresses. **agronomy**, n. 10:1255, 2020. Doi: 10.3390/agronomy10091255.

DUWADI, K.; AUSTIN, R. S.; MAINALI, H. R.; BETT, K.; MARSOLAIS, F.; DHAUBHADEL, S. Slow darkening of pinto bean seed coat is associated with significant metabolite and transcript differences related to proanthocyanidin biosynthesis. **BMC Genomics**, v. 19:260, 2018. Doi: 10.1186/s12864-018-4550-z.

ELSADR, H. T.; WRIGHT, L. C.; PAULS, K. P.; BETT, K. E. Characterization of seed coat post-harvest darkening in common bean (*Phaseolus vulgaris* L.). **Theoretical and Applied Genetics**, v. 123, p. 1467-1472, 2011. Doi: 10.1007/s00122-011-168.

FALL, A. L.; BYRNE, P. F.; JUNG, G.; COYNE, D. P.; BRICK, M. A.; SCHWARTZ, H. F. Detection and mapping of a major locus for *Fusarium* wilt resistance in common bean. **Crop Science**, v. 41, p. 1494-1498, 2001. Doi: 10.2135/cropsci2001.4151494x.

FERREIRA, C. M.; FIGUEIREDO, R. S.; LUZ, T. C. L. A. Arroz e Feijão: Intervenção multi-institucional em prol do Brasil. **Revista Política Agrícola**, v. 27, n. 1, p. 96-112. 2018.

GOMES, L. M.; PEREIRA, H. S; COSTA, J. G. C.; AGUIAR, M. S.; MELO, L. C.; SOUZA, T. L. P. O. Seleção de progênies de feijão carioca contendo o alelo *Co-Realce* de resistência à antracnose. In: XIII Seminário Jovens Talentos, 13. **Resumos**. Santo Antônio de Goiás: Embrapa Arroz e Feijão: ISBN 978-65-86056-69-3, 2019, p. 66.

GOMES-MESSIAS, L. M.; VIANELLO, R. P.; MONTEIRO-JÚNIOR, J. P.; RODRIGUES, L. A.; MOTA, A. P. S.; PEREIRA, H. S.; MELO, L. C.; RAATZ, B.; SOUZA, T. L. P. O. Molecular characterization of parental lines and validation of SNP markers for anthracnose and angular spot in common bean. **Euphytica**, n. 218:49, 2022. Doi: 10.1007/s10681-022-03002-2.

GONÇALVES-VIDIGAL, M. C.; CRUZ, A. S.; LACANALLO, G. F.; VIDIGAL FILHO, P. S.; SOUSA, L. L.; PACHECO, C. M. N. A.; MCCLEAN, P. GEPTS, P.; PASTOR-CORRALES, M. A. Co-segregation analysis and mapping of the anthracnose *Co-10* and angular leaf spot *Phg-ON* disease-resistance genes in the common bean cultivar Ouro Negro. **Theoretical and Applied Genetics**, v. 126, p. 2245-2255, 2013. Doi: 10.1007/s00122-013-2131-8.

HENRIQUE, F. H.; CARBONELL, S. A. M.; ITO, M. F.; GONÇALVES, J. G. R.; SASSERON, G. R.; CHIORATO, A. F. Classification of physiological races of *Fusarium*

oxysporum f. sp. *phaseoli* in common bean. **Bragantia**, v. 74, n. 1, p. 84-92, 2014. Doi: 10.1590/1678-4499.0265.

KELLY, J. D.; MIKLAS, P. N. The role of RAPD markers in breeding for disease resistance in common bean. **Molecular Breeding**, n. 4, p. 1-11, 1998. Doi: 10.1023/A:1009612002144.

KELLY, J. D.; VALLEJO, V. A. A comprehensive review of the major genes conditioning resistance to anthracnose in common bean. **HortScience**, v. 39, n. 6, p. 1196-1207, 2004. Doi: 10.21273/HORTSCI.39.6.1196.

KELLY, J. D.; YOUNG, R. A. Proposed symbols for anthracnose resistance genes. **Annual Reports Bean Improvement Cooperative**, v. 39, p. 20-24, 1996.

KOTUE, T. C.; MARLYNE JOSEPHINE, M.; WIRBA, L. Y.; AMALENE, S. R. H.; NKENMENI, D. C.; KWUIMGOIN, I.; DJOTE, W. N. B.; KANSCI, G.; FOKOU, E.; FOKAM, D. P. Nutritional properties and nutrients chemical analysis of common beans seed. **MOJ Biology and Medicine**, v. 3, n. 2, p. 41-47. 2018. Doi: 10.15406/mojbm.2018.03.00074.

LOBATON, J. D.; MILLER, T.; GIL, J.; ARIZA, D.; DE LA HOZ, J. F.; SOLER, A.; BEEBE, S.; DUITAMA, J.; GEPTS, P.; RAATZ, B. Resequencing of common bean identifies regions of inter–gene pool introgression and provides comprehensive resources for molecular breeding. Plant Genome, v. 11:170068, n. 2, 2018. Doi: 10.3835/plantgenome2017.08.0068.

LONG, J.; ZHANG, J.; ZHANG, X.; WU, J.; CHEN, H.; WANG, P.; WANG, Q.; DU, C. Genetic diversity of common bean (*Phaseolus vulgaris* L.) germplasm resources in chongqing, evidenced by morphological characterization. **Frontiers in Genetics**, v. 11:697. 2020. Doi: 10.3389/fgene.2020.00697.

MAINA, P. K.; WACHIRA, P. M.; OKOTH, S. A.; KIMENJU, J. W.; (2017) Cultural, morphological and pathogenic variability among *Fusarium oxysporum* f. sp. *phaseoli* causing wilt in French Bean (*Phaseolus vulgaris* L.). **Journal of Advances in Microbiology** 2 (4): 1-9. Doi: 10.9734/JAMB/2017/32684.

MELO, C. M.; PEREIRA, H. S.; FARIA, L. C.; SOUZA, T. L. P. O.; WENDLAND, A.; DÍAZ, J. L. C.; CARVALHO, H. W. L.; MELO, C. L. P.; COSTA, A. F.; MAGALDI, M. C. S.; COSTA, J. G. C. BRS FC402: high-yielding common bean cultivar with carioca grain, resistance to anthracnose and fusarium wilt. **Crop Breeding Applied Biotechnology**, **v.** 17, p. 67-71, 2017. Doi: 10.1590/1984-70332017v17n1c11.

MEZIADI, CH.; RICHARD, M. M. S.; DERQUENNES, A.; THAREAU, V.; BLANCHET, S.; GRATIAS, A.; PFLIEGER, S.; GEFFROY, V. Development of molecular markers linked to disease resistance genes in common bean based on whole genome sequence. **Plant Science**, v. 242, p. 351-357, 2016. Doi: 10.1016/j.plantsci.2015.09.006.

MIKLAS, P. N.; KELLY, J. D.; BEEBE, S. E.; BLAIR, M. W. Common bean breeding for resistance against biotic and abiotic stresses: from classical to mas breeding. **Euphytica**, v. 147, p. 105-131, 2006. Doi: 10.1007/s10681-006-4600-5.

MILLER, T.; GEPTS, P.; KIMMO, S.; ARUNGA, E.; CHILAGANE, L. A.; NCHIMBI-MSOLLA, S.; NAMUSOKE, A.; NAMAYANJA, A.; TEDLA, Y. R. Alternative markers linked to the *Phg-2* angular leaf spot resistance locus in common bean using the Phaseolus

genes marker database. **African Journal of Biotechnology**, v. 17, n. 26, p. 818-828, 2018. Doi: 10.5897/AJB2018.16493.

MORAIS, S. R. P.; VIERA, A. F.; ALMEIDA, L. C. S.; RODRIGUES, L. A.; MELO, P. G. S.; FARIA, L. C.; MELO, L. C.; PEREIRA, H. S.; SOUZA, T. L. P. O. Application of microsatellite markers to confirm controlled crosses and assess genetic identity in common bean. **Crop Breeding and Applied Biotechnology**, v. 16, p. 234-239, 2016. Doi: 10.1590/1984-70332016v16n3n35.

MUKANKUSI, C.; RAATZ, B.; NKALUBO, S.; BERHANU, F.; BINAGWA, P.; KILANGO, M.; WILLIANS, M.; ENID, K.; CHIRWA, R.; BEEBE, S. Genomics, genetics and breeding of common bean in Africa: A review of tropical legume project. **Plant Breeding**, n. 138, p. 401-414. 2019. Doi: 10.1111/pbr.12573.

NADEEM, M. A.; YEKEN, M. Z.; SHAHID, M. Q.; HABYARIMANA, E.; YILMAZ, H.; ALSALEH, A.; HATIPOĞLU, R.; ÇILESIZ, Y.; KHAWAR, K. M.; LUDIDI, N.; ERCIŞLI, S.; AASIM, M.; KARAKÖY, T.; BALOCH, F. S. Common bean as a potential crop for future food security: an overview of past, current and future contributions in genomics, transcriptomics, transgenics and proteomics. **Biotechnology & Biotechnological Equipment**, v. 35, n. 1, p. 758-786, 2021. Doi: 10.1080/13102818.2021.1920462.

NAY, M. M.; SOUZA, T. L. P. O.; RAATZ, B.; MUKANKUSI, C. M.; GONÇALVES-VIDIGAL, M. C.; ABREU, A. F. B.; MELO, L. C.; PASTOR-CORRALES, M. A. A review of angular leaf spot resistance in common bean. **Crop Science**, **v.** 59, p. 1376-1391, 2019. Doi: 10.2135/cropsci2018.09.0596.

NIETSCHE, S.; BORÉM, A.; ALZATE-MARIN, A. L.; COSTA, M.; ROCHA, R. C.; CAIXETA, E. T.; DE BARROS, E. G.; MOREIRA, M. A. Variabilidade genética da patogenicidade de Phaeoisariopsis griseola no Brasil. **Summa Phytopathologica**, v. 28, n. 4, p. 331-335, 2002.

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**, v. 128, p. 1193-1208, 2015. Doi: 10.1007/s00122-015-2500-6.

PADDER, B. A.; SHARMA, P. N.; AWALE, H. E.; KELLY, J. D. *Colletotrichum lindemuthianum*, the causal agent of bean anthracnose. **Journal of Plant Pathology**, v. 99 n. 2, p. 317-330, 2017. Doi: 10.4454/jpp.v99i2.3867.

PÁDUA, P. F. **Estudo de associação genômica ampla (GWAS) para resistência à *Pseudocercospora griseola* na linhagem MAIII-16.159**. 2022. 60 f. Tese (Doutorado em Genética e Melhoramento de Plantas) – Universidade Federal de Lavras, Lavras, 2022.

PAULINO, J. F. C.; ALMEIDA, C. P.; BUENO, C. J.; SONG, Q.; FRITSCHE-NETO, R.; CARBONELL, S. A. M.; CHIORATO, A. F.; BENCHIMOL-REIS, L. L. Genome-wide association study reveals genomic regions associated with *fusarium* wilt resistance in common bean. **Genes**, v. 12:765, 2021. Doi: 10.3390/genes12050765.

PAULINO, P. P. S.; GONÇALVES-VIDIGAL, M. C.; VAZ BISNETA, M.; VIDIGAL FILHO, P. S.; NUNES, M. P. B. A.; XAVIER, L. F. S.; MARTINS, V. S. R.; LACANALHO, G. F. Occurrence of anthracnose pathogen races and resistance genes in

common bean across 30 years in Brazil. **Agronomy Science and Biotechnology**, v. 8, p. 1-21, 2022. Doi: 10.33158/ASB.r140.v8.2022.

PEREIRA, H. S.; FARIA, L. C.; WENDLAND, A.; COSTA, J. G. C.; SOUZA, T. L. P. O.; MELO, L. C. Genotype by environment interaction for disease resistance and other important agronomic traits supporting the indication of common bean cultivars. **Euphytica**, v. 214:12, 2018. Doi: 10.1007/s10681-017-2093-z.

PEREIRA, H. S.; SOUZA, T. L. P. O.; FARIA, L. C.; AGUIAR, M. S.; WENDLAND, A.; COSTA, J. G. C.; DÍAZ, J. L. C.; MAGALDI, M. C. S.; SOUZA, N. P.; CARVALHO, H. W. L.; COSTA, A. F.; MELO, C. L. P.; ALMEIDA, V. M.; MELO, L. C. BRS FC406: Common bean cultivar with high yield in the rainy season in central Brazil. **Functional Plant Breeding Journal**, v. 3, n. 2, p. 115-120, 2021*a*. Doi: 10.35418/2526-4117/v3n2a10.

PEREIRA, S. P.; MENDONÇA, F. R.; RODRIGUES, L. L.; MELO, L. C.; MELO, P. G. S.; FARIA, L. C.; COSTA, A. F.; CARVALHO, H. W. L.; PEREIRA FILHO, I. A.; ALMEIDA, V. M. Selection of carioca common bean lines with slow darkening. **Pesquisa Agropecuária Brasileira**, v.56, e02471, 2021b. Doi: 10.1590/S1678-3921.pab2021.v56.02471.

PEREIRA, H. S.; WENDLAND, A.; MELO, L. C.: DEL PELOSO, M. J.; FARIA, L. C.; COSTA, J. G. C.; NASCENTE, A. S.; DÍAZ, J. L. C.; CARVALHO, H. W. L.; ALMEIDA, V. M.; MELO, C. L. P.; COSTA, A. F.; POSSE, S. C. P.; SOUZA, J. F.; ABREU, A. F. B.; MAGALDI, M. C. S.; GUIMARÃES, C. M.; OLIVEIRA, J. P. BRS Notável: a medium-early-maturing, disease-resistant Carioca common bean cultivar with high yield potential. **Crop Breeding and Applied Biotechnology**, v. 12, p. 220-223, 2012. Doi: 10.1590/S1984-70332012000300010.

PEREIRA, R.; ABREU, A. F. B.; NAIN, R. S.; SOUZA, E. A. Phenotyping for angular leaf spot severity and its implication in breeding common bean for resistance. **Scientia Agricola**, v. 76, n. 5, p. 415-423*, 2019*. Doi: 10.1590/1678-992X-2017-0394.

PEREIRA, R.; SOUZA, E. A.; BARCELOS, Q. L.; ABREU, A. F. B.; LIBRELON, S. S. Aggressiveness of *Pseudocercospora griseola* strains in common bean genotypes and implicatins for genetic improvement. **Genetic Molecular Research**, v. 14, n. 2, p. 5044-5053, 2015. Doi: 10.4238/2015.May.12.7.

PORCH, T. G.; BEAVER, J. S.; DEBOUCK, D. G.; JACKSON, S. A.; KELLY, J. D.; EMPEWOLF, H. Use of wild relatives and closely related species to adapt common bean to climate change. **agronomy**, v. 3, p. 433-461, 2013. doi:10.3390/agronomy3020433.

RAGAGNIN, V. A.; DE SOUZA, T. L. P. O.; SANGLARD, D. A.; ARRUDA, K. M. A.; COSTA, M. R.; ALZATE-MARIN, A. L.; CARNEIRO, J. E. DE S.; MOREIRA, M. A.; DE BARROS, E. G. Development and agronomic performance of common bean lines simultaneously resistant to anthracnose, angular leaf spot and rust. **Plant Breeding**, v. 128, p. 156-163, 2009. Doi: 10.1111/j.1439-0523.2008.01549.x.

RAMALHO, M. A. P.; ABREU, A. F. B.; SANTOS, J. B.; NUNES, J. A. R. **Aplicações da genética quantitativa no melhoramento de plantas autógamas**. 1 ed. Lavras: Editora UFLA. 2012, 566 p.

RODRIGUES, L. L.; RODRIGUES, L. A.; SOUZA, T. L. P. O.; MELO, L. C.; PEREIRA, H. S. Genetic control of seed coat darkening in common bean cultivars from three market classes. **Crop Science**, v. 59, p. 2046-2054, 2019a. Doi: 10.2135/cropsci2019.03.0161.

RODRIGUES, L. L.; TORRES, M. H. R. M.; SOUZA, T. L. P. O.; MELO, L. C.; PEREIRA, H. S. Associação entre o escurecimento dos grãos e reação à murcha de *fusarium* em feijão carioca. In: XIII Seminário Jovens Talentos, 13. **Resumos**. Santo Antônio de Goiás: Embrapa Arroz e Feijão: ISBN 978-65-86056-69-3, 2019b, p. 69.

SANGLARD, D. A. **Melhoramento do feijoeiro para resistência à mancha-angular: validação de marcadores moleculares SCAR, piramidação alélica de genes, testes de alelismo e caracterização de linhagens elites**. 2010. 109f. Tese (Doutorado em Genética e Melhoramento de Plantas) – Universidade Federal de Viçosa, Viçosa, 2010.

SHEN, G. Q.; ABDULLAH, K. G.; WANG, Q. K. (2009). The TaqMan method for SNP genotyping. In: Komar, A. (eds) **Single Nucleotide Polymorphisms. Methods in Molecular Biology™**, Totowa: Humana Press, 2009, v. 578. p. 293-306. Doi: 10.1007/978-1-60327-411-1_19.

SILVA, F. C.; PEREIRA, H. S.; MELO, P. G. S.; MELO, L. C. Selection of parents and segregating populations of common bean with high agronomic potential and slow seed-coat darkening. **Pesquisa Agropecuária Tropical**, v. 48, n. 1, p. 75-82, 2018. Doi:10.1590/1983-40632018v4849519.

SILVA, G. S.; RAMALHO, M. A. P.; ABREU, A. F. B.; SILVA, F. B. Genetic control of early grain darkening of carioca common bean. **Crop Breeding and Applied Biotechnology**, v. 8, p. 299-304, 2008. Doi: 10.12702/1984-7033.v08n04a07.

SILVA, R. S. **Melhoramento molecular do feijão-comum para resistência múltipla a viroses**. 2021. 100 f. Tese (Doutorado em Genética e Melhoramento de Plantas) – Escola de Agronomia, Universidade Federal de Goiás, Goiânia, 2021.

SINGH, S. P.; SCHWARTZ, H. F. Breeding common bean for resistance to diseases: a review. **Crop Science**, v. 50, p. 2199-2223, 2010. Doi: 10.2135/cropsci2009.03.0163.

SIQUEIRA, B. S.; PEREIRA, W. J.; BATISTA, K. A.; OOMAH, B. D.; FERNANDES, K. F.; BASSINELLO, P. Z. Influence of storage on darkening and hardening of slow- and regular-darkening carioca bean (*Phaseolus vulgaris* L.) genotypes. Journal of Agricultural Studies, v. 2, n. 2, p. 87-104, 2014. Doi: 10.5296/jas.v2i2.5859.

SOUZA, T. L. P. O.; GONÇALVES-VIDIGAL, M. C.; RAATZ, B.; MUKANKUSI, C. M.; ABREU, A. F. B.; MELO, L. C.; PASTOR-CORRALES, M. A. Major loci controlling resistance to the angular leaf spot of common bean**. Annual Report of the Bean Improvement Cooperative**, v. 58, p. xv – xviii, 2016.

SOUZA, T. L. P. O.; PEREIRA, H. S.; FARIA, L. C.; WENDLAND, A.; COSTA, J. G. C.; ABREU, Â. F. B.; DIAS, J. L. C.; MAGALDI, M. C. S.; SOUZA, N. P.; PELOSO, M. J. D.; MELO, L. C. Cultivares de feijão comum da Embrapa e parceiros disponíveis para 2013. **Comunicado Técnico**, v. 211, 2013.

SOUZA, T. L. P. O.; WENDLAND, A.; RODRIGUES, M. S.; ALMEIDA, L. C. S.; CORREIA, F. R.; RODRIGUES, L. A.; PEREIRA, H. S.; FARIA, L. C.; MELO, L. C. Anthracnose resistance sources to be explored by the common bean breeding programs in Brazil. **Annual Report of the Bean Improvement Cooperative**, **n.** 57, p. 217-218, 2014.

TERÁN, H.; JARA, C.; MAHUKU, G.; BEEBE, S.; SINGH, S. P. Simultaneous selection for resistance to five bacterial, fungal, and viral diseases in three Andean x Middle American inter-gene pool common bean populations. **Euphytica**, v. 189, p. 283-292, 2013. Doi: 10.1007/s10681-012-0803-0.

TORRES, M. H. R. M. **Melhoramento de feijão preto para resistência à murcha de fusário: identificação de marcadores moleculares e seleção de populações segregantes e linhagens**. 2020. 103 f. Tese (Doutorado em Genética e Melhoramento de Plantas) – Escola de Agronomia, Universidade Federal de Goiás, Goiânia, 2020.

VALDISSER, P. A. M. R. MOTA, A. P. S.; BUENO, L. G.; MENEZES, I. P. P.; COELHO, G. R. C.; MAGALHÃES, F. O. C.; VIANELLO, R. P. Protocolo de extração de DNA e genotipagem de SSRs em larga escala para uso no melhoramento do feijoeiro comum (*Phaseolus vulgaris* L.). **Comunicado Técnico**, n. 208, 2013. ISSN1678-961X.

VALENTINI, G.; GONÇALVES-VIDIGAL, M. C.; HURTADO-GONZALES, O. P.; CASTRO, S. A. L.; CREGAN, P. B.; SONG, Q.; PASTOR-CORRALES, M. A. High-resolution mapping reveals linkage between genes in common bean cultivar Ouro Negro conferring resistance to the rust, anthracnose, and angular leaf spot diseases. **Theorical and Applied Genetics**, v. 130, p. 1705-1722, 2017. Doi: 10.1007/s00122-017-2920-6.

VIEIRA, A. F.; ALMEIDA, L. C. S.; RODRIGUES, L. A.; COSTA, J. G. C.; MELO, L. C.; PEREIRA, H. S.; SANGLARD, D. A.; SOUZA, T. L. P. O. Selection of resistance sources to common bean anthracnose by field phenotyping and DNA marker-assisted screening. **Genetics and Molecular Research**, v. 17, n. 3, gmr18066, 2018. Doi: 10.4238/gmr18066.

WENDLAND, A.; PEREIRA, H. S.; FARIA, L. C.; SOUZA, T. L. P. O.; DEL PELOSO, M. J.; COSTA, J. G. C.; DÍAZ, J. L. C.; MAGALDI, M. C. S.; ALMEIDA, V. M.; CARVALHO, H. W. L.; MELO, C. L. P.; COSTA, A. F.; ABREU, A. F. B.; MELO, L. C. BRS Sublime: Common bean cultivar with carioca grain, resistance to angular leaf spot and high nutritional quality. **Crop Breeding and Applied Biotechnology**, v. 18, p. 440-445, 2018. Doi: 10.1590/1984-70332018v18n4c65.

WOO, S. L.; ZOINA, A.; DEL SORBO, G.; LORITO, M.; NANNI, B.; SCALA, F.; NOVIELLO, C. Characterization of *Fusarium oxysporum* f. sp. *phaseoli* by pathogenic races, VCGs, RFLPs and RAPD. **Phytopathology**, v. 86, p. 966-973, 1996. Doi: 10.1094/Phyto-86-966.

10 APÊNDICES

Apêndice 1.A. Marcadores microssatélites usados nas etapas de checagem da natureza híbridas das plantas oriundas de cruzamentos biparentais e suas respectivas fluorescências, tamanho do fragmento em pares de bases (pb), sequência dos iniciadores e o cromossomo em cada marcador foi mapeado.

Painel	Marcador	Fluorescência	Fragmento (pb)	Primer F	Primer R	Cromossomo
1	BM143	HEX	100-170	GGGAAATGAACAGAGGAAA	ATGTTGGGAACTTTTAGTGTG	Pv02
	PVBR25	6-FAM	140-180	GAGCTTCTCCGTCCTGTGT	CGAACTGAATCAGAAAGGAA	Pv09
	BM164	NED	130-190	CCACCACAAGGAGAAGCAAC	ACCATTCAGGCCGATACTCC	Pv02
	BM114	6-FAM	230-260	AGCCTGGTGAAATGCTCATAG	CATGCTTGTTGCCTAACTCTCT	Pv09
	BM138	NED	190-210	TGTCCCTAAGAACGAATATGGAATC	GAATCAAGCAACCTTGGATCATAAC	Pv05
	PVBR169	HEX	195-220	TGGAAAGTCGGAGGAGAAGA	AAAAGGGTCCCAACCAAAAC	Pv03
2	PVBR5	HEX	160-220	ATTAGACGCTGATGACAGAG	AGCAGAATCCTTTGAGTGTG	Pv06
	PVBR35	6-FAM	190-260	TCTACGCGTTCCCTCTGTCT	AGTGGATGTGTGGGAAAAGC	Pv04
	BM202	6-FAM	100-173	ATGCGAAAGAGGAACAATCG	CCTTTACCCACACGCCTTC	Pv11
	BM189	NED	80-120	CTCCCACTCTCACCCTCACT	GCGCCAAGTGAAACTAAGTAGA	Pv03
	BM210	NED	160-220	ACCACTGCAATCCTCATCTTTG	CCCTCATCCTCCATTCTTATCG	Pv07
3	PVBR113	NED	60-110	TGCATTCTTCCTCCCATCTT	TTGATTTGATTTGATCAGTGGTG	Pv06
	PVBR87	NED	150-201	CTCATTGCGTCTACCAGTGC	CCTAGGTTCCGCAGCATGT	Pv05
	PVBR272	6-FAM	70-135	CAGAACAGAAGAAGAAACAGAAAATG	GCGTGTTCCTCTGTGTGTGT	Pv02
	BM154	6-FAM	205-317	TCTTGCGACCGAGCTTCTCC	CTGAATCTGAGGAACGATGACCAG	Pv09
	PVBR13	6-FAM	159-200	TGAGAAAGTTGATGGGATTG	ACGCTGTTGAAGGCTCTAC	Pv06
4	PVBR11	HEX	175-192	AAACTCAAAGTCGTTGTTCC	CCACTGACTCTAGCTCCTCC	Pv02
	BM181	NED	170-250	CAACAGTTAAAGGTCGTCAAATT	CCACTCTTAGCATCAACTGGA	Pv05
	BM183	6-FAM	130-170	CTCAAATCTATTCACTGGTCAGC	TCTTACAGCCTTGCAGACATC	Pv07
	PVBR163	6-FAM	180-350	TGAGAGTGGAGAAGGAGAGAGA	TGACAACACTGCAAACACCA	Pv06
	BM201	NED	90-120	TGGTGCTACAGACTTGATGG	TGTCACCTCTCTCCTCCAAT	Pv01
	PVBR251	HEX	193-220	TGAAGTTGCAGCTAGGTTGG	GGTTGTGCTTGTGTTGTTGG	Pv01

Adaptado de Morais et al. (2016).

Apêndice 1.B. Condições de amplificação de cada marcador que será utilizado no estudo.

Marcador	Ciclos	Etapas	Condições de amplificação	Tempo
g796	1	Desnaturação inicial	95°C	15’
	40	Desnaturação	94 °C	30’’
		Anelamento	62°C	1’ e 30’’
		Extensão	72°C	1’ e 30’’
	1	Extensão final	60°C	30’
SE04	1	Desnaturação inicial	95°C	15’
	35	Desnaturação	94 °C	30’’
		Anelamento	65°C	1’ e 30’’
		Extensão	72°C	1’ e 30’’
	1	Extensão final	60°C	30’
Ensaios TaqMan	1	Desnaturação inicial	95°C	30’’
	50	Desnaturação	95 °C	3’’
		Anelamento	60°C	30’’
		Extensão	60°C	30’’
	1	Extensão final	72°C	5’

*Marcadores SNP em ensaios tipo TaqMan® (ThermoFisher) foram submetidos às mesmas condições de desnaturação, anelamento e extensão.

Apêndice 1.C. Perfil molecular de 35 plantas oriundas do cruzamento simples $F_{1(CD)}$ [CNFC 16902 x DM103].

Genótipo	ID_PlantaF_1	PV5_alelo1	PV5_alelo2	PV35_alelo1	PV35_alelo2
CNFC 16902	P5545_19	177[a]	177	208	208
DM103	P5543_19	175	175	214	214
$F_{1(CD)}$ [CNFC 16902♀ X DM 103♂]	P5507_19	175	177	208	214
	P5508_19	175	177	208	214
	P5509_19	175	177	208	214
	P5510_19	-	-	208	214
	P5511_19	175	177	208	214
	P5512_19	-	-	208	214
	P5513_19	-	-	208	214
	P5514_19	175	177	208	214
	P5515_19	175	177	208	214
	P5516_19	175	177	208	214
	P5517_19	175	177	208	214
	P5518_19	175	177	208	214
	P5519_19	-	-	-	-
	P5520_19	175	177	208	214
	P5521_19	-	-	-	-
	P5522_19	-	-	-	-
	P5523_19	175	177	208	214
	P5524_19	175	177	208	214
	P5525_19	175	177	208	214
	P5526_19	175	177	208	214
	P5527_19	-	-	208	214
	P5528_19	-	-	-	-
	P5529_19	175	177	208	214
	P5530_19	-	-	-	-
	P5531_19	175	177	208	214
	P5532_19	-	-	208	214
	P5533_19	175	177	208	214
	P5534_19	175	177	208	214
	P5535_19	175	177	208	214
	P5536_19	175	177	208	214
	P5537_19	175	177	208	214
	P5538_19	175	177	208	214
	P5539_19	175	177	208	214
	P5540_19	-	-	-	-
	P5541_19	175	177	208	214

[a]Tamanho da banda (pares de base); - Plantas em que os marcadores não amplificaram.

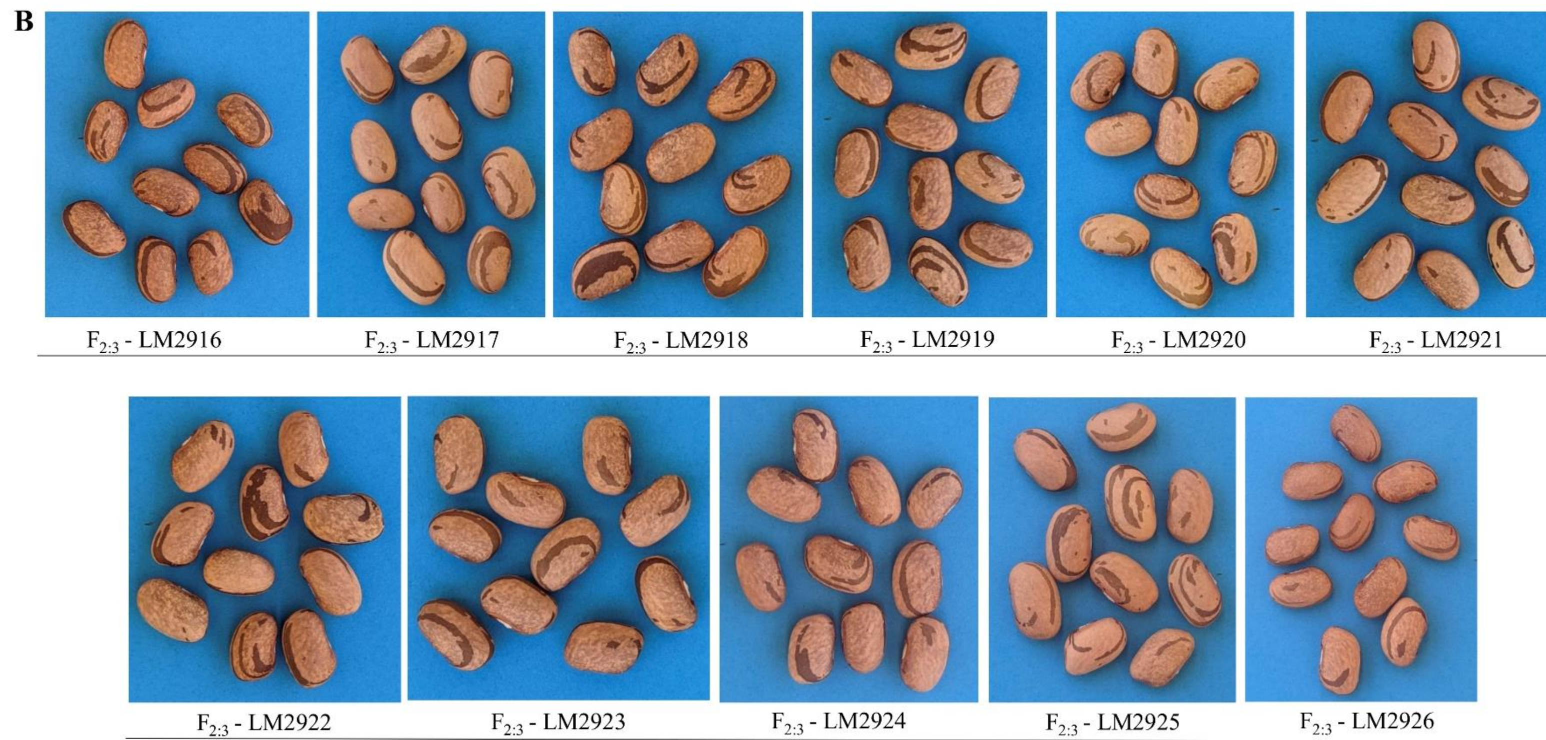
Apêndice 1.D. Genealogia do híbrido múltiplo $F_{1(ABCDEF)}$. Número de sementes F_2 dos três cruzamentos múltiplos e os alelos-alvo piramidados no híbrido múltiplo $F_{1(ABCDEF)}$.

Cruzamento múltiplo: $F_{1(ABCDEF)}$					
Híbrido Simples		Híbrido Duplo	N° sementes $F_{1(ABCDEF)}$	Código	Alelos-alvo*
$F_{1\ (EF)}$: [BRS Sublime♀ x CNFC 15826♂]_planta7	vs	$F_{1(AB)(CD)}$ _D6plt2: $F_{1(AB)}$ [Prog. 218311376♀ X K10♂)_plt4:1_P5477_19_A4] **X** $F_{1(CD)}$ [CNFC 16902♀ X DM 103♂)_plt2:1_P5511_19_B2♂]	1	LM01 (97)	*Fop* + *Co-4*^{2}
$F_{1\ (EF)}$: [BRS Sublime♀ x CNFC 15826♂]_planta6	vs	$F_{1(AB)(CD)}$ _D6plt2: $F_{1(AB)}$ [Prog. 218311376♀ X K10♂)_plt4:1_P5477_19_A4] **X** $F_{1(CD)}$ [CNFC 16902♀ X DM 103♂)_plt2:1_P5511_19_B2♂]	2	LM02 (36)	*Fop* + *sd*
				LM03 (68)	*Fop* + *Co-4*^{2}
$F_{1\ (EF)}$: [BRS Sublime♀ x CNFC 15826♂]_planta1	vs	$F_{1(CD)(AB)}$ _D78plt1: $F_{1(CD)}$ [CNFC 16902♀ X DM 103♂)_plt14_P5541_B14] **X** $F_{1(AB)}$ [Prog. 218311376♀ X K10♂)_plt6:1_P5481_19_A6}♂]	4	LM04 (71)	*Fop* + *sd*
				LM05 (36)	*Sd*
				LM06 (72)	*Co-4*^{2} + *sd*
				LM07 (14)	*Fop* + *sd*

() Número de sementes F_2 de cada planta F_1 do cruzamento múltiplo; Código - Cada semente do cruzamento múltiplo foi codificada, gerando sete códigos (LM01 ao LM07);
*Alelos-alvo piramidados no híbrido múltiplo $F_{1(ABCDEF)}$.

Apêndice 1.E. Fenótipo das sementes oriundas das onze plantas F_2 selecionadas contendo os alelos *Fop* e *sd* em homozigose.

Apêndice 1.F. (A) - Fenótipo das sementes oriundas de dez progênies $F_{2:3}$ selecionadas contendo os alelos *Co-4*^{2} e *Fop* em homozigose. G01 - LM2906 ao LM2914 e G02 – LM2915.



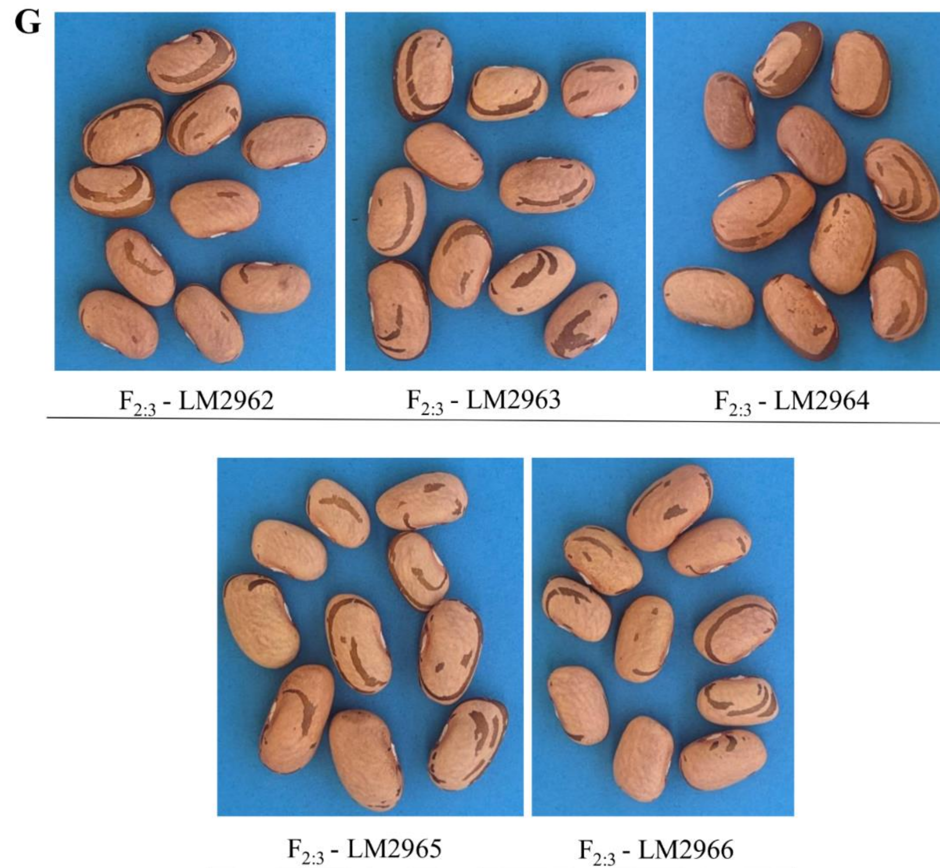
Apêndice 1.F. (B) - Fenótipo das sementes oriundas de onze progênies $F_{2:3}$ selecionadas contendo os alelos *Co-4*^{2} e *Fop* em homozigose. G03 - LM2916 ao LM2926.

Apêndice 1.F. (C) - Fenótipo das sementes oriundas de oito progênies $F_{2:3}$ selecionadas contendo os alelos *Co-4²* e *Fop* em homozigose. G04 - LM2927 ao LM2935.

Apêndice 1.F. (D) - Fenótipo das sementes oriundas de oito progênies $F_{2:3}$ selecionadas contendo os alelos *Co-4*^{2} e *Fop* em homozigose. G05 - LM2936 ao LM2943.

Apêndice 1.F. (E) - Fenótipo das sementes oriundas de seis progênies $F_{2:3}$ selecionadas contendo os alelos *Co-4*^{2} e *Fop* em homozigose. G06 - LM2944 ao LM2950.

Apêndice 1.F. (F) - Fenótipo das sementes oriundas de onze progênies $F_{2:3}$ selecionadas contendo os alelos *Co-4*^{2} e *Fop* em homozigose. G07 - LM2951 ao LM2961.



Apêndice 1.F. (G) - Fenótipo das sementes oriundas de quatro progênies $F_{2:3}$ selecionadas contendo os alelos *Co-4*^{2} e *Fop* em homozigose. G08 - LM2962 ao LM2966.

CAPÍTULO 2

11 CHARACTERIZATION AND GENETIC MAPPING OF A NEW ANDEAN ANTHRACNOSE RESISTANCE GENE PRESENT IN THE COMMON BEAN CULTIVAR BRSMG REALCE

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Characterization and genetic mapping of a new Andean anthracnose resistance gene present in the common bean cultivar BRSMG Realce

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ABSTRACT

Preliminary results revealed that the rajado seeded Andean cultivar BRSMG Realce (striped seed coat) developed by Embrapa (Brazil) shown a high level of anthracnose resistance in field and greenhouse screenings. The main goal of this study was to evaluate the inheritance of anthracnose resistance in BRSMG Realce, map the resistance locus or major gene cluster previously named as *Co-Realce*, identify resistance-related positional genes, and analyze potential markers linked to the resistance allele. F_2 plants derived from the cross BRSMG Realce × BRS FC104 (Mesoamerican) and from the cross BRSMG Realce × BRS Notável (Mesoamerican) were inoculated with the *C. lindemuthianum* pathotypes 475 and 81, respectively. The BRSMG Realce × BRS FC104 F_2 population was also genotyped using the DArTseq technology. Crosses between BRSMG Realce and BAT 93 (Mesoamerican) have also been done and resulting F_2 plants were inoculated with the *C. lindemuthianum* pathotypes 65 and 1609, individually. The results shown that anthracnose resistance in BRSMG Realce is controlled by a single locus with complete dominance. A genetic map including 1,118 SNP markers was built and shown 78% of the markers mapped at a distances less than 5.0 cM, with a total genetic length of 4,473.4 cM. A major locus (*Co-Realce*) explaining 54.6% of the phenotypic variation of symptoms caused by the pathotype 475 was identified in Pv04, flanked by the markers snp1327 and snp12782 and 4.48 cM apart each other. These SNPs are useful for marker-assisted selection, once they shown an estimated selection efficiency of 99.2%. The identified resistance allele segregates independently of the resistance allele *Co-3*^{3} (Pv04) presents in BAT 93. Allelism tests and physical mapping of Pv04 support that *Co-Realce* is different from other major loci already mapped on this same P. vulgaris chromosome (*Co-3*, *Co-15* and *Co-16*). The mapped genomic region with 704,867 bp comprising 63 putative genes, 44 of which were related to the pathogen-host interaction. Based on all these results and evidences, anthracnose resistance in BRSMG Realce should be considered as monogenic (major gene or complex gene locus), especially for breeding purpose. It is proposed that *Co-Realce* locus is unique and be officially named in accordance with the rules established by the Bean Improvement Cooperative Genetics Committee.

Keywords: *Phaseolus vulgaris* L., *Colletotrichum lindemuthianum*, molecular breeding, genetic resistance, allelism test, inheritance study.

12 INTRODUCTION

The common bean is grown in more than 120 countries under different temperatures, light intensities, relative humidity, rainfall distributions and technological levels, aspects that contribute to the unstable global production (Pereira et al., 2018; FAO, 2022). Brazil is one of the main producer countries, harvesting 2,366,527 ton in 2020, 85% of which were the carioca and black seeded cultivars (Embrapa Rice and Beans, 2022).

The soil and climate conditions in regions with tropical and subtropical climates favor the occurrence of fungal diseases such as anthracnose, caused by *Colletotrichum lindemuthianum* (Basavaraja et al., 2020). This disease, which displays wide geographic distribution and pathogenic variability (Nabi et al., 2022), is more prevalent in areas with temperatures between 15 and 22°C, associated with high relative humidity (RU ≥ 95%) and frequent rainfall (Padder et al., 2017). Depending on the susceptibility level of cultivars, favorable environmental conditions and the presence of the initial inoculum, the disease can cause losses of up to 100% (Sing and Schwartz, 2010). In Brazil, where anthracnose pathotypes from the Mesoamerican gene pool is predominant, the introgression of resistance alleles from Andean gene pool is an important strategy aiming the development of cultivars with durable and broad resistance spectrum (Miklas et al., 2006; Paulino et al., 2022). This strategy is supported by the high level of anthracnose resistance in the Andean cultivars developed by Embrapa in Brazil, particularly in BRSMG Realce, which is resistant to pathotypes 65, 73 and 81 (Melo et al., 2014; Aguiar et al., 2021). These races are the most prevalent in the main Brazilian common bean growing areas for the past 30 years (Paulino et al., 2022). The anthracnose resistance of BRSMG Realce has also shown to be stable over time, becoming one of the resistant controls in the final field trials – experiments of Value for Cultivation and Use (VCU) – conducted by the Embrapa breeding program (Aguiar et al., 2021). Thus, identifying resistance sources from the Andean gene pool and mapping the resistance alleles present in these genotypes is an indispensable target of common bean pre-breeding programs worldwide, enabling their effective use in the development of cultivars with durable and broad-spectrum resistance.

Disease integrated management and the use of resistant cultivars are considered the most promising, environmentally sustainable and economically profitable methods, in addition to being easily applied by growers (Miklas et al., 2006; Souza et al., 2013). Anthracnose resistance in common bean is largely conditioned by dominant alleles of major quantitative trait loci (QTLs), except for *co-8* (Paulino et al., 2022). Currently, 14 effective resistance loci have been identified; *Co-1* to *Co-17*, excluding *Co-7*, *Co-9* and *Co-10* which have been renamed as alleles from other loci. They were mapped in eight common bean chromosomes (Pv01, Pv02, Pv03, Pv04, Pv07, Pv08, Pv09 and Pv11). Five of these loci have been identified in resistance sources from the Andean gene pool, namely as *Co-1*, *Co-12*, *Co-13*, *Co-14* and *Co-15* (BIC, List of Genes – *Phaseolus vulgaris* L.: http://www.bic.uprm.edu/wp-content/uploads/2019/10/Bean-Genes-List-2018-v2-1.pdf). *Co-1* is from the Michigan Dark Red Kidney resistance source and it was mapped in Pv01 (Zuiderveen et al., 2016). In this same genomic region, four alleles were identified: *Co-1*^{2} (Melotto and Kelly, 2000), *Co-1*^{3} (Melotto and Kelly, 2000), *Co-1*^{4} (Gonçalves-Vidigal et al., 2011), and *Co-1*^{5} (Gonçalves-Vidigal and Kelly, 2006). *Co-12* is a non-mapped resistance allele identified in the cultivar Jalo Vermelho (Gonçalves-Vidigal et al., 2008). *Co-13* was mapped on Pv03 in the Brazilian landrace Jalo Listas Pretas (Gonçalves-Vidigal et al., 2009; Lacanallo and Gonçalves-

Vidigal, 2015). *Co-14* was mapped on Pv01, in the Pitanga resistance source (Gonçalves-Vidigal et al., 2012), while *Co-15* was mapped on Pv04 in the Brazilian landrace Corinthiano (Sousa et al., 2015).

Recent studies report new genomic regions associated with race-specific resistance to *C. lindemuthianum* in the common bean germplasm from Andean gene pool, such as the *Co-Bf* (Marcon et al., 2021), *Co-AC* (Gilio et al., 2020), $CoPv01^{CDRK}$ (Gonçalves-Vidigal et al., 2020) and *Co-Pa* alleles (Lima-Castro et al., 2017), which have not been officially named in accordance with the rules established by the Bean Improvement Cooperative Genetics Committee (BIC, Genetics Committee: http://arsftfbean.uprm.edu/bic/wp-content/uploads/2018/04/Gene_Committee_Rules.pdf).

The main goal of this study was to evaluate the inheritance of anthracnose resistance in BRSMG Realce, map the resistance locus previously named as *Co-Realce*, identify resistance-related positional genes, and analyze potential markers linked to the resistance allele. In addition, allelism tests have also been done to check if *Co-Realce* segregates independently of the resistance allele $Co\text{-}3^3$ present in BAT 93, already used by the Embrapa common bean breeding program.

13 MATERIALS AND METHODS

13.1 Genetic material and crosses

BRSMG Realce is a rajado (striped seed coat) seeded cultivar from the Andean gene pool developed by Embrapa and partners in Brazil (**Supplementary Figure 1**). This cultivar presents a type I determinate growth habit, high yield potential and it is well suited to mechanized harvesting. In addition to anthracnose resistance, it is also resistant to powdery mildew (*Erysiphe polygoni*) and bacterial wilt (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*) (Melo et al., 2014). BRS FC104 is a Mesoamerican carioca seeded cultivar also developed by Embrapa, showing a super-early maturing cycle and high yield potential (Melo et al., 2019). BRS Notável is also a Mesoamerican cultivar from carioca market class, but with a medium-early maturing cycle. It is resistant to anthracnose, fusarium wilt, common bacterial blight and bacterial wilt (Pereira et al., 2012). BAT 93 harbors the anthracnose resistance allele $Co\text{-}3^3$. It is a Mesoamerican breeding line developed by Centro International de Agricultura Tropical (CIAT, Cali, Colombia) from a double cross involving the parents Veranic 2, PI 207262, Jamapa, and Great Northern Tara (Geffroy et al., 2008).

For the inheritance studies, crosses between BRSMG Realce (female parent) and BRS FC104 (male parent) and between BRSMG Realce and BRS Notável (male parent) were carried out at Embrapa Rice and Beans (Santo Antônio de Goiás, Goiás, Brazil), under controlled conditions (greenhouse). The resulting F_1 plants were checked as true hybrids using 24 microsatellite markers, as described by Morais et al. (2016). F_1 checked plants were then advanced and F_2 seeds were obtained. For the allelism tests, using the same strategy, BRSMG Realce (female parent) was crossed with BAT 93 (male parent) and resulting F_2 seeds were obtained.

13.2 Phenotyping of F_2 populations

An inoculation test of the parents and control lines (BRSMG Realce, BRS FC104, BRS Notável, BAT 93, SEL 1308 and IPA 7419) was carried out under controlled conditions using the pathotypes 65, 73, 81, 91, 113, 475 and 1609 of *C. lindemuthianum*. The segregating F_2 populations were inoculated using the pathotypes that resulted in a better phenotypic contrast between their parents (**Supplementary Table 1**).

For the inheritance studies, 161 F_2 seedlings from the cross BRSMG Realce × BRS FC104 and 128 F_2 seedlings derived from the cross BRSMG Realce × BRS Notável were grown in expanded polystyrene trays filled with commercial substrate (Plantmax®). Each tray also contained 12 plants of the parents and the control lines (SEL 1308, resistant control; IPA 7419, susceptible control) (Sartorato et al., 2004). Before inoculation, plant tissue samples of each F_2 (BRSMG Realce × BRS FC104) plant and of their parents were collected and stored in a freezer at -20°C for genomic DNA extraction. For the allelism studies aiming to test the independence between the anthracnose resistance locus present in BRSMG Realce (*Co-Realce*) and *Co-3*^{3} present in BAT 93 (chromosome Pv04), which is already used by the Embrapa common bean breeding program, F_2 (BRSMG Realce × BAT 93) plants were independently inoculated with *C. lindemuthianum* pathotypes 65 (132 F_2 plants) and 1609 (183 F_2 plants).

Plants were inoculated seven days after sowing, in the V2 stage (fully expanded primary leaves) (Pastor-Corrales, 1992). The spore solution (1.2×10^6 spores/mL) was applied to the abaxial and adaxial leaves, using a manual atomizer (De Vilbiss, No. 15). After inoculation, the plants were incubated in a humidity chamber for 48 h, with temperature adjusted to 20±2ºC, 95% relative humidity controlled by nebulization and a 12-hour light/dark photoperiod. Later, nebulization was discontinued, and the inoculated plants were kept in a controlled environment under the same temperature and photoperiod conditions described above, where they remained until disease symptoms were screened.

Symptoms were evaluated seven days after inoculation, based on a 1-to-9 scale, where 1 = absence of symptoms and 9 = dead plants due to symptoms caused by the disease (Pastor-Corrales and Tu, 1989). Plants with scores between 1 and 3 were considered resistant and the others susceptible.

13.3 Genotyping with SNP markers and SilicoDArT

Genomic DNA extraction from parental lines and F_2 plants (BRSMG Realce × BRS FC104) was performed according to the protocol described by Ferreira and Grattapaglia (1998). DNA concentration was estimated by fluorescence, using a Qubit® 2.0 Fluorometer (Invitrogen by Life Technology), and DNA integrity was checked via 1.0% agarose gel electrophoresis. The genotyping protocol was accomplished based on DArTseq technology, developed by DArT Pty Ltd (Kilian et al., 2012), from which SNP and SilicoDArT markers were extracted, as described by Valdisser et al. (2020).

13.4 Genetic mapping with SNP markers

The polymorphic SNP markers between parental lines were tested for Mendelian segregation at an expected ratio of 1:2:1 using the chi-squared test (χ^2; P-value < 0.05), followed by FDR (False Discovery Rate, P-value < 0.05) correction proposed by Benjamin and Hocheberg (1995). The linkage groups were established using a LOD-score (logarithm of the odds) of

5 and maximum recombination fraction of 0.1. The order of markers was estimated using the RCD (Rapid Chain Delineation) method with a LOD-score of 3.0. In addition, the most likely position of each marker on the map was obtained using the safe function and later, the ripple function (5-marker windows and LOD-score of 3). Genetic distances were estimated using the Kosambi function (Kosambi, 1944). The coefficient of Spearman's correlation was estimated for the genetic marker positions and the physical marker positions on the reference genomes. The linkage map was constructed in the R software (R Core Team, 2022), using the OneMap package (Margarido et al., 2007).

13.5 QTL analysis and physical mapping

QTL (Quantitative Trait Loci) analysis was carried out using composite interval mapping (CIM) (Zeng, 1993), with a walkspeed of 0.5 cM and window size of 1.0 cM. The coefficient of determination (R^2) was calculated separately for each interval to determine the percentage of phenotypic variation explained by a single locus. The likelihood ratio values were converted into LOD values using the equation LOD = 0.2171*LTR (Churchill and Doerge, 1994). The minimum LOD value to declare the existence of a QTL was estimated using the criterion proposed by Churchill and Doerge (1994), with 1,000 permutations. Analyses were conducted using QTL-Cartographer software (Wang et al., 2012). The *Co-Realce* genomic region on the Pv04 was graphically represented using the software MapChart (Voorrips, 2002). The physical map was obtained using the positions of each marker linked with target alleles provided in base pairs (bp), according to the reference genome (Schmutz et al., 2014) and using the software MapChart (Voorrips, 2002).

13.6 Gene annotation

The genes annotated in the current version of the bean genome (Schmutz et al. 2014) were extracted from the sequences included in the locus interval identified in this study, using the Phytozome platform (*Phaseolus vulgaris* v2.1, DOE-JGI and USDA-NIFA, http://phytozome.jgi.doe.gov/).

13.7 Selection efficiency

Selection efficiency (%SE) of the SNP markers identified in the resistance locus interval was estimated according to the methodology described by Liu (1998), using the following estimator: $SE\ (\%) = (1 - 4rf^2) \times 100$, where "rf" is the recombination frequency between marker pairs.

14 RESULTS

14.1 Reaction of parents to selected *C. lindemuthianum* pathotypes

Out of the seven *C. lindemuthianum* pathotypes used to screen the parents and controls (65, 73, 81, 91, 113, 475 and 1609), BRSMG Realce was resistant to six pathotypes, with mean score of 1.0, being susceptible only to pathotype 113 (mean score of 5.2). BRS Notável was susceptible only to pathotype 81 (mean score of 9.0). As expected, the resistant control SEL 1308 was resistant to all seven pathotypes, with mean score of 1.0, and the susceptible control IPA 7419 was susceptible, with mean score of 9.0. BRS FC104 was screened with five pathotypes (73, 81, 91, 475 and 1609), showing susceptibility to the pathotypes 81, 91, 475 and 1609. For the inheritance studies and allelism tests, the *C. lindemuthianum*

pathotypes causing strongest contrasts for disease symptoms among parents were those selected and used to inoculate the segregating populations (**Supplementary Table 1**).

Table 1. Inheritance of anthracnose resistance in the Andean common bean cultivar BRSMG Realce from the rajado (striped seed coat) market class, and allelism test between BRSMG Realce (*Co-Realce*) and BAT93 (*Co-3*^{3}).

Pathotype[a]	Genotype	Hypothesis[d] R:S	Observed R	Observed S	Expected R	Expected S	χ^2	P-value
81	BRSMG Realce (*Co-Realce*)	1:0	12	0	12	0	-	-
	BRS Notável	0:1	0	12	0	12	-	-
	IPA 7419[b]	0:1	0	12	0	12	-	-
	F_2 (BRSMG Realce × BRS Notável)	3:1	101	27	96	32	1.0	0.31
475	BRSMG Realce (*Co-Realce*)	1:0	12	0	12	0	-	-
	BRS FC104	0:1	0	12	0	12	-	-
	IPA 7419	0:1	0	12	0	12	-	-
	F_2 (BRSMG Realce × BRS FC104)	3:1	127	34	121	40	1.3	0.26
65	BRSMG Realce (*Co-Realce*)	1:0	12	0	12	0	-	-
	BAT93	1:0	12	0	12	0	-	-
	IPA 7419	0:1	0	12	0	12	-	-
	F_2 (BRSMG Realce × BAT93)	3:1	121	11	99	33	19.6	$9.77e^{-06}$
		9:7	121	11	74	58	67.3	$2.36e^{-16}$
		13:3	121	11	107	25	9.4	0.002
		15:1	121	11	124	8	0.98	0.32
1609	BRSMG Realce	1:0	12	0	12	0	-	-
	BAT93 (*Co-3*^{3})	1:0	12	0	12	0	-	-
	IPA7419	0:1	0	12	0	12	-	-
	F_2 (BRSMG Realce × BAT93)	3:1	174	9	137	46	39.4	$3.52e^{-10}$
		9:7	174	9	103	80	112.1	$< 2.2e^{-16}$
		13:3	174	9	149	34	22.9	$1.64e^{-06}$
		15:1	174	9	172	11	0.55	0.46
F_2 (BRSMG Realce × BAT93) – Joint analysis[c]		3:1	295	20	236	79	58.4	$2.10e^{-14}$
		9:7	295	20	177	138	179.1	$< 2.2e^{-16}$
		13:3	295	20	256	59	31.8	$1.71e^{-08}$
		15:1	295	20	295	20	0.01	0.94

[a]Pathotype of *Colletotrichum lindemuthianum*;
[b]Susceptible control;
[c]Joint allelism test performed using all resistant (121+174) and susceptible (11+9) F_2 (BRSMG Realce × BAT93) plants, considering the reaction to pathotypes 65 and 1609;
[d]R – Number of resistant plants, and S – Number of susceptible plants.

14.2 Inheritance studies and allelism tests

The screening of 161 F_2 (BRSMG Realce × BRS FC104) plants with the pathotype 475 shown 127 resistant (scores 1-to-3) and 34 susceptible plants (scores 4-to-9), resulting in a segregation ratio of 3R:1S (χ^2 = 1.29; P-value = 26%). A total of 128 F_2 (BRSMG Realce × BRS Notável) plants were inoculated with the *C. lindemuthianum* pathotype 81. The segregation ratio observed was also 3R:1S (χ^2 = 1.04 and P-value = 31%) (**Tables 1 and 2**).

A total of 132 and 183 F_2 (BRSMG Realce × BAT 93) plants were inoculated with the *C. lindemuthianum* pathotypes 65 and 1609, respectively. In both cases, the segregation ratio

observed was 15R:1S (χ^2 = 0.98 and P-value = 32%, and χ^2 = 0.55; P-value = 46%). The joint analysis using data from all 315 F_2 (BRSMG Realce × BAT 93) also shown a segregation ratio of 15R:1S (χ^2 = 0.005 and P-value = 94%) (**Table 1**).

These results strongly suggest that anthracnose resistance in BRSMG Realce is controlled by a single locus with complete dominance. In addition, that the resistance allele present in BRSMG Realce segregates independently of the resistance allele *Co-3*^{3} presents in BAT 93 and mapped in Pv04.

Table 2. Reaction of F_2 plants derived from the cross BRSMG Realce × BRS FC104 to the *C. lindemuthianum* pathotype 475.

Class	Grade scale[a]									
	1	2	3	4	5	6	7	8	9	Total
Resistant	103	13	11	0	0	0	0	0	0	127
Susceptible	0	0	0	14	0	0	4	1	15	34
Total	103	13	11	14	0	0	4	1	15	161

[a]Number of plants evaluated as showing each one of the reaction scores from the 1-to-9 grade scale used for disease symptom screening.

14.3 Genetic map

The genotyping approach based on DArTseq technology resulted in 13,083 SNP and 16,186 DArT markers (**Supplementary Table 2**), with call rates ranging from 0.68 to 1.00 and from 0.56 to 1.00, respectively (**Supplementary Table 3**). A total of 6,304 (48.2%) SNP markers were polymorphic in the F_2 (BRSMG Realce × BRS FC104) population. The segregation test identified 4,175 (31.9%) of these markers as undistorted SNPs, once they fit to the segregation ratio of 1:2:1 (FDR ≥ 5%) and therefore were used for genetic mapping. Out of these markers, 4,129 (31.6%) performed well for linkage analysis. Among them, 395 and 60 markers were positioned in contigs and scaffolds, respectively (**Supplementary Figure 2; Supplementary Table 3**).

A linkage map was built including 4,074 SNP markers covering all common bean genome. The linkage groups with the largest and smallest number of markers were Pv02 and Pv04, with 505 and 152 SNP markers, respectively. The average number of markers per linkage group was 370 (**Supplementary Table 4**). The SNPs mapped on contigs and scaffolds were allocated to the 11 chromosomes (**Supplementary Table 5*: Pv01 – Pv11***). When keeping only the markers with high statistical support (SAFE map), a total of 1,315 markers were mapped and well distributed in the common bean genome (**Supplementary Figure 2**), with an average of 120 markers per linkage group. The total genetic linkage distance of the SAFE map was 4,473.44 cM, with an average of 406.68 cM. Pv01 was the largest linkage group, with 561.32 cM, and the smallest one was Pv04, with 196.82 cM. In average, 78.1% of the markers were mapped at distances less than or equal to 5.0 cM, with an average distance of 4.07 cM between markers along the 11 chromosomes (**Supplementary Table 4**). Pv02 shown highest density (**Supplementary Figure 2**), with an average distance of 2.91 cM

between markers and 89.7% of the markers were mapped at ≤ 5.0 cM (**Supplementary Table 4**). Markers ordered with a LOD-score < 3.0 were represented as accessory markers in their most likely position **(Supplementary Table 5*:* Pv01 – Pv11)**. The Spearman's correlation coefficients (ρ) between the positions of the markers on linkage map and physical map were positive (0.996-to-0.999) and highly significant (p-value < $2.2e^{-16}$), with an average of 0.999 (**Supplementary Table 4**).

14.4 Major locus associated with anthracnose resistance

The QTL analysis identified a major locus associated with anthracnose resistance in the Andean common bean cultivar BRS Realce on Pv04 (*Co-Realce*), with a LOD-score of 15.3 and explaining 54.60% of the phenotypic variation considering the symptoms incited by the *C. lindemuthianum* pathotype 475. The size of this QTL was 4.48 cM flanked by the SNP markers snp1327 (position 477,285 bp) and snp12782 (1,182,123 bp) (**Table 3**; **Figure 1**). Simple linear regression analysis shown that markers snp1327 and snp12782 explain, respectively, 29% and 33% of the phenotypic variation (**Table 4**). The homozygous plants for the snp1327 reference allele (TT) associated with disease resistance shown a mean severity score of 1.62, while the mean score of homozygous plants for the respective susceptibility allele (CC) was 4.54 (**Figure 2**). Considering the locus snp12782, the homozygous plants for the resistance allele (CC) shown a mean severity score of 1.59, while the mean score of homozygous plants for the respective susceptibility allele (TT) was 4.86 (**Figure 2**). The joint selection of homozygous and heterozygous plants for *Co-Realce* using the markers snp1327 and snp12782 resulted in a set of plants showing a mean severity score of 1.54. The size of *Co-Realce* genomic region was 704,867 bp long (Pv04: 477,217 bp…1,182,084 bp) (**Table 3**) and a total of 63 genes were observed to be locates in this interval, of which 44 are involved in signaling pathways of response to pathogen attack (**Supplementary Table 6**).

Table 3. SNP and DArT markers flanking the major locus (*Co-Realce*) controlling anthracnose resistance in the Andean common bean cultivar BRSMG Realce, recombination frequency between the pair of markers flanking *Co-Realce*, interval size of the *Co-Realce* region, LOD-score and percentage of phenotypic variation explained by major locus *Co-Realce*.

Interval[a]	Pair of markers[b]	rf	Interval size	LOD-score	R^2 (%)
Pv04: 477,217...1,182,084	snp1327 and snp12782	4.48 cM	704,867 pb	15.3	54.60
Pv04: 485,246...505,651	dart9817 and snp3308	2.91 cM	20,405 pb	16.3	54.02

[a]Chromosome Pv04 (Chr04)
[b]Markers flanking the major locus *Co-Realce*; rf – recombination frequency between the markers flanking *Co-Realce*.

14.5 Increasing of mapping resolution in *Co-Realce* genomic region

In order to increase the mapping resolution in the genomic region containing the major locus *Co-Realce*, an additional set of 246 markers, including 135 SNPs and 111 SilicoDArTs previously known as located on Pv04 and with call rate of 0.58-to-1.0, were included in the

genetic linkage analysis. The recombination fraction was estimated and 229 markers were mapped (**Supplementary Table 7**). By increasing markers density in the *Co-Realce* genomic region, its interval reduced from 704,867 bp to 20,405 pb (LOD of 16.3) and the phenotypic variation explained was 54% (**Table 3**). After this new approach, the closest and significantly markers identified as associated with *Co-Realce* were dart9817 (position 485,246 bp) and snp3308 markers (position 505,696 bp) spanning 2.9 cM (**Table 3; Figure 1**). A total two putative candidate genes associated with cell membrane processes were identified in the *Co-Realce* region. The Phvul.004G006800 transcript encodes proteins from the nuclear pore complex involved in the membrane transport system (Nuclear Pore Complex NPC - Nup210 GP210), and the transcript Phvul.004G006900 that encodes a protein from the glycosylphosphatidylinositol transamidase complex (Glycosylphosphatidylinositol transamidase-GAA1; Phvul.004G006900-GAA1; Phvul.004G006900), which generally act as membrane anchors for many cell surface proteins (**Supplementary Table 6**).

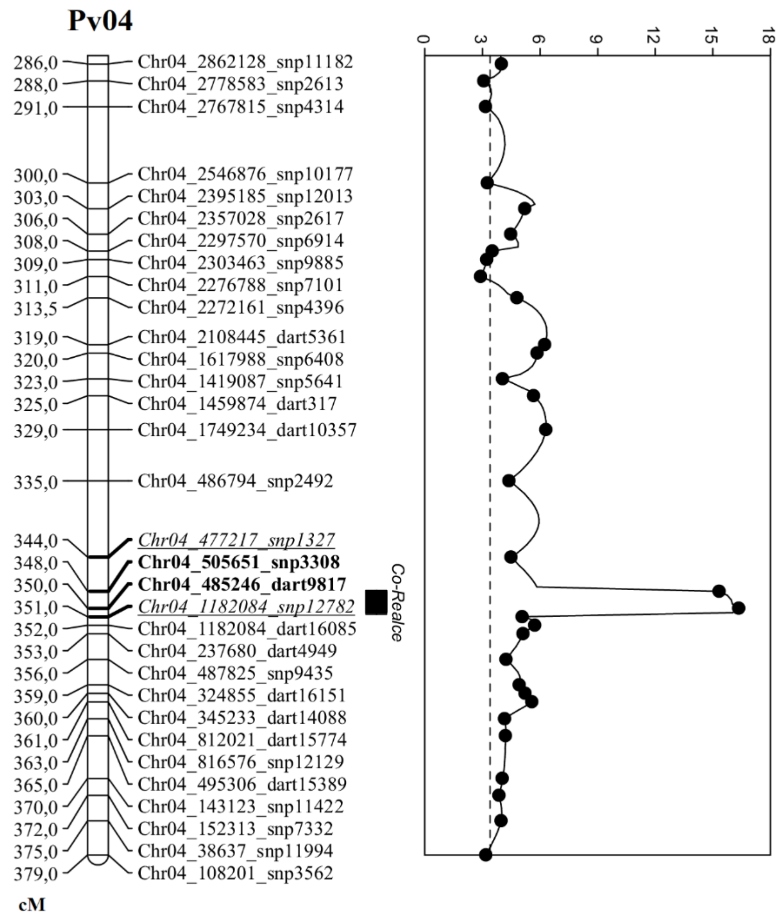

Figure 1. Genetic map of the *Co-Realce* genomic region on the common bean chromosome Pv04. QTL analysis was used to increase the mapping resolution in *Co-Realce* genomic region, performed using the F_2 population derived from the cross BRSMG Realce × BRS FC104 phenotyped with the *Colletotrichum lindemuthianum* pathotype 475 and genotyped with SNP and SilicoDArT markers. The two underlined and italicized markers delimit the *Co-Realce* genomic region. The two bold markers delimit the *Co-Realce* genomic region after increasing the mapping resolution. The highest peak on Pv4 represents the major locus

in the *Co-Realce* genomic region and the horizontal dashed line is the LOD-score threshold estimated after 1,000 permutations.

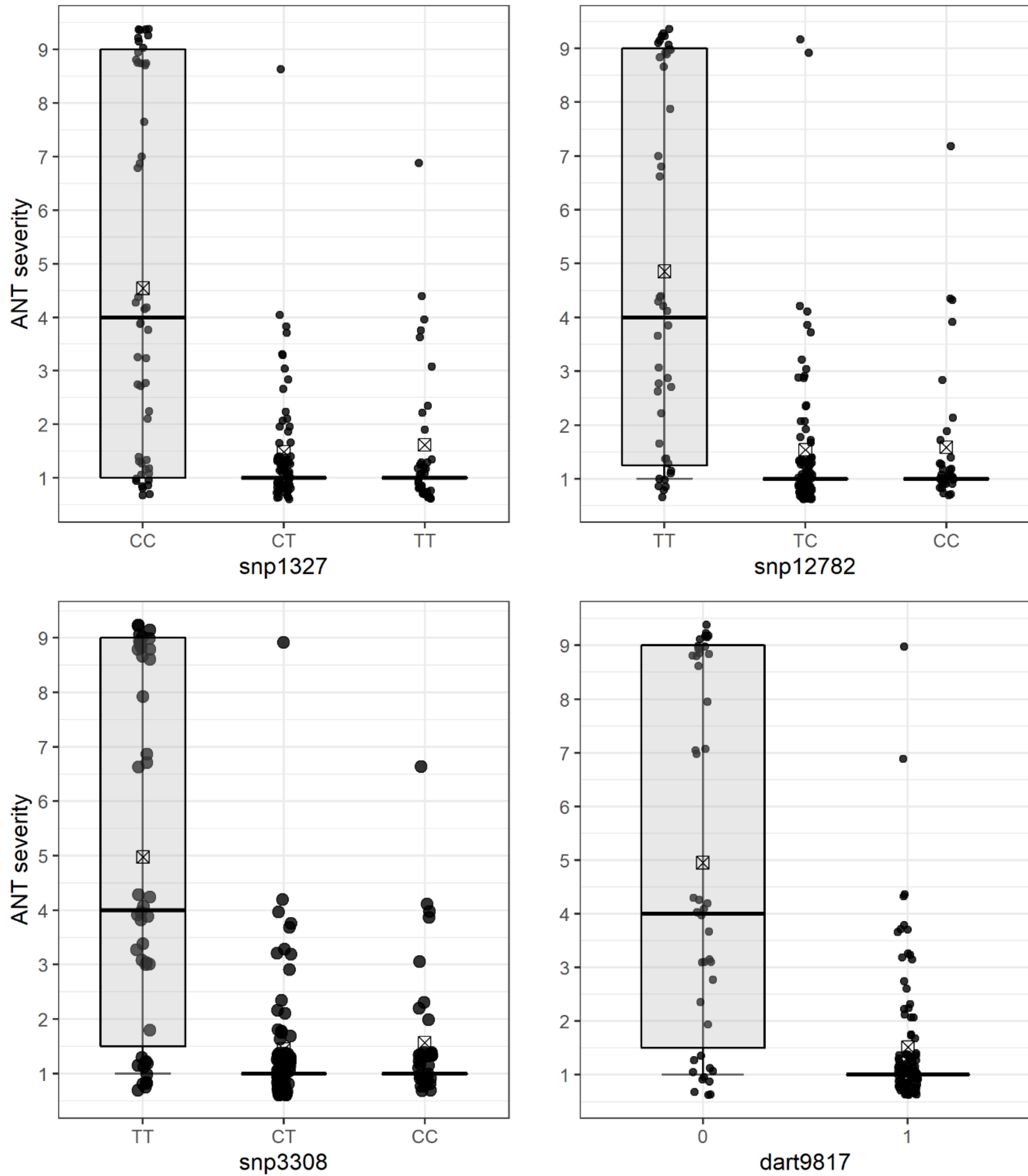

Figure 2. Differential reaction of F_2 (BRSMG Realce × BRS FC104) plants to *Colletotrichum lindemuthianum* pathotype 475 for each molecular genotype class of SNP markers flanking the *Co-Realce* genomic region: snp1327 (CC, CT and TT), snp12782 (TT, TC and CC), snp3308 (TT, CT and CC) and dart9817 (0 and 1). The mean phenotypic scores are represented by a rectangle inside each box plot.

15 DISCUSSION

Based on inheritance and allelism studies, and considering addition information from genetic and physical mapping, this study identified a major anthracnose resistance locus in the Andean common bean cultivar BRSMG Realce developed by Embrapa and partners in Brazil. This rajado seeded cultivar shows several important agronomic traits (Melo et al., 2014), including a high level, wide and durable resistance to anthracnose disease caused by the fungus *C. lindemuthianum*. It has being used as parent in crosses and as a resistant control in final field trials conducted by the Embrapa breeding program at least for the last decade (Aguiar et al., 2021), and its resistance has shown to be stable and durable over time. The use of genetic resistance is the most effective and sustainable tool to manage plant pathogens (Assefa et al., 2019). The potential to exploit resistance increases when the genetic control of the trait is well known, as well as its effects (Vollmann and Buerstmayr, 2016). For these reasons, and considering that the majority of anthracnose resistance genes described and mapped in common bean are from Mesoamerican gene pool, the efforts of the present work on characterization and mapping a new resistance alleles in the Andean cultivar BRSMG Realce should be of great interest to the bean research community worldwide.

The recent advances of genotyping by sequencing (GBS) methods resulted in the consequent development of high-density genetic maps using SNP markers. This approach allowed the identification of a large number of associations between genetic markers and genomic regions (major genes or QTLs), broadening the perspectives for marker-assisted selection (MAS) (Cobb et al., 2019). Just as examples, Berry et al. (2020) developed a linkage map for common bean containing 1,951 SNPs, with an average density of one marker every 0.52 cM and a total size of 1,011.7 cM, from a total of 48,244 SNPs and n =146 RILs. Almeida et al. (2021) used a population of 91 BC_2F_3 individuals and an initial set of 791,361 SNPs to develop a P. vulgaris genetic map with 1,091 markers and a total size of 1,923.16 cM, with an average distance between markers of 1.90 cM. In the present study, 13,083 SNPs were identified and a linkage map with 1,118 SNPs (n =161 F_2) was developed, with a total size of 4,473.4 cM and an average distance of 4.07 cM (**Supplementary Table 4**). However, it is important to highlight that in the present study only high quality not-distorted markers were used and that the markers' orders correlated well with their physical map positions (Spearman's coefficient > 99%) (**Supplementary Table 4**).

Table 4. Simple linear regression analysis between molecular markers (snp1327, snp12782, snp3308 and dart9817) flanking the genomic region of the major locus *Co-Realce* and the phenotype of F_2 (BRSMG Realce × BRS FC104) plants inoculated with the *C. lindemuthianum* pathotype 475.

Chr04_477285_snp1327[a]							
Source of variation	Df	SS	MS	F-value	p-value	R^2	Inclination[c]
Genotype	2	300.4	150.2	32.7	1.48E-12	0.29	-
TT vs CC[b]	1	203.5	203.5	44.33	4.68E-10	-	-0.13
CT vs CC	1	96.9	96.9	21.11	9.02E-06	-	-2.12
Residual	153	702.4	4.59	-	-	-	-
Total	155	1002.8	154.79	-	-	-	-
Chr04_1182123_snp12782							
Source of variation	Df	SS	MS	F-value	p-value	R^2	Inclination
Genotype	2	338.6	169.2	39.7	1.10E-14	0.33	-
CC vs TT	1	225.9	225.9	52.91	1.53E-11	-	-3.32
TC vs TT	1	112.8	112.8	26.42	8.02E-07	-	-2.24
Residual	158	674.5	4.27	-	-	-	-
Total	160	1013.1	173.47				
Chr04_505696_snp3308							
Source of variation	Df	SS	MS	F-value	p-value	R^2	Inclination
Genotype	2	381.5	190.8	47.1	< 2e-16	0.37	-
CC vs TT	1	250.8	250.8	61.97	5.64E-13	-	-3.53
TC vs TT	1	130.7	130.7	32.29	6.39E-08	-	-2.43
Residual	155	627.3	4.05	-	-	-	-
Total	157	1008.8	194.81				
Chr04_485246_dart9817							
Source of variation	Df	SS	MS	F-value	p-value	R^2	Inclination
1 vs 0	1	356.7	356.7	81.56	1.06E-15	0.36	-1.72
Residual	143	625.4	4.4	-	-	-	-
Total	144	982.1	361.1	-	-	-	-

[a]Df – degree of freedom, SS – sum of squares, MS – mean squares; the underline alleles are linked to disease resistance;
[b]Contrast considered in the regression analysis between marker alleles and the disease severity of *C. lindemuthianum* pathotype 475;
[c]Angular coefficient of the linear regression equation; the negative sign on the inclination score indicates that the allele is associated with disease resistance.

The resolution of a genetic map depends directly on the number of recombination events between the marker loci and potential target loci, what can be limited by the population size (Liu, 1998). In the perspective of value and usefulness for plant breeding, a low genetic distance could be redressed by the identification of markers flanking the target locus and explaining a significant part of the phenotypic variation (Ferreira et al., 2006). In this study, the initial genetic map built by linkage analysis shown a limitation of the population size to identify recombinant individuals, once the inclusion of *Co-Realce* locus inflated the genetic distances in its genomic region on Pv04. In addition, regarding the phenotypic data from the F_2 mapping population, the categorization of nine symptom-scores into only two phenotypic classes (1-to-3, resistance; and 4-to-9, susceptibility) may also explain the no precision in positioning *Co-Realce* locus in the initial linkage map. For these reasons, and considering that the *Co-Realce* locus segregates as a major gene (**Table 1**) and that is has shown a real value for the common bean breeding programs in Brazil, the QTL analysis was the approach used to map the major locus in the genomic region associated to anthracnose resistance and to identify useful SNP markers for MAS.

Using a panel of 189 common bean genotypes inoculated with the isolates Lv134 and Lv238 of the *C. lindemuthianum* pathotype 65, Costa et al. (2021) identified by association study two genomic regions on Pv04 related with the resistance to Lv134 and Lv238. The SNP marker ss715649771 (96,165 bp) associated with the resistance to Lv134 and explaining 64.4% of the phenotypic variation and ss715646893 (1,165,722 bp) associated with the resistance to Lv238 and explaining 72.2% of the phenotypic variation. Mungalu et al. (2020) also report a major QTL (ANT02.1^{UC,SA}) for anthracnose resistance on Pv02, which explained 79.0 and 76.8% of the phenotypic variation. In both cases, major loci for resistance to anthracnose were identified by mapping using quantitative approaches.

The major anthracnose resistance locus (*Co-Realce*) identified in BRSMG Realce on over an interval of 704,867 bp (477,217-to-1,182,084 bp) of the *P. vulgaris* chromosome Pv04 explained 54.6% of the total phenotypic variation (**Table 3**). For this reason, anthracnose resistance in BRSMG Realce should be considered as a major gene or complex gene locus for breeding purpose. It was also verified that *Co-Realce* segregates independently from *Co-3* (**Table 1**), the physically closest anthracnose resistance locus on Pv04. Still considering physical map evidences, the positions of *Co-3* (1,286,490 bp) (Murube et al., 2019), *Co-15* (9,432,376 bp) (Sousa et al., 2015) and *Co-16* (1,537,169 bp) (Coimbra-Gonçalves et al., 2016) on Pv04 shown that those anthracnose resistance loci are distant from *Co-Realce* by 780,839 bp, 8,926,725 bp and 1,031,518 bp, respectively (**Figure 3**). The locus *Co-3* is the physically closest to *Co-Realce* but allelism tests demonstrated that they are distinct and independent from each other (**Table 1**). This evidence also indicates that the physically more distant loci *Co-15* and *Co-16* are also distinct and independent of *Co-Realce* (**Figure 3**). These results corroborate the hypothesis that BRSMG Realce harbors a new anthracnose resistance locus on Pv04. As already reported by Souza et al. (2016) and Nay et al. (2019b), physical position analysis using information from molecular markers linked to known resistance genes and the reference genome sequence of *P. vulgaris* has been used as an additional criterion to support the characterization of new disease resistance loci in common bean, as for angular leaf spot caused by *Pseudocercospora griseola*. Other disease resistance genes have been mapped on Pv04, such as *Pse-6* for resistance to *Pseudomonas syringae*, *Ur-5* for resistance to *Uromyces appendiculatus*, *Phg-3* for resistance to *P. griseola*, and *Pm-2* for resistance to *Erysiphe difusa* (Pérez-Vega et al., 2013; Gonçalves-Vidigal et al.,

2013; Cabrera, 2020). Some of these genes were mapped close to the genomic position of *Co-Realce* on Pv04, showing that this region is an important gene cluster for the coevolution between *P. vulgaris* and some of its relevant pathogen species.

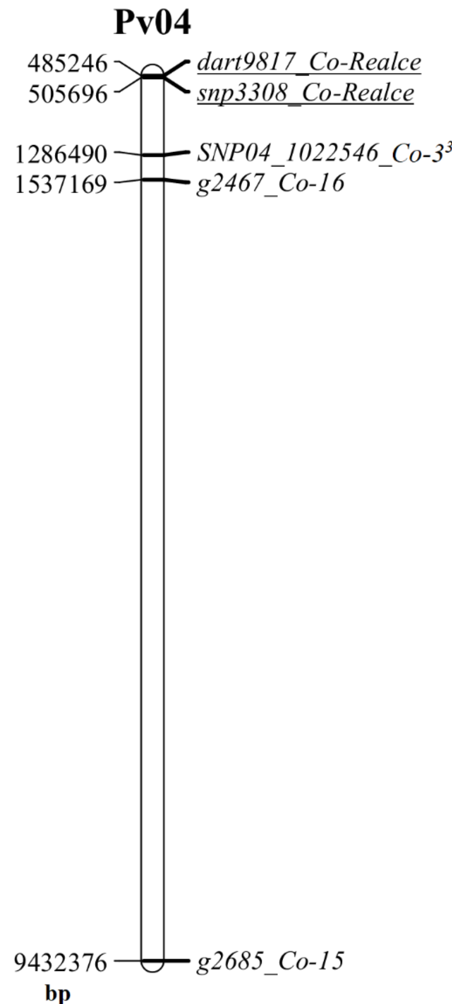

Figure 3. Physical map of the common bean chromosome Pv04 highlighting the location of the anthracnose resistance loci *Co-3*, *Co-15*, *Co-16* and *Co-Realce*, and their respective linked markers SNP04_1022546 (*Co-3^3*), g2685 (*Co-15*), g2467 (*Co-16*), dart9817 and snp3308 (*Co-Realce*). This physical map was built using the physical position of markers at the reference genome of *Phaseolus vulgaris* v2.1, available at www.phytozome.net (Paulino et al., 2022), using the software MapChart (Voorrips, 2002).

Forty-four candidate genes related to pathogen-host interaction were annotated on Co-Realce genomic region (**Supplementary Table 6**). Among these genes, it is important to highlight those associated with response mechanisms to pathogen attack, including immunological receptors (Bent and Mackey, 2007), cellular communication between cytoplasm and nucleus (Vidigal Filho et al., 2020; Zuiderveen et al., 2016), association with kinase receptors (Zhou, 2019), elicitor molecule recognition and degradation (Craig et al., 2009), post-transitional processing (Manna, 2015), phosphate transport (Dong et al., 2019), transcription regulation and translation (Woloshen et al., 2011; Grafi et al., 2007), and

extracellular pH modulation (Elmore and Coaker, 2011). There were also candidate genes that encode LRR proteins in different common bean chromosomes and that are associated with defense against fungi (Nabi et al., 2022; Mungalu et al., 2020; Nay et al., 2019a), bacteria (Wu et al., 2017) and virus (Seo et al., 2006). Furthermore, the upper portion of Pv04 contains a large cluster of resistance genes (Meziadi et al., 2016), over an interval of ~650 kb (from 345,784-to-993,499 bp) and including 28 genes related to resistance mechanisms in beans (Phytozome v11.0; *Phaseolus vulgaris* v2.1).

Three SNP markers linked to *Co-Realce* were identified by the QTL analysis (**Figure 2**). The snp12782 (position 1,182,123 pb) is positioned at around 5,164 pb from the Phvul.004G009500 gene (LRR), and the presence of the reference allele C (C/T) in homozygosis resulted in the selection of F_2 plants with an average score three times lower than that of plants without this allele ($p < 0.05$) (**Figure 2**). In addition, we assigned the markers snp1327 (position 477,285 pb) and dart9817 (position 485,246 pb) close to the Phvul.004G006800 gene region. This gene encodes the glycoprotein (NUP210) of the nuclear pore complex (NPC) and it has already been reported as associated with P. vulgaris resistance to anthracnose (Vidigal Filho et al., 2020; Shafi et al., 2022). It plays an important role in plant defense mechanisms, since they depend on the communication between the cytoplasm and the cell nucleus to be activated (Fang and Gu, 2021). NPC glycoproteins are necessary to make the nuclear envelope permeable to signaling macromolecules (Tamura and Hara-Nishimura, 2013). The snp3308 (position 505,696 pb) was mapped in the region of the Phvul.004G006900 (GAA1), which encodes the protein glycosylphosphatidylinositol transferase and helps recognize extracellular signals by associating with receptor-like kinases (Zhou, 2019). There are other candidate genes positioned in the *Co-Realce* genomic region, such as the Phvul.004G007600 and Phvul.004G009401 protein-encoding genes (RBP-RNA binding proteins) (**Supplementary Table 6**), essential to activate the defense response to pathogen attack in plants (Woloshen et al., 2011; Albà and Pagès, 1998). The main activities performed by RBP occur in the post-transcriptional processing of pre-RNA, and act to control splicing, polyadenylation of 3'extremity of RNA in the cap (modified guanine) added to the 5' extremity (Woloshen et al., 2011; Albà and Pagès, 1998). The Phvul.004G007600 gene is associated with P. vulgaris resistance to pathotype 6 of *Pseudomonas syringae* pv. *phaseolicola* (Tock et al., 2017). Recently, Vidigal Filho et al. (2020) identified the gene Phvul.004G020900, which encodes RBP associated with *P. vulgaris* resistance to anthracnose pathotype 65 (R^2=15%), corroborating the results of the present study.

The markers snp1327 (position 477,285 pb) and dart9817 explained 29 and 36% of phenotypic variation, respectively (**Table 4**). Selecting efficiency of the marker pairs snp1327/snp12782, snp1327/snp3308 and snp12782/snp3308 flanking the *Co-Realce* genomic region was 98.9%, 99.1% and 99.6%, respectively. This result support the high potential of theses markers for MAS of *Co-Realce* during its introgression in elite lines and cultivars (**Table 5; Supplementary Table 8**). They are already being used by the Embrapa common bean breeding program in an allele pyramiding approach aiming to stack *Co-Realce* and the Mesoamerican resistance allele *Co-4*^{2}, present in the SEL 1308 (**Supplementary Table 1**), in carioca seeded advanced lines. This breeding strategy aims to broadening the genetic resistance to anthracnose in the Brazilian common bean elite germplasm.

Table 5. Selection efficiency and recombination frequency of SNP markers positioned in the genomic interval of the major locus *Co-Realce*.

	snp1327	snp3308	snp10195	snp11894	snp12782	
snp1327	-	99.1	99.0	99.4	98.9	ES (%)[a]
snp3308	0.047	-	99.8	99.9	99.6	
snp10195	0.046	0.022	-	99.9	99.8	
snp11894	0.039	0.016	0.006	-	99.9	
snp12782	0.053	0.032	0.022	0.016	-	
rf (cM)[a]						

[a]rf – Recombination frequency; ES – Selection efficiency.

16 CONCLUSIONS

Results obtained by the present work from inheritance studies, allelism tests, genetic and physical mapping shown that anthracnose resistance in the Andean common bean cultivar BRSMG Realce is controlled by a major locus (or complex gene locus) on Pv04, which has been previously named as *Co-Realce*. SNP markers useful for marker-assisted selection have been identified as linked to the dominant allele of this locus, showing a selection efficiency higher than 99.0%. Allelism tests and physical mapping of *Co-Realce* genomic region on Pv04 support that *Co-Realce* is different from other major loci already mapped on this same chromosome (*Co-3*, *Co-15* and *Co-16*). The mapped genomic region included candidate genes related to pathogen-host interaction. Based on all these results and evidences, anthracnose resistance in BRSMG Realce should be considered as monogenic (major gene or complex gene locus) for breeding purpose. It is proposed that *Co-Realce* locus is unique and be officially named in accordance with the rules established by the Bean Improvement Cooperative Genetics Committee.

The cultivar BRSMG Realce is being already used by the Embrapa common bean breeding program as an anthracnose resistant donor parent from the Andean gene pool. This is because its resistance has shown to be stable and durable over time, even on final field trials conducted by the Embrapa in Brazil at least for the last 10 years. After the fully characterization of the anthracnose resistance in BRSMG Realce by the present work, this cultivar can now be used as a relevant donor source of an Andean resistance allele by common bean breeding programs worldwide, once it is already been successfully used for this propose in Brazil.

Data availability statement

The relevant datasets used to support the conclusions of this study can be found in the online repositories as supplementary material and under request to the corresponding author.

Author contributions

LMG-M, RPV, HSP, LCM, and TLPOS contributed to the conception and design of the study. LMG-M and LAR were in charge of laboratory analysis on DNA extraction and

samples preparation and shipment. LMG-M, GRM, and TLPOS carried out crosses, plant material development, and the phenotyping assays. LMG-M, ASGC, and TLPOS performed the statistical analysis and elaborated graphs and figures. RPV, HSP, LCM, and TLPOS contributed with research grant funding application and management. LMG-M and RVP wrote the first draft of the manuscript. LMG-M and TLPOS wrote the final version of the manuscript. All authors reviewed and contributed to the article, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

The supplementary material for this article can be found online.

17 REFERENCES

Aguiar, M.S., Pereira, H.S., Faria, L.C., Souza, T.L.P.O., Da Costa, J.G.C., Contadora, N.P.S. et al. (2021). BRS FS311: common bean cultivar with striped seed coat, high yield, and commercial quality. CBAB 21, e388121412. doi: 10.1590/1984-70332021v21n4c61

Albà, M.M. and Pagès, M. (1998). Plant proteins containing the RNA-recognition motif. Trends Plant Sci. 3, 15-21. doi: 10.1016/S1360-1385(97)01151-5

Almeida, C.P., De Carvalho Paulino, J.F., Bonfante, G.F.J., Perseguini, J.M.K.C., Santos, I.L., Gonçalves, J.G.R. et al. (2021). Angular leaf spot resistance loci associated with different plant growth stages in common bean. Front. Plant Sci. 12, 647043. doi:10.3389/fpls.2021.647043

Alvares, R.C., Stonehouse, R., Souza, T.L.P.O., Melo, P.G.S., Miklas, P.N., Bett, K.E. et al. (2019). Generation and validation of genetic markers for the selection of carioca dry bean

genotypes with the slow-darkening seed coat trait. Euphytica 215, 141. doi: 10.1007/s10681-019-2461-y

Assefa, T., Mahama, A., Brown, A.V., Cannon, E.K.S., Rubyogo, J.C., Rao, I.M., Blair, M.W., Cannon, S.B. (2019). A review of breeding objectives, genomic resources, and marker-assisted methods in common bean (Phaseolus vulgaris L.). Mol. Breed. 39, 1-23. doi: 10.1007/s11032-018-0920-0

Banoo, A., Nabi, A., Rasool, R.S., Mahiya-Farooq, Shah, M.D., Ahmad, M. et al. (2020). North-Western Himalayan common beans: population structure and mapping of quantitative anthracnose resistance through genome wide association study. Front. Plant Sci. 11, 571618. doi: 10.3389/fpls.2020.571618

Basavaraja, T., Pratap, A., Dubey, V., Gurumurthy, S., Gupta, S., Singh, N.P. (2020). “Molecular and conventional breeding strategies for improving biotic stress resistance in common bean”, in Accelerated Plant Breeding, eds. S. S. Gosal, S. H. Wani (3rd Springer Nature, Switzerland), 389-421.

Benjamini, Y. and Hochberg, Y. (1995). Controlling the False Discovery Rate: a practical and powerful approach to multiple testing. J. R. Statist. Soc. 57, 289-300. doi: 10.1111/j.2517-6161.1995.tb02031.x

Bent, A. F. and Mackey, D. (2007). Elicitors, Effectors, and R Genes: the new paradigm and a lifetime supply of questions. Annu. Rev. Phytopathol. 45, 399-436. doi: 10.1146/annurev.phyto.45.062806.094427

Berry, M., Izquierdo, P., Jeffery, H., Shaw, S., Nchimbi-Msolla, S., Cichy, K. (2020). QTL analysis of cooking time and quality traits in dry bean (Phaseolus vulgaris L.). Theor. Appl. Genet. 133, 2291-2305. doi:10.1007/s00122-020-03598-w

Cabrera, E.R.I. (2020). Mapping the Ur-5 gene conferring resistance to common bean rust. Master Dissertation submitted for the degree of Master of Sciences to Department of Plant Science, North Dakota State University, Fargo. pp. 55.

Churchill, G.A. and Doerge, R.W. (1994). Empirical threshold values for quantitative trait mapping. Genetics 138, 963-971. doi: 10.1093/genetics/138.3.963

Cobb, J.N., Biswas, P.S., Platten, J.D. (2019). Back to the future: revisiting MAS as a tool for modern plant breeding. Theor. Appl. Genet. 132, 647-667. doi: 10.1007/s00122-018-3266-4

Coimbra-Gonçalves, G.K., Gonçalves-Vidigal, M.C., Coelho, R.T., Valentini, G., Vidigal Filho, P.S., Lacanallo, G.F. (2016). Characterization and mapping of anthracnose resistance gene in mesoamerican common bean cultivar Crioulo 159. Crop Sci. 56, 2904-2915. doi: 10.2135/cropsci2015.10.0651

Costa, L.C., Nalin, R.S., Dias, M.A., Ferreira, M.E., Song, Q., Pastor-Corrales, M.A. et al. (2021). Different loci control resistance to different isolates of the same race of *Colletotrichum lindemuthianum* in common bean. Theor. Appl. Genet. 134, 543-556. doi: 10.1007/s00122-020-03713-x

Craig, A., Ewan, R., Mesmar, J., Gudipati, V., Sadanandom, A. (2009). E3 ubiquitin ligases and plant innate immunity. J. Exp. Bot. 60, 1123-1132. doi: 10.1093/jxb/erp059

Dong, Z., Li, W., Liu, J., Li, L., Pan, S., Liu, S. (2019). The rice phosphate transporter protein OsPT8 regulates disease resistance and plant growth. Sci. Rep. 9:5408. doi: 10.1038/s41598-019-41718-9

Elmore, J. M. and Coaker, G. (2011). The Role of the Plasma Membrane H1-ATPase in Plant–Microbe Interactions. Mol. Plant 4, 416-427. doi: 10.1093/mp/ssq083

Embrapa Rice and Beans (2022). Dados conjunturais da produção de feijão comum (*Phaseolus vulgaris* L.) e caupi (*Vigna unguiculata* (L.) Walp) no Brasil (1985 a 2020): área, produção e rendimento. http://www.cnpaf.embrapa.br/socioeconomia/index.htm. [Accessed August 7, 2022].

Fang, Y. and Gu, Y. (2021). Regulation of Plant Immunity by Nuclear Membrane-Associated Mechanisms. Front. Immunol. 12, 771065. doi: 10.3389/fimmu.2021.771065

FAO - Food and Agriculture Organization of the United Nations -Statistical Database (2022). Disponível em: http://faostat.fao.org/site/291/default.aspx. [Accessed June 14, 2022].

Ferreira, A., Silva, M.F., Costa e Silva, L., Cruz, C.D. (2006). Estimating the effects of population size and type on the accuracy of genetic maps. Genet. Mol. Biol. 29, 187-192. doi: 10.1590/S1415-47572006000100033

Ferreira, M. E. and Grattapaglia, D. (1998). Introdução ao uso de marcadores moleculares em análise genética. Brasília: Embrapa Cenargen, Documento 20.

Geffroy, V., Sévignac, M., Billant, P., Dron, M., Langin, T. (2008). Resistance to *Colletotrichum lindemuthianum* in Phaseolus vulgaris: a case study for mapping two independent genes. Theor. Appl. Genet. 116, 407-415. doi: 10.1007/s00122-007-0678-y

Gilio, T.A.S., Hurtado-Gonzales, O.P., Gonçalves-Vidigal, M.C., Valentini, G., Ferreira Elias, J.C, Song, Q. et al. (2020). Fine mapping of an anthracnose-resistance locus in Andean common bean cultivar Amendoim Cavalo. PLoS ONE 15, e0239763. doi: 10.1371/journal.pone.0239763

Gomes-Messias, L.M., Vianello, R.P., Monteiro-Júnior, J.P., Rodrigues, L.A., Mota, A.P.S., Pereira, H.S. et al. (2022). Molecular characterization of parental lines and validation of SNP markers for anthracnose and angular leaf spot in common bean. Euphytica 218, 49. doi: 10.1007/s10681-022-03002-2

Gonçalves-Vidigal, M.C., Cruz, A.S., Garcia, A., Kami, J., Vidigal Filho, P.S., Sousa, L.L., et al. (2011). 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. Theor. Appl. Genet. 122, 893-903. doi: 10.1007/s00122-010-1496-1

Gonçalves-Vidigal, M.C., Cruz, A.S., Lacanallo, G.F., Vidigal Filho, P.S., Sousa, L.L., Pacheco, C.M.N.A. et al. (2013). Co-segregation analysis and mapping of the anthracnose

Co-10 and angular leaf spot *Phg-ON* disease-resistance genes in the common bean cultivar Ouro Negro. Theor. Appl. Genet. 126, 2245-2255. doi: 10.1007/s00122-013-2131-8

Gonçalves-Vidigal, M.C., Gilio, T.A.S., Valentini, G., Vaz-Bisneta, M., Vidigal Filho, P.S., Song, Q. et al. (2020). New Andean source of resistance to anthracnose and angular leaf spot: Fine-mapping of disease-resistance genes in California Dark Red Kidney common bean cultivar. PLoS ONE 15, e0235215. doi: 10.1371/journal.pone.0235215

Gonçalves-Vidigal, M.C., Lacanallo, G.F., Vidigal Filho, P.S. (2008). A new gene conferring resistance to anthracnose in Andean common bean (*Phaseolus vulgaris* L.) cultivar 'Jalo Vermelho'. Plant Breed. 127, 592-596. doi: 10.1111/j.1439-0523.2008.01530.x

Gonçalves-Vidigal, M.C., Meirelles, A.C., Poletine, J.P., Sousa, L.L., Cruz, A.S., Nunes, M.P. et al. (2012). Genetic analysis of anthracnose resistance in 'Pitanga' dry bean cultivar. Plant Breed. 131, 423-429. doi: 10.1111/j.1439-0523.2011.01939.x

Gonçalves-Vidigal, M.C., Vidigal Filho, P.S., Medeiros, A.F., Pastor-Corrales, M.A. (2009). Common bean landrace Jalo Listras Pretas is the source of a new Andean anthracnose resistance gene. Crop Sci. 49, 133-138. doi: 10.2135/cropsci2008.01.0004

Gonçalves-Vidigal, M.C. and Kelly, J. D. (2006). Inheritance of anthracnose resistance in the common bean cultivar Widusa. Euphytica 151, 411-419. doi: 10.1007/s10681-006-9164-x

Grafi, G., Zemach, A., Pitto, L. (2007). Methyl-CpG-binding domain (MBD) proteins in plants. BBA 1769, 287-294. doi: 10.1016/j.bbaexp.2007.02.004

Hasan, N., Choudhary, S., Naaz, N., Sharma, N., Laskar, R.A. (2021). Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. JGEB 19:128 doi: 10.1186/s43141-021-00231-1

Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H. et al. (2012). "Diversity Arrays Technology: A generic genome profiling technology on open platforms", in Data Production and Analysis in Population Genomics: Methods and Protocols, Methods in Molecular Biology, eds. François Pompanon and Aurélie Bonin (New York: Springer Science+Business Media), 67-89.

Kiryowa, M., Nkalubo, S.T., Mukankusi, C., Male, A., Gibson, P., Tukamuhabwa, P. et al. (2021). Effectiveness of pyramided genes in conferring resistance to anthracnose disease in common bean populations. J. Plant Breed. Crop Sci. 13, 1-13. doi: 10.5897/JPBCS2019.0808

Kosambi, D.D. (1944). The Estimation of Map Distances from Recombination Values. Ann. of Eugen. 12, 172-175. doi: 10.1111/j.1469-1809.1943.tb02321.x

Lacanallo, G.F. and Gonçalves-Vidigal, M.C. (2015). Mapping of an Andean gene for anthracnose resistance (*Co-13*) in common bean (*Phaseolus vulgaris* L.) Jalo Listras Pretas landrace. AJCS 9, 394-400

Lima-Castro, S.A., Gonçalves-Vidigal, M.C., Gilio, T.A.S., Lacanallo, G.F., Valentini, G., Martins, V.S.R. et al. (2017). Genetics and mapping of a new anthracnose resistance locus in Andean common bean Paloma. BMC Genom. 18, 306. doi: 10.1186/s12864-017-3685-7

Liu, B.H. (1998). Statistical Genomics: Linkage, Mapping and QTL analysis. Boca Raton. CRC press.

Lobaton, J.D., Miller, T., Gil, J., Ariza, D., La Hoz, J.F., Soler, A. et al. (2018). Resequencing of common bean identifies regions of inter–gene pool introgression and provides comprehensive resources for molecular breeding. Plant Genome 11, 170068. doi: 10.3835/plantgenome2017.08.0068

Manna, S. (2015). An overview of pentatricopeptide repeat proteins and their applications. Biochimie 113, 93e99. doi: 10.1016/j.biochi.2015.04.004

Marcon, J.R.S., Gonçalves-Vidigal, M.C., Paulino, J.F.C., Vidigal Filho, P.S., Coêlho, M. (2021). Genetic resistance of common bean cultivar Beija Flor to *Colletotrichum lindemuthianum*. Acta Sci. Agron. 43, e44910. doi: 10.4025/actasciagron.v43i1.44910

Margarido, G.R.A., Souza A.P., Garcia A.A.F. (2007). OneMap: software for genetic mapping in outcrossing species. Hereditas 144, 78-79. doi: 10.1111/j.2007.0018-0661.02000.x

Melo, L.C., Abreu, Â.F.B., Ramalho, M.A.P., Carneiro, J.E.S., de Paula Júnior, T.J., Del Peloso, M.J. et al. (2014). BRSMG Realce: Common bean cultivar with striped grains for the state of Minas Gerais. CBAB 14, 61-64. doi: 10.1590/S1984-70332014000100010

Melo, L. C., Pereira, H. S., de Faria, L. C., Aguiar, M. S., da Costa, J. G. C., Wendland, A. et al. (2019). BRS FC104 – Super-early carioca seeded common bean cultivar with high yield potential. CBAB 19, 471-475. doi: 10.1590/1984-70332019v19n4c67

Melotto, M. and Kelly, J.D. (2000). An allelic series at the Co-1 locus conditioning resistance to anthracnose in common bean of Andean origin. Euphytica 116, 143-149. doi: 10.1023/A:1004005001049

Meziadi, C., Richard, M.M.S., Derquennes, A., Thareau, V., Blanchet, S., Gratias, A., Pflieger, S., Geffroy, V. (2016). Development of molecular markers linked to disease resistance genes in common bean based on whole genome sequence. Plant Sci. 242, 351-357. doi: 10.1016/j.plantsci.2015.09.006

Miklas, P.N., Fourie, D., Trapp, J., Davis, J., Myers, R. (2014). New loci including Pse-6 conferring resistance to halo bacterial blight on chromosome Pv04 in common bean. Crop Sci. 54, 2099-2108. doi: 10.2135/cropsci2014.03.0213

Miklas, P.N., Kelly, J.D., Beebe, S.E., Blair, M.W. (2006). Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. Euphytica 147, 105-131. doi: 10.1007/s10681-006-4600-5

Morais, S.R.P., Vieira, A.F., Almeida, L.C.S., Rodrigues, L.A., Melo, P.G.S., de Faria, L.C. et al. (2016). Application of microsatellite markers to confirm controlled crosses and assess

genetic identity in common bean. CBAB 16, 234-239. doi: 10.1590/1984-70332016v16n3n35

Mungalu, H., Sansala, M., Hamabwe, S., Mukuma, C., Gepts, P., Kelly, J.D. et al. (2020). Identification of race-specific quantitative trait loci for resistance to *Colletotrichum lindemuthianum* in an Andean population of common bean. Crop Sci. 60, 2843-2856. doi: 10.1002/csc2.20191

Murube, E., Campa, A., Ferreira, J.J. (2019). Integrating genetic and physical positions of the anthracnose resistance genes described in bean chromosomes Pv01 and Pv04. PLoS ONE 14, e0212298. doi: 10.1371/journal.pone.0212298

Nabi, A., Lateef, I., Nisa, Q., Banoo, A., Rasool, R.S., Shah, M.D., Ahmad, M., Padder, B.A. (2022). *Phaseolus vulgaris-Colletotrichum lindemuthianum* pathosystem in the post-genomic era: an update. Curr. Microbiol. 79, 36. doi: 10.1007/s00284-021-02711-6

Nay, M.M., Mukankusi, C.M., Studer, B., Raatz, B. (2019a). Haplotypes at the Phg-2 locus are determining pathotype-specificity of angular leaf spot resistance in common bean. Front. Plant Sci. 10, 1126. doi: 10.3389/fpls.2019.01126

Nay, M.M., Souza, T.L.P.O., Raatz, B., Mukankusi, C.M., Gonçalves-Vidigal, M.C., Abreu, A.F.B., Melo, L.C., Pastor-Corrales, M.A (2019b). A review of angular leaf spot resistance in common bean. Crop Sci. 59, 1376–1391.

Oblessuc, P.R., Baroni, R.M., Pereira, G. da Silva., Chiorato, A.F., Carbonell, S.A.M., Briñez, B. et al. (2014). Quantitative analysis of race-specific resistance to *Colletotrichum lindemuthianum* in common bean. Mol. Breeding 34, 1313-1329. doi: 10.1007/s11032-014-0118-z

Oblessuc, P.R., Francisco, C., Melotto, M. (2015). 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. Theor. Appl. Genet. 128, 1193-1208. doi: 10.1007/s00122-015-2500-6

Padder, B.A., Sharma, P.N., Awale, H.E., Kelly, J.D. (2017). Colletotrichum lindemuthianum, the causal agent of bean anthracnose. J. Plant Pathol. 99, 317-330. doi: 10.4454/jpp.v99i2.3867

Pastor-Corrales, M.A. (1992). La Antracnosis del fríjol común, Phaseolus vulgaris, en América Latina. Cali, CIAT Documento de trabajo n°113.

Pastor-Corrales, M.A., and Tu, J. C. (1989). “Anthracnose,” in Bean production problems in the tropics, eds. H. F. Schwartz and M. A. Pastor-Corrales. (Cali: CIAT), 77-104.

Paulino, P.P.S., Gonçalves-Vidigal, M. C., Vaz Bisneta, M., Vidigal Filho, P. S., Nunes, M.P.B.A., Xavier, L.F.S. et al. (2022). Occurrence of anthracnose pathogen races and resistance genes in common bean across 30 years in Brazil. ABS, 8, 1-21. doi: 10.33158/ASB.r140.v8.2022

Pereira, H.S., Faria, L.C., Wendland, A., Costa, J.G.C., Souza, T.L.P.O., Melo, L.C. (2018). Genotype by environment interaction for disease resistanc and other important agronomic

traits supporting the indication of common bean cultivars. Euphytica 214, 12. doi: 10.1007/s10681-017-2093-z

Pereira, H.S., Wendland, A., Melo, L.M., Del Peloso, M.J., Faria, L.C., Costa, J.G.C. et al. (2012). BRS Notável: a medium-early-maturing, disease-resistant Carioca common bean cultivar with high yield potential. CBAB 12, 220-223. doi: 10.1590/S1984-70332012000300010

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

R Core Team (2022). R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/ [Accessed March 10, 2022].

Sartorato, A., Del Peloso, M.J., Rava, C.A., da Costa, J.G.C., de Faria, L.C., Melo, L.C. (2004). Bean reactions to 24 pathotypes of *Colletotrichum lindemuthianum*. Annu. Rep. Bean Improv. Coop. 47, 247-248.

Schmutz, J., McClean, P.E., Mamidi, S., Wu, G.A., Cannon, S.B., Grimwood, J., 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

Seo, Young-Su, Rojas, M., Lee, Jung-Youn, Lee, Sang-Won, Jeon, Jong-Seong, Ronald, P. et al. (2006). A viral resistance gene from common bean functions across plant families and is up-regulated in a non-virus-specific manner. PNAS 103, 11856-11861. doi: 10.1073/pnas.0604815103

Shafi, S., Saini, D.K., Khan, M.A., Bawa, V., Choudhary, N., Dar, W.A., et al. (2022). Delineating meta-quantitative trait loci for anthracnose resistance in common bean (*Phaseolus vulgaris* L.). Front. Plant Sci. 13, 966339. doi: 10.3389/fpls.2022.966339

Silva, R.S., Faria, J.C., Knupp, A.M., Aguiar, M.S., Pereira, H.S., Ferreira, A.L. et al. (2022). Development and selection of transgenic advanced lines of carioca seeded common bean with multiple resistance to viruses. Euphytica 218, 67. doi: 10.1007/s10681-022-03017-9

Singh, S. P., and Schwartz, H.F. (2010). Breeding common bean for resistance to diseases: a review. Crop Sci. 50, 2199-2223. doi: 10.2135/cropsci2009.03.0163

Sousa, L.L., Gonçalves, A.O., Gonçalves-Vidigal, M.C., Lacanallo, G.F., Fernandez, A. C., Awale, H., Kelly, J.D. (2015). Genetic characterization and mapping of anthracnose resistance of common bean landrace cultivar Corinthiano. Crop Sci. 55, 1900-1910. doi: 10.2135/cropsci2014.09.0604

Souza, T.L.P.O., Faleiro, F.G., Dessaune, S.N., Paula-Júnior, T.J., Moreira, M.A., Barros, E.G. (2013). Breeding for common bean (*Phaseolus vulgaris* L.) rust resistance in Brazil. Trop. Plant Pathol. 38, 361-374. doi: 10.1590/S1982-56762013005000027

Souza, T.L.P.O., Gonçalves-Vidigal, M.C., Raatz, B., Mukankusi, C.M., Abreu, Â.F.B., Melo, L.C., Pastor-Corrales, M.A. (2016). Major loci controlling resistance to the angular leaf spot of common bean. Annu. Rep. Bean Improv. Coop. 59, xv–xviii.

Tamura, K. and Hara-Nishimura, I. (2013). The molecular architecture of the plant nuclear pore complex. J. Exp. Bot. 64, 695-709. doi: 10.1093/jxb/ers258

Tock, A.J., Fourie, D., Walley, P.G., Holub, E.B., Soler, A., Cichy, K.A. et al. (2017). Genome-wide linkage and association mapping of halo blight resistance in common bean to race 6 of the globally important bacterial pathogen. Front. Plant Sci. 8, 1170. doi: 10.3389/fpls.2017.01170

Valdisser, P.A.M.R., Müller, B.S.F., Almeida Filho, J.E., Morais Júnior, O.P., Guimarães, C.M., Borba, T.C.O. et al. (2020) Genome-wide association studies detect multiple QTLs for productivity in Mesoamerican diversity panel of common bean under drought stress. Front. Plant Sci. 11, 574674. doi: 10.3389/fpls.2020.574674

Vidigal Filho, P.S., Gonçalves-Vidigal, M.C., Bisneta, M.V., Souza, V.B., Gilio, T.A.S., Calvi, A.A. et al. (2020). Genome-wide association study of resistance to anthracnose and angular leaf spot in Brazilian Mesoamerican and Andean common bean cultivars. Crop Sci. 60, 2931-2950. doi: 10.1002/csc2.20308

Vollmann, J. and Buerstmayr, H. (2016). From phenotype to genotype: celebrating 150 years of Mendelian genetics in plant breeding research. Theor. Appl. Genet. 129, 2237-2239. doi: 10.1007/s00122-016-2817-9

Voorrips, R.E. (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Hered. 93, 77-78. doi: 10.1093/jhered/93.1.77

Wang, S., Basten, C.J., Zeng, Z.B. (2012). Windows QTL Cartographer 2.5. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm [Accessed March 29, 2022]

Woloshen, V., Huang, S., Li, X. (2011). RNA-Binding Proteins in Plant Immunity. J. Pathog. 2011, 278697. doi: 10.4061/2011/278697

Wu, J., Zhu, J., Wang, L., Wang, S. (2017). Genome-wide association study identifies NBS-LRR-encoding genes related with anthracnose and common bacterial blight in the common bean. Front. Plant Sci. 8, 1398. doi: 10.3389/fpls.2017.01398

Zeng, Z.B. (1993). Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. PNAS 90, 10972-10976. doi: 10.1073/pnas.90.23.10972

Zhou, K. (2019). Glycosylphosphatidylinositol-anchored proteins in Arabidopsis and one of their common roles in signaling transduction. Front. Plant Sci. 10:1022. doi: 10.3389/fpls.2019.01022

Zuiderveen, G.H., Padder, B.A., Kamfwa, K., Song, Q., Kelly, J.D. (2016). Genome-wide association study of anthracnose resistance in Andean Beans (*Phaseolus vulgaris*). PLoS ONE 11:e0156391. doi: 10.1371/journal.pone.0156391

18 SUPPLEMENTARY FILES

Supplementary Figure 1. Seeds of BRSMG Realce, an Andean common bean rajado (striped seed coat) seeded cultivar developed by Embrapa and partners in Brazil (Melo et al., 2014).

Supplementary Table 1. Parental and control common bean lines screened with seven different *Colletotrichum lindemuthianum* pathotypes.

Pathotype	Genotype	Grade scale[b]									NEP[c]	Mean score	Reaction class[d]
		1	2	3	4	5	6	7	8	9			
65	BRSMG Realce	12									12	1.0	R
73	BRSMG Realce	9									9	1.0	R
81	BRSMG Realce	11									11	1.0	R
91	BRSMG Realce	11									11	1.0	R
113	BRSMG Realce				5		3	2			10	5.2	S
475	BRSMG Realce	12									12	1.0	R
1609	BRSMG Realce	12									12	1.0	R
65	BRS FC104										0	-	-
73	BRS FC104	12									12	1.0	R
81	BRS FC104		1		5			2			8	4.5	S
91	BRS FC104				1			3	6		10	7.3	S
113	BRS FC104										0	-	-
475	BRS FC104									12	12	9.0	S
1609	BRS FC104	7								3	10	3.4	S
65	BRS Notável	12									12	1.0	R
73	BRS Notável	12									12	1.0	R
81	BRS Notável									12	12	9.0	S
91	BRS Notável	12									12	1.0	R
113	BRS Notável	12									12	1.0	R
475	BRS Notável	12									12	1.0	R
1609	BRS Notável	12									12	1.0	R
65	BAT 93	18									18	1.0	R
73	BAT 93									10	10	9.0	S
81	BAT 93										0	-	-
91	BAT 93									8	8	9.0	S
113	BAT 93	7									7	1.0	R
475	BAT 93									9	9	9.0	S
1609	BAT 93	16									16	1.0	R
65	SEL1308[a]	12									12	1.0	R
73	SEL1308	12									12	1.0	R
81	SEL1308	12									12	1.0	R
91	SEL1308	12									12	1.0	R
113	SEL1308	12									12	1.0	R
475	SEL1308	12									12	1.0	R
1609	SEL1308	12									12	1.0	R
65	IPA 7419[a]									12	12	9.0	S
73	IPA 7419									12	12	9.0	S
81	IPA 7419									12	12	9.0	S
91	IPA 7419									12	12	9.0	S
113	IPA 7419									12	12	9.0	S
475	IPA 7419									12	12	9.0	S
1609	IPA 7419									12	12	9.0	S

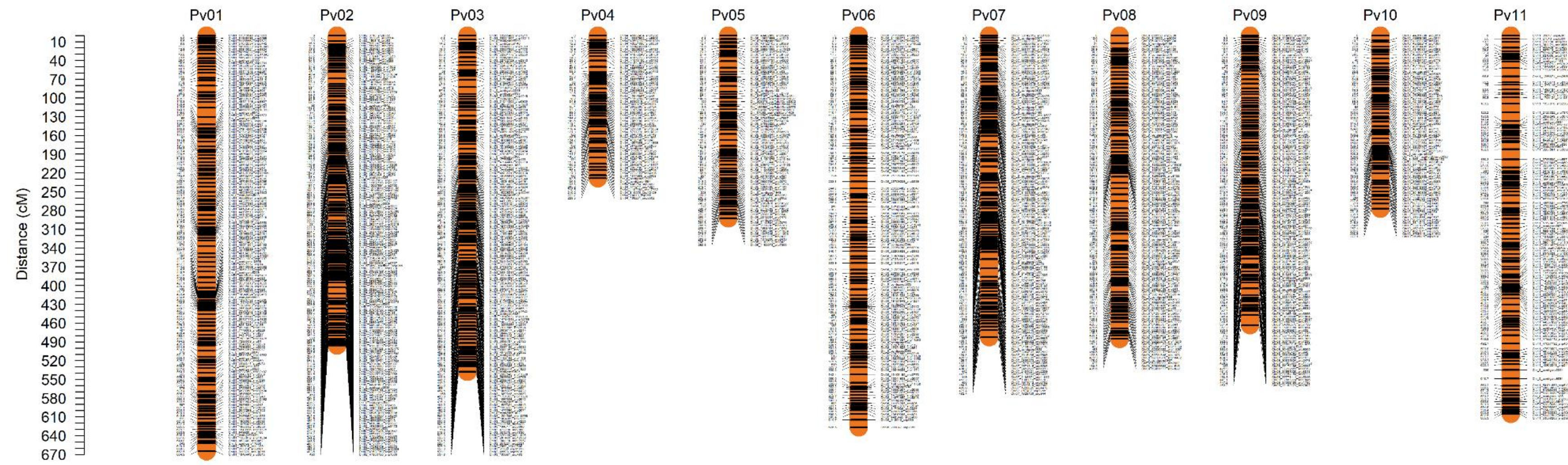
[a]SEL1308 – Resistant control line, and IPA 7419 – Susceptible control line.
[b]Number of plants evaluated as showing each one of the reaction scores from the 1-to-9 grade scale used for disease symptom screening; [c]NEP – Number of evaluated plants;
[d]R – Resistant (mean score 1.0-to-3.0), and S – Susceptible (mean score > 3.0).

Supplementary Table 2. Genotyping summary of the F_2 population derived from the cross BRSMG Realce × BRS FC104 with the DArTseq technology.

Marker type	Number of marker	Polymorphic markers	Monomorphic markers	Distorted markers	Undistorted markers
DArT	16186	11180	5006	5804	5376
SNP	13083	6304	6779	2129	4175
Total	29269	17484	11785	7933	9551

Supplementary Table 3. Genotyping summary by chromosome of the F_2 population derived from the cross BRSMG Realce × BRS FC104 with the DArTseq technology.

Chromosome	SNP	SNP–Call rate	DArT	DArT–Call rate	Total
Pv01	418	0.75 – 1.00	455	0.61 – 0.98	873
Pv02	457	0.89 – 1.00	473	0.58 – 0.99	930
Pv03	422	0.90 – 1.00	415	0.58 – 0.99	837
Pv04	160	0.81 – 1.00	282	0.58 – 1.00	442
Pv05	238	0.68 – 1.00	256	0.57 – 0.98	494
Pv06	355	0.84 – 1.00	383	0.58 – 0.99	738
Pv07	416	0.89 – 1.00	417	0.61 – 0.99	833
Pv08	345	0.86 – 1.00	408	0.56 – 0.99	753
Pv09	346	0.81 – 1.00	287	0.57 – 0.99	633
Pv10	224	0.88 – 1.00	315	0.58 – 0.99	539
Pv11	339	0.91 – 1.00	468	0.59 – 0.99	807
Scaffolds	60	0.81 – 1.00	61	0.65 – 0.99	121
Contigs	395	0.77 – 1.00	1156	0.56 – 0.99	1551
Total	4175	-	5376	-	9551



Supplementary Figure 2. Genetic map of the F_2 (BRSMG Realce × BRS FC104) population containing 1,118 SNP markers distributed across all 11 common bean chromosomes (Pv01-to-Pv11).

Supplementary Table 4. Summary of the genetic mapping for the F_2 population derived from the cross BRSMG Realce × BRS FC104 using SNP markers.

Chr[a]	SNPs[b]	SNPs[c]	SNPs[d]	SNPs[e]	Linkage map size (cM)[f]	Larger distance (cM)[g]	Minor distance (cM)[g]	Mean distance (cM)[g]	Distance ≤ 5 cM (%)[h]	ρ***
Pv01	417	457	153	126	561.32	14.69	0.62	4.49	70.6	0.998
Pv02	457	505	163	136	392.20	10.18	0.31	2.91	89.7	0.998
Pv03	422	459	153	131	471.00	12.04	0.61	3.62	87.8	0.999
Pv04	135	152	60	50	196.82	10.61	0.93	4.02	78.0	0.998
Pv05	233	286	77	64	263.64	10.32	1.55	4.18	81.3	0.999
Pv06	353	404	131	111	559.29	13.15	0.94	5.08	63.1	0.999
Pv07	416	464	131	118	434.73	14.85	0.62	3.72	83.1	0.999
Pv08	340	361	122	93	396.71	11.28	0.62	4.31	74.2	0.996
Pv09	345	382	128	118	415.01	11.21	0.31	3.55	86.4	0.999
Pv10	224	241	74	58	222.92	9.62	0.62	3.91	75.9	0.999
Pv11	338	363	123	113	559.80	16.06	0.31	5.00	69.0	0.998
Total	3680	4074	1315	1118	4473.44	-	-	-	-	-
Mean	335	370	120	102	406.68	12.18	0.68	4.07	78.09	0.999

[a]Chromosome/linkage group of common bean (*Phaseolus vulgaris*);
[b]Number of undistorted markers (Call rate ≥ 65% and Correction by FDR ≥ 5%), no scaffolds and contigs;
[c]Number of SNPs after obtaining the linkage groups, containing 57 scaffolds and 392 contigs;
[d]Number of SNPs in the SAFE map [R Software; OneMap package (Margarido et al., 2007; Core Team, 2022)], with LOD-score of 3.0;
[e]Number of SNPs of the SAFE map that approved by the "ripple_seq" function, with LOD-score of 3.0;
[f]Linkage map obtained by SAFE map and order confirmation with "ripple_seq" function, with LOD-score of 3.0;
[g]Larger, minor, and mean distance between markers;
[h]Percentage of distances values less than or equal to 5.0 cM;
***All Spearman's correlation coefficients (ρ) for order positions of the markers on linkage map and physical map were significant (P-value < $2.2e^{-16}$).

Supplementary Table 5*:* Pv01 – Pv11. Genetic map of 161 F_2 plants derived from the cross between BRSMG Realce and BRS FC104 with 1,315 SNP markers obtained after linkage analysis using 4,074 SNP markers. Accessory marks are underlined and in italics.

Pv01

Marker	cM	Marker	cM	Marker	cM	Marker	cM
Chr01_51319646_snp7653	0	Chr01_46298914_snp249	228.82	Chr01_12975738_snp5235	435.86	Chr01_949920_snp1160	633.86
Chr01_51335493_snp2599	3.47	Chr01_46198638_snp2340	235.98	Chr01_13124290_snp7495	436.48	Chr_0_contigsnp9391	636.06
Chr01_51155567_snp1571	6.62	Chr01_46176764_snp11666	241.74	Chr01_13856189_snp2787	437.72	Chr01_1507872_snp11034	637.61
Chr01_51072534_snp5442	8.81	Chr01_45530392_snp6714	250.51	Chr01_25539468_snp9779	440.23	Chr01_863349_snp8067	640.77
Chr01_50969279_snp10845	11.33	Chr01_45616931_snp1865	257.05	Chr01_33943508_snp3708	445.68	Chr_0_contigsnp11658	642.97
Chr01_50943621_snp3834	12.89	Chr01_45705456_snp8095	262.7	Chr01_32470646_snp8185	453.48	Chr01_150616_snp11851	648.08
Chr01_50768327_snp4175	17.21	Chr01_45392118_snp1806	265.32	Chr01_28356145_snp8551	459.25	Chr01_317314_snp3981	650.28
Chr01_50754297_snp11345	19.84	Chr01_45356215_snp845	269.61	Chr01_26157175_snp1828	464.36	Chr01_319379_snp10055	652.48
Chr01_50674178_snp6132	23.64	Chr01_44960090_snp2312	274.24	Chr01_10602118_snp1939	471.8	Chr01_1848449_snp3915	664.91
Chr_0_contigsnp6156	29.4	Chr01_45005462_snp1383	276.76	Chr01_9281889_snp1391	479.15	Number of markers: 153	
Chr01_50548065_snp4204	36.5	Chr_0_contigsnp4893	279.68	Chr01_8535427_snp6715	484.51	Log-likelihood: -7653.43	
Chr01_50533630_snp294	40.62	Chr01_45099937_snp3096	287.38	Chr01_7779363_snp9229	487.02		
Chr01_50357244_snp4374	44.1	Chr01_44386395_snp9412	294.83	Chr01_7764667_snp829	490.38		
Chr01_50111678_snp2597	49.55	Chr01_44027395_snp3666	300.94	Chr01_7724715_snp361	492.37		
Chr01_49980955_snp7738	54.68	Chr01_44146794_snp4425	306.72	Chr01_7501367_snp11475	493.3		
Chr01_50017716_snp2814	59.8	Chr01_44125479_snp3011	309.55	Chr01_7288569_snp81	494.23		
Chr01_49834315_snp1816	67.95	Chr01_44156510_snp861	310.79	Chr01_6946328_snp2678	496.11		
Chr01_49809393_snp2876	71.76	Chr01_44146728_snp451	312.35	Chr01_7693580_snp2252	504.27		
Chr01_49613751_snp2970	80.34	Chr01_44032225_snp9218	314.55	Chr01_6550261_snp3217	514.08		
Chr01_49544474_snp7543	91.05	Chr_0_contigsnp5190	316.42	Chr_0_contigsnp9647	519		
Chr01_49468979_snp366	95.45	Chr01_43426547_snp8858	317.67	Chr01_6510426_snp11563	521.2		
Chr01_49263580_snp4498	98.29	Chr01_43500262_snp6638	319.55	Chr_0_contigsnp5060	523.08		
Chr01_48986494_snp3774	101.46	Chr01_43118244_snp1134	324.01	Chr01_6454578_snp959	526.88		
Chr01_48986219_snp783	106.7	Chr01_43047735_snp2904	328.46	Chr01_5998571_snp4615	531.99		
Chr01_49171149_snp9907	115.77	Chr01_43495852_snp2181	337.26	Chr_0_contigsnp1837	539.81		
Chr01_48533615_snp6543	130.45	Chr01_42638915_snp3948	348.85	Chr_0_contigsnp6320	547.63		
Chr01_48536080_snp8461	142.48	Chr01_42688852_snp860	354.77	Chr01_4737353_snp12482	550.15		
Chr01_48662190_snp10333	148.08	Chr01_40083402_snp301	362.07	Chr01_5045781_snp6908	552.99		
Chr_0_contigsnp9042	149.64	Chr01_40571204_snp6452	370.05	Chr01_5003367_snp802	559.06		
Chr01_48273485_snp1573	152.8	Chr01_41132335_snp7606	377.33	Chr01_4499632_snp1095	569.49		
Chr_0_contigsnp5675	155	Chr01_40486597_snp813	387.17	Chr01_4327402_snp2721	574.88		
Chr01_48442697_snp8390	156.56	Chr01_40194336_snp4262	395.29	Chr01_4085905_snp1963	576.13		
Chr01_48184176_snp57	159.72	Chr01_42105654_snp11824	403.97	Chr01_3725663_snp3043	578.96		
Chr01_47956315_snp1013	161.91	Chr01_42311904_snp9797	409.55	Chr01_4629368_snp10698	583.42		
Chr01_47914060_snp2329	163.8	Chr_0_contigsnp10959	411.43	Chr01_3516606_snp422	585.3		
Chr01_47721498_snp1848	169.78	Chr01_41672357_snp14	412.67	Chr01_3363630_snp7249	585.92		
Chr01_47645324_snp3101	177.74	Chr01_39446069_snp5092	414.23	Chr01_3286701_snp12192	588.12		
Chr01_47590317_snp325	182.98	Chr01_38591349_snp6097	415.48	Chr01_3070504_snp283	593.55		
Chr01_47459138_snp364	184.86	Chr_0_contigsnp5229	416.71	Chr01_2989684_snp318	597.03		
Chr01_47407597_snp7063	186.74	Chr01_40066268_snp5413	417.64	Chr01_2853275_snp3890	601.15		
Chr01_47353925_snp957	191.52	Chr01_38612096_snp583	418.89	Chr01_2647299_snp7102	606.1		
Chr01_47368713_snp3993	196.95	Chr01_36985483_snp9795	423.67	Chr01_2478748_snp70	608.14		
Chr01_47118158_snp5654	203.38	Chr01_35315019_snp1859	427.15	Chr01_2489092_snp1886	612.92		
Chr01_46950008_snp701	208.49	Chr01_36805824_snp1974	428.39	Chr01_2220386_snp8198	619.02		
Chr01_46854453_snp1881	212.95	Chr01_34154068_snp622	429.95	Chr01_1963054_snp5538	621.22		
Chr01_46736597_snp3932	216.1	Chr01_33455833_snp1644	431.82	Chr01_1895407_snp6063	622.15		
Chr01_46617378_snp9211	220.56	Chr01_18948524_snp9413	433.38	Chr01_1860592_snp8259	625.29		
Chr01_46588920_snp1687	223.72	Chr_0_contigsnp5580	434.62	Chr01_1649996_snp11175	629.74		

Pv02							
Marker	cM	Marker	cM	Marker	cM	Marker	cM
Chr02_9710_snp8762	0	Chr02_4016866_snp97	172.16	Chr02_35437880_snp2163	310.58	Chr02_44911687_snp1336	436.65
Chr02_327825_snp6564	9.18	Chr02_4121766_snp5668	175.06	Chr02_35437243_snp5001	314.18	Chr02_45239350_snp881	444.81
Chr02_196816_snp86	13.96	Chr02_4318361_snp10865	176.94	Chr02_35927110_snp951	316.05	Chr02_45461099_snp3006	448.61
Chr02_520142_snp6074	16.8	Chr02_4848053_snp4493	178.49	Chr02_36109252_snp7345	317.28	Chr02_45786966_snp8246	451.45
Chr02_564543_snp2954	18.36	Chr02_4998965_snp10354	180.04	Chr02_36251686_snp489	319.16	Chr02_45688984_snp887	454.92
Chr02_659621_snp4914	19.29	Chr02_6243785_snp6626	182.56	Chr02_36267632_snp3879	322.95	Chr02_45960693_snp443	460.35
Chr02_675420_snp9533	20.85	Chr02_8060664_snp8214	185.07	Chr02_36353459_snp2569	326.74	Chr02_46100837_snp1537	463.83
Chr02_701428_snp2922	22.72	Chr_0_contigsnp329	188.22	Chr02_36867708_snp12078	328.29	Chr02_46352555_snp8472	467.78
Chr02_778487_snp11215	25.24	Chr02_11637097_snp3303	192.99	Chr_0_contigsnp8863	329.85	Chr_0_contigsnp880	472.07
Chr02_819644_snp1360	27.75	Chr02_13197515_snp5337	196.14	Chr02_37263069_snp5780	332.04	Chr02_46482486_snp3642	473.94
Chr02_993411_snp3098	30.26	Chr02_13667991_snp2758	197.7	Chr02_37616750_snp2542	333.91	Chr02_46644130_snp01	475.19
Chr_0_contigsnp6647	33.73	Chr02_13979814_snp11198	198.95	Chr02_38213716_snp738	336.1	Chr02_47006480_snp5689	476.44
Chr02_1293620_snp1902	37.21	Chr02_15945940_snp10090	200.19	Chr02_38245936_snp3884	337.66	Chr02_46967335_snp2894	479.91
Chr02_1335823_snp1738	40.36	Chr02_17399294_snp8301	201.74	Chr02_38571492_snp1088	339.21	Chr02_47168978_snp3970	485.34
Chr02_1567497_snp4221	45.14	Chr02_20811480_snp10934	203.29	Chr02_38574796_snp6828	340.77	Chr02_47253391_snp9777	488.17
Chr02_1585045_snp11834	46.39	Chr02_21012633_snp2786	204.22	Chr02_39954672_snp5170	342.01	Chr02_47292159_snp8270	490.36
Chr02_1819842_snp1294	48.9	Chr_0_contigsnp1129	206.1	Chr02_40638628_snp680	345.8	Chr02_47505855_snp12289	492.56
Chr_0_contigsnp6264	52.06	Chr_0_contigsnp5669	209.89	Chr02_40993346_snp226	349.26	Chr02_47631763_snp6843	493.58
Chr02_1895441_snp9845	55.86	Chr02_22526764_snp1541	214.65	Chr02_41265906_snp9072	351.46	Chr02_47806195_snp1509	496.01
Chr02_1896774_snp9634	58.69	Chr02_22527573_snp1475	219.42	Chr02_41365511_snp3920	351.77	Number of markers: 163	
Chr02_2238165_snp473	69.12	Chr02_23578888_snp10008	222.9	Chr02_41024821_snp3389	352.37	Log-likelihood: -6390.34	
Chr02_2326582_snp3471	78.13	Chr02_27017830_snp4495	225.09	Chr02_41419790_snp7167	352.97		
Chr02_2444255_snp10776	81.61	Chr02_26077828_snp2195	227.6	Chr02_41606433_snp7184	353.9		
Chr02_2524596_snp176	84.76	Chr02_28434590_snp9205	228.53	Chr02_41749615_snp2390	355.14		
Chr02_2684216_snp7526	88.89	Chr02_29411172_snp10823	229.46	Chr02_41808275_snp5106	356.38		
Chr02_2639311_snp1087	92.68	Chr02_25217107_snp10219	231.01	Chr02_41797128_snp10476	357.93		
Chr02_2926069_snp3627	96.16	Chr02_24858855_snp2529	231.95	Chr02_42073091_snp236	359.17		
Chr_0_contigsnp4942	98.04	Chr02_25166689_snp1758	232.87	Chr02_42209801_snp11588	360.73		
Chr02_2968517_snp9080	103.8	Chr02_25847686_snp10444	234.11	Chr02_42248991_snp8324	362.92		
Chr02_3205689_snp3559	110.89	Chr02_24949841_snp7525	234.73	Chr02_42558520_snp1565	367.69		
Chr02_3408014_snp3349	115.02	Chr02_26728133_snp3727	237.24	Chr02_42618714_snp11285	371.8		
Chr02_3468437_snp956	117.21	Chr02_26291192_snp863	242.83	Chr02_42963805_snp6902	373.37		
Chr02_3518768_snp4424	119.4	Chr02_28954170_snp2748	248.76	Chr02_43129560_snp999	376.83		
Chr02_3791263_snp390	123.85	Chr02_30866789_snp3867	253.21	Chr02_43317800_snp307	379.66		
Chr02_3663161_snp5989	128.95	Chr_0_contigsnp10972	255.4	Chr02_43438494_snp4337	380.29		
Chr02_3653697_snp8617	132.1	Chr02_30320681_snp10338	256.34	Chr02_43476736_snp3765	381.53		
Chr02_3629044_snp5457	133.66	Chr02_26927596_snp4058	260.13	Chr02_43484760_snp5808	383.4		
Chr02_3664843_snp11042	134.88	Chr02_31555333_snp938	266.54	Chr02_43885285_snp5133	386.23		
Chr02_3664978_snp8623	138.49	Chr02_31437474_snp6485	270.66	Chr02_43921025_snp1309	388.11		
Chr02_3902734_snp3552	143.45	Chr_0_contigsnp539	272.86	Chr02_44241809_snp230	389.67		
Chr02_3926849_snp15	146.59	Chr02_32438328_snp6727	276.65	Chr_0_contigsnp3538	391.54		
Chr02_4017902_snp2742	147.84	Chr_588658_scaffold17snp365	279.8	Chr02_44236738_snp7524	395.34		
Chr02_4422253_snp1773	149.41	Chr02_33577531_snp1314	283.27	Chr02_43011207_snp4103	402.78		
Chr02_4359288_snp3343	152.24	Chr02_33555129_snp1253	288.37	Chr02_44376116_snp4349	412.96		
Chr02_4294077_snp1594	156.68	Chr02_34257163_snp2980	296.14	Chr02_44712141_snp8927	420.39		
Chr02_4421074_snp1528	160.48	Chr02_34721554_snp6474	300.26	Chr02_44584478_snp827	424.19		
Chr02_4381330_snp2182	162.84	Chr02_35024902_snp879	302.14	Chr_0_contigsnp9900	426.71		
Chr02_4878091_snp322	167.26	Chr02_35387994_snp2441	305.29	Chr02_44885258_snp455	429.86		

Pv03

Marker	cM	Marker	cM	Marker	cM	Marker	cM
Chr03_53265486_snp1215	0	Chr03_47731336_snp2609	197.09	Chr03_31428865_snp3621	335.3	Chr03_551275_snp7836	518.5
Chr03_53221007_snp11611	6.83	Chr03_47235273_snp10264	200.43	Chr03_31414609_snp2877	343.83	Chr03_429448_snp11035	522.3
Chr_0_contigsnp5379	11.96	Chr03_46835319_snp9108	202.31	Chr03_31373502_snp7159	350.76	Chr03_287463_snp12959	523.54
Chr_0_contigsnp9146	14.16	Chr03_46466519_snp9891	203.87	Chr03_30830631_snp6511	356.18	Chr03_380818_snp12957	524.46
Chr03_53146273_snp1353	17	Chr03_46531159_snp1340	204.8	Chr03_30047024_snp1425	361.6	Chr03_351221_snp2521	525.08
Chr03_53067056_snp7776	18.57	Chr03_46091559_snp4491	206.68	Chr_0_contigsnp8894	363.16	Chr03_7958_snp12606	526.33
Chr03_52984501_snp4388	20.77	Chr03_46034471_snp6666	212.11	Chr03_13549977_snp2189	364.71	Chr03_31398_snp11317	527.26
Chr03_52961178_snp9256	23.29	Chr03_45813897_snp1264	216.89	Chr03_14734313_snp12759	365.64	Chr03_45500_snp5516	529.14
Chr_0_contigsnp9337	27.38	Chr03_45065289_snp432	220.04	Chr03_10077154_snp9910	366.88	Chr03_183997_snp493	537.82
Chr03_52702729_snp2343	34.83	Chr03_45229213_snp10044	222.55	Chr03_10856297_snp816	369.19	Number of markers: 153	
Chr03_52495203_snp195	42.27	Chr_0_contigsnp6120	223.48	Chr03_7930606_snp2624	372.85	Log-likelihood: -6669.11	
Chr03_52472749_snp634	47.39	Chr03_44548545_snp10667	225.03	Chr03_6909717_snp2214	376.32		
Chr03_52145937_snp384	55.55	Chr03_44139607_snp2295	226.59	Chr03_6844720_snp2307	377.26		
Chr03_52030188_snp7383	59.04	Chr03_44105124_snp8304	228.78	Chr03_6511010_snp902	380.41		
Chr03_51877763_snp1480	60.6	Chr03_43878139_snp9645	233.55	Chr03_6080420_snp7861	384.85		
Chr03_51811604_snp5320	62.48	Chr03_43524101_snp8157	238.99	Chr03_5998787_snp2135	389.61		
Chr03_51719901_snp2231	65.31	Chr03_43360545_snp4006	241.82	Chr_0_contigsnp5349	392.76		
Chr03_51823471_snp512	70.73	Chr03_43319670_snp228	243.07	Chr03_5037106_snp3632	395.91		
Chr03_51620208_snp4665	82.73	Chr03_43026312_snp5698	245.26	Chr03_4980357_snp1234	398.42		
Chr03_51348127_snp1513	94.02	Chr03_42598487_snp11452	247.14	Chr03_4973638_snp2032	402.22		
Chr_0_contigsnp6319	97.83	Chr03_42598270_snp5273	249.01	Chr_0_contigsnp7100	407.65		
Chr_0_contigsnp8933	101.97	Chr03_42147039_snp10385	251.52	Chr03_4152102_snp1108	413.75		
Chr03_51062254_snp1249	106.42	Chr03_42003787_snp3315	254.68	Chr03_3971566_snp1973	417.54		
Chr_0_contigsnp2227	107.99	Chr03_42057446_snp8477	258.15	Chr03_3897839_snp818	420.06		
Chr03_51044306_snp6950	109.55	Chr03_41564994_snp8028	262.91	Chr03_3329365_snp2535	429.92		
Chr03_50879132_snp7903	112.78	Chr03_40750245_snp1085	268.01	Chr03_3352414_snp1925	435.01		
Chr03_50769029_snp639	114.74	Chr03_40408283_snp3388	272.12	Chr_0_contigsnp8525	437.52		
Chr03_50660548_snp6550	119.53	Chr03_40356462_snp973	275.6	Chr03_2900943_snp11560	439.08		
Chr03_50707315_snp9774	128.67	Chr03_40098967_snp4434	278.43	Chr03_2939382_snp3769	441.27		
Chr03_50352208_snp12145	135.94	Chr03_39233072_snp3858	282.54	Chr03_2698447_snp8207	444.74		
Chr03_50345414_snp2483	137.5	Chr03_39619999_snp2025	284.73	Chr03_2532833_snp3603	449.19		
Chr03_50174945_snp9712	139.07	Chr03_39742605_snp4260	287.56	Chr03_2390315_snp1282	453.63		
Chr03_50262586_snp6101	142.22	Chr03_39585262_snp333	290.07	Chr03_1978565_snp6601	459.07		
Chr03_49799440_snp3794	147.99	Chr03_39534586_snp11111	291	Chr03_1886025_snp4430	464.51		
Chr03_49511161_snp12260	153.76	Chr03_38113425_snp3193	292.53	Chr03_1862048_snp3880	468.96		
Chr03_49651386_snp435	156.28	Chr03_37393613_snp10866	294.09	Chr03_1751953_snp11506	469.9		
Chr_0_contigsnp3066	158.8	Chr03_37085764_snp753	295.96	Chr03_1697389_snp2540	471.15		
Chr03_49333395_snp3323	161.63	Chr03_36500271_snp8579	300.08	Chr_0_contigsnp10814	476.26		
Chr03_49159615_snp5181	163.19	Chr03_36342215_snp9024	303.23	Chr03_1587024_snp6940	481.21		
Chr03_48622879_snp5427	164.75	Chr03_35953876_snp11504	305.11	Chr03_1509223_snp6609	485.81		
Chr03_48728528_snp5020	166.63	Chr03_35334891_snp692	306.66	Chr03_1477596_snp2571	492.23		
Chr03_48482765_snp6865	169.46	Chr03_35203719_snp3570	309.6	Chr_0_contigsnp10921	497.66		
Chr03_48346852_snp2464	173.59	Chr03_33540606_snp8379	315.24	Chr03_1204606_snp10899	500.18		
Chr03_47836777_snp4113	181.7	Chr03_34376352_snp466	320.01	Chr03_1004069_snp7951	503.65		
Chr_0_contigsnp5090	189.12	Chr03_34480110_snp1057	322.84	Chr03_1005303_snp4225	505.21		
Chr03_47766961_snp138	192.59	Chr03_32121844_snp9607	325.67	Chr03_812065_snp6429	507.41		
Chr03_47944812_snp2710	193.52	Chr03_32669346_snp6491	327.86	Chr03_104017_snp3686	510.39		
Chr_0_contigsnp10991	194.77	Chr03_32329192_snp2077	330.06	Chr03_472230_snp7000	514.05		

Pv04

Marker	cM	Marker	cM
Chr04_45082434_snp5292	0	Chr04_2395185_snp12013	169.05
Chr04_43887952_snp9908	7.14	Chr04_2327020_snp9746	170.3
Chr04_43597198_snp11931	11.26	Chr04_2272161_snp4396	171.86
Chr04_43521680_snp6050	12.19	Chr04_1617988_snp6408	180.01
Chr_0_contigsnp6079	14.07	Chr_0_contigsnp12170	183.49
Chr04_42229881_snp6436	16.89	Chr_0_contigsnp4594	186.33
Chr04_42159718_snp11301	18.45	Chr04_486794_snp2492	195.48
Chr04_41878249_snp8700	20.96	Chr04_477217_snp1327	205.22
Chr04_41857223_snp5563	24.75	Chr04_1182084_snp12782	210.47
Chr04_41573180_snp3736	28.54	Chr04_816576_snp12129	215.25
Chr04_41631087_snp4347	33.3	Chr04_78013_snp266	223.74
Chr04_18562099_snp1725	40.54	Chr04_108201_snp3562	228.54
Chr04_39975280_snp4258	50.31	Number of markers: 60	
Chr04_40380695_snp1473	58.46	Log-likelihood: -2839.12	
Chr_0_contigsnp10772	60.97		
Chr04_39379369_snp3990	62.22		
Chr04_39377577_snp5662	63.15		
Chr04_39384063_snp2818	65.02		
Chr04_37552665_snp1405	66.57		
Chr04_35472743_snp3250	68.45		
Chr04_20589888_snp2471	71.28		
Chr04_32571940_snp158	73.46		
Chr04_12046097_snp45	75.98		
Chr04_11692988_snp4154	78.25		
Chr04_9336619_snp11473	84.91		
Chr04_9010476_snp5284	91.99		
Chr04_9032609_snp9134	95.14		
Chr04_8785208_snp8342	97.34		
Chr04_8655981_snp4961	99.53		
Chr04_8471848_snp1858	101.4		
Chr04_8250605_snp6634	104.87		
Chr04_7069188_snp11912	109.63		
Chr04_5988895_snp316	113.1		
Chr04_5362219_snp8266	115.61		
Chr04_5299507_snp12074	118.44		
Chr04_5211278_snp10827	121.26		
Chr04_5045180_snp5743	125.05		
Chr04_5016343_snp1998	128.51		
Chr04_4128140_snp847	134.25		
Chr04_4257359_snp11939	136.44		
Chr04_3812634_snp5855	138.01		
Chr_0_contigsnp10825	139.57		
Chr04_3196575_snp9526	140.81		
Chr04_3200604_snp7950	141.74		
Chr04_3454386_snp10344	145.53		
Chr04_2860174_snp768	153.97		
Chr04_2862128_snp11182	157.44		
Chr04_2546876_snp10177	165.24		

Pv05

Marker	cM	Marker	cM
Chr05_40482868_snp9248	0	Chr05_11079666_snp982	191.83
Chr05_40551999_snp1012	5.79	Chr05_11806620_snp5866	193.08
Chr_0_contigsnp10980	10.24	Chr05_11216880_snp2375	196.55
Chr05_40722165_snp8389	12.12	Chr05_23187363_snp424	200.99
Chr05_40060828_snp1812	16.58	Chr05_4843403_snp2968	207.07
Chr05_39934393_snp11333	19.09	Chr05_5980766_snp2872	213.15
Chr05_39721415_snp7547	24.2	Chr05_4432973_snp11628	216.62
Chr05_39720871_snp8759	27.35	Chr05_4081795_snp1144	219.13
Chr05_39672766_snp8015	29.23	Chr05_3949464_snp4150	223.24
Chr05_39566784_snp4356	31.12	Chr_0_contigsnp4949	228.01
Chr05_39441198_snp7250	33.77	Chr05_3462666_snp2262	231.16
Chr05_39555089_snp7675	41.29	Chr05_3273616_snp9962	235.28
Chr05_39330832_snp7624	51.62	Chr_0_contigsnp5112	239.72
Chr05_39259263_snp63	58.56	Chr05_2962470_snp10322	243.2
Chr05_38996140_snp2357	62.36	Chr05_2916711_snp8857	246.99
Chr05_38884894_snp3404	66.82	Chr05_2778002_snp10038	248.87
Chr05_38730524_snp3730	69.33	Chr05_2729126_snp10597	250.42
Chr05_38649942_snp8973	70.89	Chr05_2616136_snp1648	253.24
Chr05_38627819_snp2391	73.4	Chr05_2512054_snp7470	256.39
Chr05_38611717_snp929	80.87	Chr_0_contigsnp9350	259.54
Chr_0_contigsnp438	90.68	Chr05_2110904_snp2875	264.3
Chr05_38418717_snp2779	93.69	Chr05_2111203_snp5789	266.5
Chr_627467_scaffold15snp1811	97.4	Chr05_1958484_snp1646	268.38
Chr_496001_scaffold15snp3827	100.56	Chr05_1808588_snp11804	270.57
Chr_358373_scaffold15snp3302	105.99	Chr05_1647245_snp11803	273.72
Chr_277619_scaffold15snp9390	110.43	Chr05_1419119_snp2020	279.13
Chr_198596_scaffold15snp3576	113.58	Chr05_1313266_snp3415	283.25
Chr_204500_scaffold15snp609	118.02	Chr05_1184014_snp892	286.08
Chr_67098_scaffold30snp1459	122.79	Chr05_1152673_snp7366	292.31
Chr_137592_scaffold30snp4990	124.35	Number of markers: 77	
Chr05_37885286_snp7987	126.54	Log-likelihood: -3662.73	
Chr05_37844659_snp4394	128.1		
Chr05_37885220_snp851	129.98		
Chr_0_contigsnp9074	133.13		
Chr_0_contigsnp11291	137.9		
Chr05_37387196_snp6579	141.7		
Chr05_36905647_snp10646	144.21		
Chr05_36668913_snp3239	146.72		
Chr05_36687744_snp172	150.84		
Chr05_35160913_snp1453	157.61		
Chr05_36108500_snp5873	167.16		
Chr05_12163460_snp1390	176.71		
Chr05_14927401_snp1579	182.14		
Chr05_25758821_snp2301	184.65		
Chr05_31230288_snp5371	185.9		
Chr05_31191876_snp11464	186.83		
Chr05_32733448_snp7877	189.33		
Chr05_30970177_snp11463	191.21		

Pv06

Marker	cM	Marker	cM	Marker	cM
Chr06_31196836_snp5025	0	Chr06_25851183_snp2114	217.41	Chr06_18301825_snp7401	457.99
Chr06_31133671_snp6875	1.88	Chr06_25736784_snp1945	224.02	Chr06_18280187_snp6608	461.47
Chr06_30877454_snp4454	4.07	Chr06_25709289_snp8539	233.83	Chr06_18014473_snp203	470.51
Chr06_30759777_snp10022	6.26	Chr06_25529482_snp3647	244.81	Chr06_17934267_snp2298	477.94
Chr_0_contigsnp5736	8.45	Chr06_25613118_snp5802	253.67	Chr06_17514588_snp2744	487.3
Chr06_30473371_snp2951	11.29	Chr06_25238042_snp8871	254.92	Chr06_17574027_snp11283	493.74
Chr06_30267048_snp2379	14.12	Chr06_25195936_snp9853	256.49	Chr_0_contigsnp5842	495.62
Chr06_30237792_snp8226	17.74	Chr06_25080693_snp276	259.65	Chr06_17961629_snp113	498.13
Chr_0_contigsnp6029	22.05	Chr06_24830737_snp2873	266.44	Chr06_17321505_snp991	501.28
Chr06_29931154_snp4277	26.31	Chr06_24712483_snp3540	274	Chr_0_contigsnp9560	506.07
Chr06_29827467_snp7182	32.28	Chr_0_contigsnp655	278.73	Chr06_17031689_snp2246	509.88
Chr06_29790718_snp6797	36.41	Chr06_24069364_snp1070	284.87	Chr06_16642779_snp11128	513.03
Chr06_29678522_snp6487	45.11	Chr06_24168330_snp3366	291.67	Chr06_16625306_snp11190	513.97
Chr06_29648280_snp11186	52.41	Chr06_23798000_snp6042	295.15	Chr06_16421094_snp6173	515.53
Chr06_29630662_snp10596	55.25	Chr06_23586496_snp1794	297.67	Chr06_16373980_snp6558	519.01
Chr06_29434349_snp8233	59.71	Chr06_23301079_snp11049	300.51	Chr06_16271123_snp7092	523.88
Chr06_29382365_snp10540	65.48	Chr06_23261086_snp823	306.63	Chr06_16649228_snp1174	530.21
Chr06_29340538_snp7258	70.29	Chr06_22969808_snp3499	313.75	Chr06_16158195_snp3776	542.37
Chr06_29335780_snp3532	74.39	Chr06_22930239_snp4078	318.55	Chr06_15844903_snp2657	555.53
Chr06_29294273_snp3901	76.59	Chr06_22992523_snp2225	322.53	Chr06_15973112_snp7713	561.65
Chr06_29224698_snp1505	78.78	Chr06_22731688_snp8078	325.84	Chr06_15873518_snp11758	562.58
Chr06_29153612_snp8109	82.26	Chr06_22495835_snp42	328.04	Chr_0_contigsnp6792	563.2
Chr06_28833478_snp7076	86.07	Chr_0_contigsnp6008	332.83	Chr06_15080267_snp2538	565.39
Chr06_28759194_snp8178	93.2	Chr06_22264280_snp9794	338.63	Chr06_14963325_snp8212	569.84
Chr06_28495708_snp3983	103.12	Chr06_22263928_snp2382	345.12	Chr06_14799490_snp2724	575.27
Chr_0_contigsnp11710	107.58	Chr06_21506681_snp7456	353.32	Chr06_14671650_snp9848	580.38
Chr06_28705124_snp31	110.1	Chr06_21313551_snp259	357.13	Chr_0_contigsnp5880	583.86
Chr06_28430436_snp1210	113.91	Chr06_21313617_snp3448	363.91	Chr06_14194295_snp926	587.98
Chr06_28305172_snp1151	116.43	Chr06_21282885_snp1155	374.48	Chr06_14057976_snp3741	591.13
Chr06_28200623_snp12153	117.68	Chr06_20093133_snp5089	380.92	Chr06_5174906_snp2027	594.28
Chr06_28374114_snp1306	123.03	Chr06_20658087_snp1507	386.38	Chr06_4298536_snp1266	596.48
Chr06_28099739_snp6538	134	Chr06_20688911_snp4074	390.19	Chr06_10619386_snp821	599.31
Chr06_28085201_snp6709	145.1	Chr06_20417385_snp11916	392.07	Chr06_4403442_snp5496	605.4
Chr06_27850346_snp7657	149.66	Chr_0_contigsnp5933	396.21	Chr06_512612_snp633	614.71
Chr06_27707249_snp810	151.52	Chr06_20084823_snp2258	406.27	Chr06_203047_snp2859	626.51
Chr06_27398130_snp5937	152.77	Chr06_19859413_snp6806	415.62	Number of markers: 131	
Chr_0_contigsnp4447	154.33	Chr06_19941307_snp66	421.42	Log-likelihood: -7042.31	
Chr06_27342267_snp346	159.47	Chr06_19164338_snp8299	425.55		
Chr06_27173567_snp1469	166.61	Chr06_18743470_snp11705	428.71		
Chr06_27149976_snp1113	173.08	Chr06_19127949_snp1224	431.54		
Chr_0_contigsnp8887	176.57	Chr_0_contigsnp5549	432.78		
Chr06_26793406_snp1228	180.71	Chr06_18887643_snp2112	433.71		
Chr06_26745178_snp3059	188.19	Chr06_18853710_snp12386	434.33		
Chr06_26423472_snp805	194.33	Chr06_18882006_snp5167	435.25		
Chr06_26290143_snp5024	197.17	Chr06_18498375_snp11859	440.04		
Chr06_26238587_snp5642	200.33	Chr06_18288130_snp3470	447.7		
Chr06_26077225_snp6812	204.8	Chr06_18050055_snp2087	452.95		
Chr06_26003849_snp2211	212.29	Chr06_18070453_snp1554	455.79		

Pv07

Marker	cM	Marker	cM	Marker	cM
Chr07_39742952_snp3706	0	Chr07_32658845_snp2203	146.33	Chr07_5884353_snp11766	324.9
Chr_0_contigsnp6587	3.47	Chr07_32772618_snp588	148.21	Chr07_5317111_snp6484	327.73
Chr07_39422123_snp11571	7.91	Chr07_32290068_snp3737	151.37	Chr07_4913375_snp2438	329.61
Chr07_39256792_snp1097	12.03	Chr_0_contigsnp5951	153.57	Chr07_4325052_snp10507	331.17
Chr07_38989781_snp943	16.16	Chr07_32611203_snp9035	154.51	Chr07_4325240_snp1959	333.37
Chr07_38817136_snp6009	17.09	Chr07_32263931_snp3164	156.38	Chr07_4004502_snp12797	335.88
Chr07_38839354_snp1225	18.01	Chr07_32090447_snp592	160.51	Chr07_3960937_snp2917	337.44
Chr07_38658803_snp1286	19.26	Chr07_32056485_snp3643	170.05	Chr07_3960871_snp6713	339
Chr07_38560307_snp2528	22.09	Chr_0_contigsnp5517	179.97	Chr07_3655944_snp6654	341.84
Chr07_38393886_snp8487	24.93	Chr07_31803669_snp8954	182.81	Chr07_3840020_snp6533	346.31
Chr07_38274020_snp2741	28.72	Chr07_30910550_snp2452	186.3	Chr07_4000572_snp12365	358.06
Chr07_38194178_snp8201	32.19	Chr07_30580048_snp2960	192.08	Chr07_4267446_snp6599	372.91
Chr07_38016538_snp255	37.95	Chr07_30169329_snp1302	197.55	Chr07_3686644_snp9830	386.28
Chr07_38016472_snp4441	44.12	Chr07_30008288_snp5809	199.11	Chr07_3703472_snp11132	393.17
Chr07_37799025_snp7465	47.69	Chr07_29880751_snp6188	201.63	Chr07_3419851_snp9903	396.97
Chr07_37832729_snp1189	49.25	Chr07_29862290_snp4268	209.98	Chr07_3329682_snp8235	400.13
Chr07_37736852_snp4411	51.44	Chr07_28443427_snp927	220.12	Chr07_3169176_snp7923	403.93
Chr07_37521342_snp6432	53.95	Chr07_29141620_snp3089	223.6	Chr07_3194721_snp3710	409.36
Chr07_37513015_snp7939	55.51	Chr07_28778350_snp1404	226.12	Chr_0_contigsnp5868	414.8
Chr07_37347565_snp5466	57.39	Chr_0_contigsnp6880	227.88	Chr07_3064881_snp2532	417
Chr_0_contigsnp5833	63.82	Chr07_28137892_snp10442	228.92	Chr07_3015485_snp7906	420.8
Chr_0_contigsnp5386	68.29	Chr07_27920223_snp10550	231.76	Chr07_2895889_snp2586	426.91
Chr07_37056602_snp8426	69.54	Chr07_27768306_snp2568	236.02	Chr07_2866273_snp1820	429.74
Chr07_36905563_snp7663	74.01	Chr07_27033716_snp11867	243.43	Chr07_2571083_snp2056	433.22
Chr07_36800044_snp3654	80.79	Chr07_26754479_snp784	251.31	Chr07_2571315_snp9206	436.06
Chr07_36713155_snp1337	84.27	Chr07_25762274_snp3589	254.47	Chr07_2452622_snp6919	438.25
Chr_0_contigsnp11095	85.83	Chr07_25743739_snp7685	255.09	Chr07_2401021_snp2239	442.05
Chr_0_contigsnp9516	87.39	Chr07_24917157_snp737	256.03	Chr07_2178933_snp3439	447.17
Chr_0_contigsnp5490	90.54	Chr07_23873808_snp3003	261.48	Chr07_2186371_snp11012	449.04
Chr07_36222942_snp10587	92.42	Chr07_12047444_snp1414	266.61	Chr07_1909019_snp1703	452.19
Chr07_36256663_snp8872	93.35	Chr07_12179355_snp7316	269.77	Chr07_1971012_snp6782	456
Chr07_36141160_snp6486	94.28	Chr07_11977356_snp6030	271.97	Chr07_1623618_snp7898	460.46
Chr_0_contigsnp9515	96.8	Chr07_10383651_snp426	276.77	Chr07_1226025_snp4470	465.58
Chr07_36039673_snp510	99.96	Chr07_9774532_snp972	282.56	Chr07_867402_snp7755	471.35
Chr07_35974956_snp1140	103.44	Chr_0_contigsnp5622	285.39	Chr07_1829150_snp944	482.37
Chr07_35501104_snp5741	107.9	Chr07_9356802_snp20	286.32	Number of markers: 131	
Chr07_35815329_snp96	109.78	Chr07_9329582_snp7198	287.57	Log-likelihood: -5861.72	
Chr07_34997128_snp884	112.62	Chr07_8633367_snp3141	289.45		
Chr07_34800638_snp741	115.45	Chr_0_contigsnp5369	291.01		
Chr_0_contigsnp5776	118.61	Chr07_8174549_snp1385	293.83		
Chr07_34535922_snp917	121.77	Chr07_7899715_snp27	296.35		
Chr07_34211947_snp6547	125.98	Chr07_7742557_snp765	298.23		
Chr07_34168635_snp1761	131.18	Chr07_7384050_snp2073	300.74		
Chr07_33913820_snp3210	136.3	Chr07_7039167_snp3180	303.9		
Chr07_33399997_snp9311	138.81	Chr07_6925900_snp2681	308.35		
Chr_0_contigsnp4970	140.37	Chr07_6308191_snp3516	314.13		
Chr07_33119027_snp10406	141.3	Chr07_5809928_snp7976	317.62		
Chr07_33115630_snp6640	143.18	Chr07_5961440_snp2978	321.42		

Pv08

Marker	cM	Marker	cM	Marker	cM
Chr08_866998_snp3830	0	Chr08_14023096_snp4012	186.57	Chr08_59816043_snp5702	392.89
Chr08_810665_snp3664	12.59	Chr08_13513148_snp1659	191.31	Chr08_59995868_snp2165	394.45
Chr08_630313_snp5300	21.05	Chr_0_contigsnp9335	192.87	Chr08_60187490_snp4507	397.6
Chr_0_contigsnp5465	24.03	Chr08_12993946_snp9198	194.75	Chr08_60287357_snp50	400.12
Chr08_750860_snp3721	24.65	Chr08_12993998_snp52	197.27	Chr08_61178349_snp11287	410.34
Chr08_873557_snp6426	25.9	Chr08_48258452_snp6193	199.46	Chr08_61237394_snp9697	415.12
Chr08_980582_snp12231	30.2	Chr08_26940191_snp9264	200.71	Chr08_61408701_snp2014	419.89
Chr_0_contigsnp5370	34.16	Chr08_41879734_snp6748	202.9	Chr08_61662321_snp5350	420.82
Chr08_1080429_snp6946	35.72	Chr08_36213724_snp3626	205.56	Chr08_61918725_snp11377	422.7
Chr08_1097956_snp1995	36.65	Chr08_53686527_snp1700	208.89	Chr08_61888757_snp641	425.53
Chr08_1207785_snp10405	39.48	Chr08_51070149_snp4045	211.73	Chr08_62081768_snp1807	429.65
Chr_0_contigsnp10909	41.68	Chr08_44258742_snp1363	213.93	Chr08_62201631_snp9267	432.8
Chr08_1424362_snp4943	42.93	Chr08_30757679_snp6926	216.44	Chr08_62278459_snp8697	435.31
Chr08_1630077_snp11233	44.17	Chr08_32087539_snp9062	217.69	Chr08_62389452_snp4138	439.11
Chr08_1630546_snp3597	47.33	Chr08_52962538_snp7070	218.61	Chr08_62395167_snp9984	441.94
Chr08_1680365_snp546	51.78	Chr08_52664081_snp520	220.49	Chr08_62525279_snp11275	446.71
Chr08_2013167_snp4034	58.24	Chr08_51951737_snp3409	226.23	Chr08_62572124_snp10281	451.15
Chr08_2001133_snp1243	63.19	Chr08_47335170_snp4171	236.06	Chr08_62645111_snp11750	453.03
Chr08_2015509_snp1233	69.44	Chr08_47833732_snp2031	244.7	Chr08_62630622_snp11697	457.96
Chr08_2113831_snp374	77.23	Chr08_29246560_snp4550	254.22	Chr08_62727137_snp8287	468.55
Chr08_2152565_snp99	83.33	Chr08_20992083_snp12101	265.42	Chr08_62734846_snp1403	472.34
Chr08_2366604_snp1832	88.44	Chr08_54888965_snp1841	276.62	Chr08_62721370_snp10402	473.9
Chr08_2309321_snp12023	91.27	Chr_0_contigsnp5488	284.57	Chr_0_contigsnp5927	475.46
Chr08_2680813_snp602	92.83	Chr08_55815020_snp7853	289.04	Chr08_62754013_snp2988	477.02
Chr08_2679083_snp8323	96.31	Chr08_55630794_snp7087	294.17	Chr08_62939668_snp1546	480.16
Chr08_2843805_snp8614	101.42	Chr08_56027865_snp4466	298.63	Chr08_63011117_snp7822	485.58
Chr08_2999290_snp9154	104.9	Chr_0_contigsnp6015	301.97	Number of markers: 122	
Chr08_2978296_snp11777	105.52	Chr08_56360768_snp9065	304.64	Log-likelihood: -5743.17	
Chr08_3053293_snp173	106.77	Chr08_56969575_snp80	307.16		
Chr08_3711888_snp8888	109.29	Chr08_57092262_snp928	309.67		
Chr08_3692265_snp9740	109.92	Chr08_57384239_snp4332	311.23		
Chr08_3763716_snp610	112.43	Chr08_57194867_snp4144	317.36		
Chr08_4031469_snp7365	117.21	Chr08_57280012_snp3500	328.64		
Chr08_4015122_snp9849	118.77	Chr08_57440976_snp415	335.78		
Chr08_4182684_snp3014	120.65	Chr08_57496887_snp8631	337.98		
Chr08_4371771_snp2058	124.77	Chr_0_contigsnp5460	340.5		
Chr_0_contigsnp8879	128.57	Chr08_57864281_snp3934	343.34		
Chr08_4965386_snp6176	133.02	Chr08_53260737_snp3306	349.96		
Chr08_5340854_snp2632	138.14	Chr08_53389251_snp3163	356.58		
Chr08_5441052_snp11985	139.7	Chr08_58506704_snp7734	362.69		
Chr08_5691770_snp667	144.16	Chr08_58401812_snp8104	366.49		
Chr08_6712649_snp2364	152.31	Chr08_58430086_snp7718	370.94		
Chr08_6380025_snp8916	157.76	Chr08_58829797_snp8651	378.39		
Chr08_6642766_snp11683	158.7	Chr08_58964507_snp1681	382.52		
Chr08_6868604_snp7841	159.95	Chr08_59252138_snp341	387.29		
Chr08_8890026_snp11781	169.18	Chr08_59176568_snp9982	389.17		
Chr08_9053545_snp684	174.63	Chr08_59274216_snp3374	390.09		
Chr08_13622388_snp10358	180.23	Chr_0_contigsnp6889	391.34		

Pv09					
Marker	cM	Marker	cM	Marker	cM
Chr09_984100_snp6056	0	Chr09_19108919_snp5667	180.55	Chr09_33442471_snp841	334.51
Chr09_1129105_snp10126	2.83	Chr09_19091481_snp5749	180.87	Chr09_33586457_snp9031	338.63
Chr09_3189265_snp2443	6.62	Chr09_19107775_snp1002	183.06	Chr09_33665121_snp8047	340.82
Chr09_7344029_snp1864	10.41	Chr09_19421698_snp1870	188.83	Chr09_33807397_snp9535	341.74
Chr09_7892079_snp4173	12.91	Chr09_19778981_snp953	194.26	Chr_0_contigsnp7421	342.67
Chr09_8651006_snp8146	16.69	Chr09_20132959_snp6459	196.46	Chr09_34056439_snp6656	344.23
Chr09_8756781_snp7716	18.89	Chr_0_contigsnp9586	198.97	Chr09_34126686_snp1084	346.42
Chr_0_contigsnp5375	21.08	Chr09_21164786_snp1060	203.74	Chr09_34582586_snp12587	348.61
Chr09_10495915_snp11657	23.59	Chr09_21324069_snp11274	207.54	Chr09_34688687_snp7512	349.54
Chr09_10807775_snp12824	25.78	Chr09_21366245_snp1226	210.7	Chr09_34696575_snp7799	351.42
Chr09_11251634_snp2878	29.89	Chr_0_contigsnp9334	216.14	Chr09_35017060_snp1814	357.17
Chr09_11635580_snp3328	34	Chr09_22354190_snp3490	223.56	Chr09_35409981_snp302	366.29
Chr09_12278887_snp5138	37.15	Chr09_22333926_snp6669	228.34	Chr09_35570579_snp12191	369.45
Chr09_12264810_snp362	39.02	Chr09_22736997_snp2821	230.85	Chr09_35713762_snp1647	371.96
Chr09_12762477_snp9123	40.27	Chr09_23488570_snp5184	232.73	Chr09_35935551_snp1389	377.57
Chr09_13002923_snp1913	44.39	Chr09_23944144_snp7025	234.29	Chr09_36078767_snp7230	382.5
Chr09_12916976_snp3907	48.51	Chr09_23812940_snp22	237.44	Chr09_35942715_snp10361	383.12
Chr09_12908447_snp7231	50.38	Chr09_24646173_snp4999	242.21	Chr_0_contigsnp1635	384.68
Chr09_13311983_snp1429	51.63	Chr09_24427324_snp7574	247.31	Chr09_36482340_snp9055	388.48
Chr09_13332465_snp10035	52.87	Chr09_25546440_snp2064	252.08	Chr09_36795421_snp940	391.31
Chr09_13611037_snp8606	56.67	Chr09_25120431_snp8352	255.88	Chr09_36619666_snp439	395.12
Chr09_13924701_snp7245	62.43	Chr09_25749686_snp367	261.3	Chr09_36619600_snp1673	404.51
Chr09_13715939_snp3420	70.2	Chr09_25828154_snp12292	263.82	Chr09_36881824_snp2478	415.72
Chr09_13941357_snp4118	76.95	Chr09_26194023_snp3215	266.01	Chr09_37065961_snp11753	420.18
Chr09_13716769_snp1829	84.7	Chr09_26667764_snp4325	269.81	Chr09_37174495_snp5288	423.02
Chr09_14084765_snp6495	89.8	Chr_0_contigsnp5399	272	Chr09_37222661_snp6163	425.85
Chr09_13975984_snp2522	93.59	Chr09_26791155_snp8556	272.31	Chr09_37991626_snp8513	431.65
Chr09_13922957_snp7355	100	Chr09_27890010_snp12212	272.94	Chr09_37754150_snp5722	435.15
Chr09_14009375_snp6731	104.44	Chr09_28122777_snp5061	273.55	Chr09_37771411_snp5316	438.28
Chr09_14298154_snp11944	107.91	Chr09_28253207_snp2038	274.79	Chr09_37667224_snp1046	441.12
Chr09_14538821_snp3742	112.03	Chr09_28627032_snp5367	276.99	Chr09_37368190_snp1780	447.23
Chr09_14719948_snp2830	114.86	Chr09_29068179_snp3157	281.1	Chr09_38192024_snp7007	463.21
Chr09_14909561_snp1283	118.97	Chr09_29468026_snp3074	285.55	Number of markers: 128	
Chr09_15382452_snp7487	123.09	Chr09_29468264_snp8126	288.7	Log-likelihood: -5721.03	
Chr_0_contigsnp5414	125.28	Chr09_30374741_snp232	294.14		
Chr09_15562385_snp3178	130.05	Chr09_29988349_snp116	297.29		
Chr09_15655603_snp646	135.15	Chr09_30543704_snp4212	298.53		
Chr09_15712417_snp8510	139.27	Chr09_30782487_snp2596	300.41		
Chr09_16132586_snp2215	143.39	Chr09_31013617_snp580	302.6		
Chr09_16125757_snp4160	145.9	Chr09_31323133_snp10910	306.72		
Chr09_16259863_snp3600	149.05	Chr09_31383306_snp9268	310.52		
Chr09_16780016_snp785	154.47	Chr_0_contigsnp10888	311.77		
Chr09_17428667_snp3677	157.94	Chr09_31898628_snp2589	314.92		
Chr09_18041543_snp2693	160.45	Chr09_32265446_snp7451	318.72		
Chr09_17530161_snp7243	164.7	Chr_0_contigsnp5692	322.84		
Chr09_18302648_snp2474	171.18	Chr09_32924896_snp4417	324.72		
Chr09_18218224_snp624	175.81	Chr09_33056047_snp7867	327.55		
Chr09_18797172_snp2936	179.62	Chr09_33314857_snp5266	329.74		

Pv10

Marker	cM
Chr10_44261780_snp3651	0
Chr10_43969198_snp2514	7.86
Chr10_43919780_snp401	10.7
Chr10_43628214_snp1397	17.81
Chr10_43614406_snp12	22.59
Chr10_43496361_snp6253	24.79
Chr10_43434436_snp3863	26.98
Chr10_43399319_snp2417	30.46
Chr10_43281515_snp1237	37.04
Chr10_43204137_snp3778	44.99
Chr10_43162582_snp133	51.07
Chr10_43118827_snp7737	54.54
Chr10_43129878_snp7439	55.17
Chr_0_contigsnp5014	55.78
Chr10_42963394_snp6305	57.66
Chr10_42835291_snp5347	61.45
Chr10_42767915_snp2749	64.93
Chr10_42666902_snp10937	66.49
Chr10_42531291_snp5553	68.36
Chr10_42410969_snp8154	71.51
Chr10_42475017_snp1590	75.3
Chr10_42429530_snp932	78.45
Chr10_42275553_snp11023	84.19
Chr10_42191723_snp11003	86.7
Chr10_42138725_snp1229	89.34
Chr10_41922479_snp7211	95.07
Chr10_41681015_snp772	98.01
Chr10_41643072_snp5558	99.57
Chr10_41686622_snp921	100.5
Chr10_41476438_snp6216	103.33
Chr10_41185849_snp9886	106.16
Chr10_41067189_snp1003	109.95
Chr10_40972087_snp3036	114.07
Chr_0_contigsnp3461	118.18
Chr10_40433192_snp119	124.59
Chr10_40190962_snp2627	130.01
Chr10_40108358_snp8210	133.17
Chr10_39995457_snp557	137.74
Chr10_39723620_snp10140	145.38
Chr10_39723554_snp1212	150.81
Chr10_39738894_snp5046	152.06
Chr10_39334101_snp04	153.62
Chr10_39107543_snp11210	155.5
Chr_0_contigsnp5527	158.33
Chr_115127_scaffold59snp6636	164.77
Chr_81366_scaffold59snp463	172.22
Chr10_37121229_snp10674	176.67
Chr10_37110616_snp1116	177.29
Chr10_37048336_snp3857	178.23
Chr10_36755777_snp1168	179.79
Chr10_36496731_snp6749	181.98
Chr10_36695335_snp4101	184.81
Chr10_35795027_snp4279	187.63
Chr10_35960574_snp3853	188.56
Chr10_36453900_snp872	188.87
Chr10_32918964_snp1073	190.44
Chr10_33547669_snp5130	192.95
Chr10_30196856_snp3942	195.78
Chr10_30699926_snp1597	199.25
Chr10_7728766_snp6278	204.03
Chr10_7277836_snp1524	207.83
Chr10_6812777_snp1489	210.98
Chr10_6309203_snp5102	213.5
Chr10_6060378_snp9502	220.56
Chr10_3178793_snp280	231.62
Chr_0_contigsnp9120	235.1
Chr10_1581655_snp2537	238.58
Chr10_2021290_snp11865	243.7
Chr10_3311040_snp8203	249.81
Chr10_3307120_snp13037	254.59
Chr10_4671091_snp9553	257.43
Chr10_4627811_snp7672	262.21
Chr10_3785145_snp11542	268.94
Chr10_3685085_snp4510	277.14

Number of markers: 74

Log-likelihood: -3450.35

Pv11

Marker	cM	Marker	cM	Marker	cM
Chr11_39817_snp4207	0	Chr11_4439415_snp6721	249.92	Chr11_46181412_snp9460	462.78
Chr11_27513_snp8931	5.46	Chr11_4656238_snp1876	255.76	Chr11_46913027_snp2194	466.27
Chr11_220190_snp4311	10.27	Chr11_4663804_snp7762	267.97	Chr11_46840644_snp9327	471.52
Chr11_384121_snp3696	19.95	Chr11_4700862_snp3578	276.93	Chr11_47925715_snp9948	479.79
Chr11_468428_snp8428	25.42	Chr11_4827465_snp10075	279.45	Chr11_47037732_snp4548	492.71
Chr11_567013_snp6420	29.55	Chr11_4894360_snp6653	281.64	Chr11_47623903_snp1979	503.81
Chr11_625424_snp8955	31.43	Chr11_4956421_snp5534	283.21	Chr11_48372762_snp3447	508.27
Chr11_821665_snp5161	33.95	Chr11_5214313_snp4904	287.35	Chr11_48470655_snp4251	510.15
Chr11_821611_snp9538	35.19	Chr11_5531531_snp3330	293.48	Chr11_48786287_snp9231	513.31
Chr_0_contigsnp1103	36.76	Chr11_5767807_snp3069	300.94	Chr11_49025116_snp9935	516.15
Chr11_1015713_snp7825	38.64	Chr_0_contigsnp5671	307.71	Chr11_48931280_snp5477	520.62
Chr11_1049064_snp839	41.81	Chr11_5991104_snp3007	317.2	Chr11_48902843_snp2413	526.06
Chr11_1159754_snp3107	52.17	Chr11_5991829_snp3967	326.01	Chr_0_contigsnp6314	534.96
Chr11_1256874_snp2556	65.61	Chr11_6525452_snp3770	331.46	Chr_0_contigsnp9694	548.71
Chr11_1375571_snp8153	75.23	Chr11_1919207_snp5073	333.97	Chr11_50191669_snp5409	560.31
Chr11_1379116_snp5403	79.05	Chr_0_contigsnp10150	336.81	Chr11_50009095_snp289	566.21
Chr11_1495197_snp1851	86.56	Chr11_6964160_snp11489	338.69	Chr11_51696483_snp2660	571.34
Chr11_1593671_snp9325	92.37	Chr11_7698957_snp327	342.49	Chr11_51886961_snp6932	575.81
Chr11_1644776_snp10837	93.93	Chr_5605_scaffold240snp1265	349.62	Chr_0_contigsnp1435	580.27
Chr11_1706124_snp10841	98.86	Chr11_8657479_snp6825	356.4	Chr11_52195251_snp5080	583.11
Chr11_1637078_snp2302	109.18	Chr11_8843628_snp10856	359.56	Chr_0_contigsnp3946	585.63
Chr11_2120622_snp7876	123.16	Chr11_8148163_snp8298	362.08	Chr11_52829968_snp7971	589.11
Chr11_2098248_snp3673	136.75	Chr11_8972965_snp8225	369.87	Chr11_53099362_snp169	594.57
Chr11_2080693_snp533	143.37	Chr11_37693547_snp6885	374.21	Chr11_53429330_snp53	598.39
Chr_0_contigsnp6684	144.61	Chr11_10891757_snp3854	382.45	Chr11_53436885_snp10926	600.27
Chr11_2320217_snp11409	145.55	Chr11_10890818_snp162	385.29	Chr11_53477502_snp11719	602.47
Chr11_2412256_snp10860	148.06	Chr_0_contigsnp5165	386.54	Chr11_53538205_snp3986	605.31
Chr11_2470275_snp10092	152.52	Chr11_13599464_snp7642	388.1	Number of markers: 123	
Chr11_2470275_snp10091	155.36	Chr11_11730647_snp6022	393.06	Log-likelihood: -6586.69	
Chr11_2715078_snp5920	157.56	Chr11_11606235_snp125	397.68		
Chr11_2776179_snp5628	160.19	Chr11_14342353_snp7105	399.24		
Chr11_2737484_snp7661	164.53	Chr11_14263150_snp4296	400.8		
Chr11_2901988_snp820	168.66	Chr11_14692667_snp9308	402.04		
Chr_0_contigsnp9585	170.87	Chr11_15078832_snp3100	402.35		
Chr11_3207881_snp3398	182.27	Chr11_15250231_snp10461	402.66		
Chr11_3756580_snp597	198.33	Chr11_30297851_snp11635	403.59		
Chr11_3312985_snp8504	211.1	Chr11_30716388_snp3233	404.83		
Chr11_3259876_snp4206	217.58	Chr11_44395110_snp37	406.71		
Chr11_3453959_snp11079	219.15	Chr11_42748984_snp4032	408.27		
Chr11_3478127_snp6997	221.03	Chr11_38370400_snp581	410.79		
Chr11_3567188_snp10858	224.51	Chr11_36386431_snp7121	414.92		
Chr11_3646388_snp4423	227.68	Chr11_40680320_snp9942	420.37		
Chr11_3663020_snp2100	230.2	Chr11_41090265_snp12583	430.61		
Chr_0_contigsnp3889	234.34	Chr11_45734948_snp8062	441.56		
Chr11_3987608_snp3535	237.5	Chr11_45113805_snp1892	448.67		
Chr11_4094331_snp472	238.75	Chr11_44557896_snp8282	452.73		
Chr11_4315400_snp1835	240.32	Chr11_45090229_snp8633	456.15		
Chr11_4294350_snp10179	244.46	Chr_0_contigsnp10557	459.3		

Supplementary Table 6. Functional annotation of 44 candidate genes identified in the genomic region interval (Pv04: 477,217 bp...1,182,084 bp) of the major locus *Co-Realce* which are related to disease resistance metabolic pathways in plants.

Candidate gene	Functional annotation
Phvul.004G007750	Leucine-rich repeat (LRR)
Phvul.004G007900	
Phvul.004G008001	
Phvul.004G008101	
Phvul.004G008200	
Phvul.004G008351	
Phvul.004G008400	
Phvul.004G008450	
Phvul.004G008560	
Phvul.004G008620	
Phvul.004G008680	
Phvul.004G008740	
Phvul.004G008981	
Phvul.004G009041	
Phvul.004G009281	
Phvul.004G009461	
Phvul.004G009521	
Phvul.004G009821	
Phvul.004G009909	
Phvul.004G009918	
Phvul.004G009936	
Phvul.004G009100	
Phvul.004G009136	
Phvul.004G009154	
Phvul.004G008900	
Phvul.004G008909	
Phvul.004G008918	
Phvul.004G009300	
Phvul.004G009500	
Phvul.004G009341	Pentatricopeptide repeat (PPR)
Phvul.004G009641	
Phvul.004G009900	
Phvul.004G009945	
Phvul.004G008500	
Phvul.004G007300	Phosphate-transporting ATPase/ABC
Phvul.004G008301	Phospholipid-transporting ATPase 10-related
Phvul.004G009145	Phospholipid-transporting ATPase 8-related
Phvul.004G006800[a]	*Nuclear pore complex protein - Nup210, GP210*
Phvul.004G006900[a]	*glycosylphosphatidylinositol transamidase (GAA1)*
Phvul.004G007100	Galactinol-sucrose galactosyltransferase 5-Related
Phvul.004G009400	E3 Ubiquitin-protein ligase UPL6
Phvul.004G007200	Methyl-CPG-binding domain
Phvul.004G009401	RNA recognition motif (RRM or RNP domain)
Phvul.004G007600	RNA-binding protein 26 (RBM26)

[a]Candidate genes also annotated in the refinement analysis of the major locus *Co-Realce* (Pv04: 485,246...505,651).

Supplementary Table 7. Genetic map with 135 SNP and 111 DArT markers used in the interval refinement analysis of the major locus (*Co-Realce*) controlling anthracnose resistance in the Andean common bean cultivar BRSMG Realce. The underlined marks are those safely positioned using the LOD-score of 3.0 and the 'safe' function as ordering criteria.

Pv04

Marker	Position, pb	Position, cM	Marker	Position, pb	Position, cM	Marker	Position, pb	Position, cM	Marker	Position, pb	Position, cM	Marker	Position, pb	Position, cM
Chr04_47114974_dart5529	47114974	0	Chr04_41573180_snp3736	41573180	111.77	Chr04_16109942_snp8418	16109942	195.99	Chr04_5362219_snp8266	5362219	288.96	Chr04_2108445_dart5361	2108445	413.46
Chr04_47181272_dart14642	47181272	9.45	Chr04_43247001_dart14465	43247001	113.12	Chr04_26377145_snp5543	26377145	197.55	Chr04_5299507_snp12074	5299507	291.79	Chr04_1617988_snp6408	1617988	414.99
Chr04_47147470_dart2538	47147470	9.48	Chr04_43382572_dart6913	43382572	114.89	Chr04_15216109_dart808	15216109	197.55	Chr04_5299507_dart15734	5299507	291.79	Chr04_1419087_snp5641	1419087	418.64
Chr04_47517559_dart5339	47517559	18.24	Chr04_42151157_snp4070	42151157	117.56	Chr04_27697606_snp6949	27697606	200.37	Chr04_5211278_snp10827	5211278	294.61	Chr04_1743652_dart16072	1743652	421.13
Chr04_47440758_dart4613	47440758	22.11	Chr04_43222578_dart13426	43222578	119.37	Chr04_14329142_snp3638	14329142	202.24	Chr04_5019371_snp3048	5019371	299.04	Chr04_1674485_dart13430	1674485	421.95
Chr04_47496448_dart14805	47496448	25.04	Chr04_43377188_dart11471	43377188	121.4	Chr04_30182627_snp379	30182627	203.48	Chr04_5045180_snp5743	5045180	302.83	Chr04_1990110_snp10425	1990110	421.95
Chr04_47325157_dart12188	47325157	26.01	Chr04_40176294_snp9637	40176294	124.95	Chr04_18442905_snp7449	18442905	203.48	Chr04_5016277_snp11234	5016277	305.65	Chr04_1991584_snp6424	1991584	422.24
Chr04_46817253_dart11172	46817253	27.22	Chr04_32475203_dart5874	32475203	124.95	Chr04_20563311_snp3335	20563311	204.73	Chr04_4965430_dart4619	4965430	307.54	Chr04_1372295_snp11880	1372295	423.16
Chr04_47173153_dart10729	47173153	27.22	Chr04_40380695_snp1473	40380695	128.52	Chr04_11692988_snp4154	11692988	208.2	Chr04_5016021_snp12448	5016021	308.17	Chr04_1459874_dart317	1459874	423.76
Chr04_46964055_dart6010	46964055	28.4	Chr04_39377259_dart10619	39377259	130.68	Chr04_11380085_dart11372	11380085	209.78	Chr04_5016343_snp1998	5016343	310.68	Chr04_1749234_dart10357	1749234	427.8
Chr04_46855391_dart5697	46855391	30.83	Chr04_39980732_snp10097	39980732	132.66	Chr04_12364074_snp8010	12364074	211.36	Chr04_4660298_snp8723	4660298	314.14	Chr04_486794_snp2492	486794	434.56
Chr04_47147117_dart5285	47147117	32.63	Chr04_39384063_snp2818	39384063	136.44	Chr04_12042681_snp9178	12042681	212.92	Chr04_3487898_dart4429	3487898	317.82	Chr04_477217_snp1327	477217	444.07
Chr04_47091122_dart14018	47091122	32.63	Chr04_34899602_dart8651	34899602	136.44	Chr04_12049157_snp7756	12049157	214.47	Chr04_3869119_snp2383	3869119	319.43	Chr04_505651_snp3308	505651	448.77
Chr04_46643162_dart10220	46643162	37.2	Chr04_31645913_dart12016	31645913	136.44	Chr04_12046097_snp45	12046097	215.39	Chr04_4228326_dart12057	4228326	322.1	Chr04_432893_snp10195	432893	451.06
Chr04_46643096_dart4340	46643096	40.1	Chr04_39377577_snp5662	39377577	138.32	Chr04_12934980_dart5553	12934980	215.39	Chr04_4257359_snp11939	4257359	322.1	Chr04_507903_snp11894	507903	451.68
Chr04_46374308_dart16045	46374308	43.72	Chr04_39377325_snp3339	39377325	140.82	Chr04_11620998_snp6943	11620998	216.01	Chr04_4128140_snp847	4128140	324.29	Chr04_916777_dart16086	916777	451.68
Chr04_46352305_dart10649	46352305	46.84	Chr04_39379369_snp3990	39379369	142.38	Chr04_11380137_snp2236	11380137	216.93	Chr04_4035446_snp2998	4035446	329.38	Chr04_1293563_dart11022	1293563	451.68
Chr04_45499123_dart5194	45499123	53.68	Chr04_39387115_snp10956	39387115	143.31	Chr04_11380085_snp5497	11380085	217.85	Chr04_4266707_dart13487	4266707	331.45	Chr04_714306_dart16076	714306	451.68
Chr04_45501936_dart14595	45501936	53.68	Chr04_38030162_snp5062	38030162	147.1	Chr04_12935922_snp5605	12935922	218.45	Chr04_3782333_dart4868	3782333	333.52	Chr04_485246_dart9817	485246	451.68
Chr04_45449150_dart5963	45449150	56.4	Chr04_35472743_snp3250	35472743	150.57	Chr04_11271888_snp2649	11271888	219.06	Chr04_3509238_dart5997	3509238	333.52	Chr04_1182084_snp12782	1182084	453.24
Chr04_45082434_snp5292	45082434	59.77	Chr04_38875960_snp5678	38875960	151.5	Chr04_9710566_dart14193	9710566	219.37	Chr04_4030700_snp8645	4030700	333.52	Chr04_1182084_dart16085	1182084	454.49
Chr04_44925079_dart6053	44925079	60.36	Chr04_37552665_snp1405	37552665	152.43	Chr04_9032609_snp9134	9032609	219.68	Chr04_4228272_dart12059	4228272	335.56	Chr04_237680_dart4949	237680	456.11
Chr04_44680215_dart5195	44680215	61.54	Chr04_25600593_snp3648	25600593	153.99	Chr04_8785208_snp8342	8785208	221.87	Chr04_3521439_dart5797	3521439	336.57	Chr04_487825_snp9435	487825	459.66
Chr04_44474382_dart5366	44474382	61.54	Chr04_36750811_snp3005	36750811	158.43	Chr04_8751664_dart12576	8751664	222.81	Chr04_4001115_snp5953	4001115	337.32	Chr04_324855_dart16151	324855	463.29
Chr04_44429169_dart5973	44429169	66.34	Chr04_31645960_snp11566	31645960	162.54	Chr04_8751664_snp6845	8751664	223.75	Chr04_3923830_snp9353	3923830	340.15	Chr04_345233_dart14088	345233	464.85
Chr04_43887952_snp9908	43887952	72.29	Chr04_29499490_snp6621	29499490	164.41	Chr04_9328169_dart11096	9328169	226.21	Chr04_3812634_snp5855	3812634	342.02	Chr04_812021_dart15774	812021	465.88
Chr04_44660494_dart10774	44660494	74.66	Chr04_15788223_snp709	15788223	165.03	Chr04_9336619_snp11473	9336619	230.55	Chr04_3326593_snp10953	3326593	344.53	Chr04_816576_snp12129	816576	467.88
Chr04_44572826_dart10022	44572826	76.07	Chr04_39613690_dart5862	39613690	166.27	Chr04_9010476_snp5284	9010476	237.63	Chr04_3196575_snp9526	3196575	345.46	Chr04_495306_dart15389	495306	470.48
Chr04_43597198_snp11931	43597198	78.44	Chr04_31406511_dart9223	31406511	166.27	Chr04_9748590_snp1051	9748590	242.39	Chr04_3200604_dart13095	3200604	346.38	Chr04_143123_snp11422	143123	475.74
Chr04_43706659_dart10767	43706659	79.37	Chr04_31406511_snp2072	31406511	166.27	Chr04_8861620_snp335	8861620	248.14	Chr04_3200604_snp7950	3200604	346.38	Chr04_152313_snp7332	152313	478.26
Chr04_43521680_snp6050	43521680	79.37	Chr04_20393238_snp8569	20393238	167.83	Chr04_9016844_dart15729	9016844	253.35	Chr04_3026374_dart13522	3026374	347.1	Chr04_38637_snp11994	38637	482.05

Chr04_43056803_dart6317	43056803	80.41	Chr04_33166274_dart4383	33166274	170.51	Chr04_9194338_dart6163	9194338	256.49	Chr04_3539233_dart10887	3539233	349.98	Chr04_108201_snp3562	108201	486.17
Chr04_42323086_dart5132	42323086	80.92	Chr04_18562099_snp1725	18562099	172.3	Chr04_9121629_snp6993	9121629	257.71	Chr04_3454386_snp10344	3454386	352.86	Chr04_78013_snp266	78013	490.95
Chr04_43299358_snp11116	43299358	80.92	Chr04_33339058_dart9370	33339058	175.39	Chr04_8471848_snp1858	8471848	260.22	Chr04_3012290_dart6204	3012290	359.57	Number of markers: 229		
Chr04_42279548_dart5636	42279548	81.44	Chr04_36272890_dart5806	36272890	175.39	Chr04_8421348_snp9107	8421348	261.77	Chr04_2860174_snp768	2860174	363.46	Log-likelihood: -6065.945		
Chr04_42522696_dart11727	42522696	83.02	Chr04_33959235_dart5213	33959235	175.39	Chr04_8464023_dart15676	8464023	263.33	Chr04_2862194_snp10594	2862194	369.87			
Chr04_42433408_snp51	42433408	84.07	Chr04_20589888_snp2471	20589888	177.41	Chr04_8655981_snp4961	8655981	263.33	Chr04_2862128_snp11182	2862128	373.99			
Chr04_43061809_dart5388	43061809	85.15	Chr04_21352551_dart15560	21352551	177.42	Chr04_8250605_snp6634	8250605	266.16	Chr04_2778583_snp2613	2778583	376.51			
Chr04_42664345_dart11854	42664345	85.83	Chr04_33952344_dart5960	33952344	178.37	Chr04_8095615_dart9163	8095615	268.22	Chr04_2767815_snp4314	2767815	379.99			
Chr04_42229881_snp6436	42229881	87.87	Chr04_40676334_dart13067	40676334	178.94	Chr04_8032107_snp9751	8032107	270.29	Chr04_2546876_snp10177	2546876	389.43			
Chr04_42280900_snp10764	42280900	88.79	Chr04_30291647_snp393	30291647	181.23	Chr04_7429730_dart5219	7429730	270.29	Chr04_2395185_snp12013	2395185	393.33			
Chr04_42285143_snp5264	42285143	89.72	Chr04_39571986_dart5809	39571986	183.11	Chr04_7069188_snp11912	7069188	274.73	Chr04_2357028_snp2617	2357028	396.49			
Chr04_41631087_snp4347	41631087	93.51	Chr04_33731013_snp3226	33731013	183.11	Chr04_6960028_dart4896	6960028	277.3	Chr04_2327020_snp9746	2327020	399			
Chr04_42159718_snp11301	42159718	96.33	Chr04_22285053_dart12226	22285053	183.83	Chr04_6959618_snp1951	6959618	279.86	Chr04_2272161_dart10202	2272161	399			
Chr04_42159718_snp11300	42159718	97.58	Chr04_14159466_snp1137	14159466	185.3	Chr04_5988895_snp316	5988895	280.79	Chr04_2303529_snp9548	2303529	399.93			
Chr04_41878249_snp8700	41878249	99.45	Chr04_14159526_snp19	14159526	188.44	Chr04_5698607_snp5462	5698607	282.03	Chr04_2297570_snp6914	2297570	400.55			
Chr04_41878249_dart13448	41878249	99.45	Chr04_14685796_snp8144	14685796	190.81	Chr04_5698541_snp8445	5698541	283.58	Chr04_2303463_snp9885	2303463	402.42			
Chr04_42451042_dart14212	42451042	103.32	Chr04_18562165_dart5796	18562165	192.79	Chr04_5796216_snp3796	5796216	284.83	Chr04_2276788_snp7101	2276788	404.61			
Chr04_41857223_snp5563	41857223	107.98	Chr04_32571940_snp158	32571940	194.11	Chr04_5485376_dart14144	5485376	287.19	Chr04_2272161_snp4396	2272161	406.81			

Supplementary Table 8. Nucleotide sequence of the SNP and DArT markers flanking the major locus (*Co-Realce*) controlling anthracnose resistance in the Andean common bean cultivar BRSMG Realce.

Marker	Position on Pv04	Polymorphism[a]		Sequence
snp12782[b]	1,182,123	39:C>T	C/T	TGCAGGGTCTGATGCTATAAAATTCTATAACTTGTCATA**[C/T]**ATAAATCAAAATGTCATGAAAATCATGAA
snp3308	505,696	45:C>T	C/T	TGCAGGTTCTGCAAACAACATGCTCTCTACTCACCATGTCTCAGA[**C/T]**GCAAATAAATTCATCAAGGACTT
dart9817	485,246	-	0/1	TGCAGTAGAGATAATCTTTATTAGATAAACTGTAGTGGTAAAAGGAAAATCCTAGAAATTACAGATCGG
snp1327	477,285	68:T>C	T/C	TGCAGATCTGAAAAAACAAACATTCAGAACTGAATCACAGATTCCTCAACCCTATGTTTGAAATTGTT**[T/C]**

[a]The allele associated with anthracnose resistance is underlined; 0 – absence, and 1 – presence. [b]For snp12782, the alignment at the common bean reference genome v2.1 (Phytozome-*Phaseolus vulgaris* v2.1) should be made with the reverse complement sequence.

CAPÍTULO 3

19 MOLECULAR CHARACTERIZATION OF PARENTAL LINES AND VALIDATION OF SNP MARKERS FOR ANTHRACNOSE AND ANGULAR LEAF SPOT IN COMMON BEAN*

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Molecular characterization of parental lines and validation of SNP markers for anthracnose and angular leaf spot in common bean

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Abstract The implementation of molecular tools that help the early selection of genotypes carrying target alleles increases efficiency and reduces the time and costs of breeding programs. The present study aimed the molecular characterization and validation of Single Nucleotide Polymorphism (SNP) targeting disease resistance alleles for assisted selection. A total of 376 common bean lines with contrasting responses for anthracnose and angular leaf spot resistance were used, as well as 149 F_2 plants from the cross between BRS Cometa × SEL 1308 (carrying the Anthracnose resistance gene *Co-4*^{2}). Seven of the ten SNP markers evaluated showed potential for assisted breeding: snpPV0025 (*Phg-2*), snpPV0027 (*Phg-5*), snpPV0079 (*Phg-5*), snpPV0046 (*Co-u*), snpPV0068 (*Co-4*^{2}), snpPV0070 (*Co-4*^{2}) and snpP8282v3-817 (*Co-4*^{2}). Markers snpPV0070 and snpP8282v3-817 showed high efficiency of selection (99.7 and 99.8%, respectively). These markers exhibit great potential to assist in the selection at different stages of the breeding program and may be readily incorporated into marker-assisted selection.

Keywords *Phaseolus vulgaris* · *Colletotrichum lindemuthianum* · *Pseudocercospora griseola* · Disease resistance · Marker-assisted selection · Molecular breeding

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Introduction

Brazil is the world's largest producer and consumer country of common beans (*Phaseolus vulgaris*), an important nutritional source and socioeconomically significant crop (Kotue et al. 2018; Embrapa Arroz e Feijão 2021). This legume is widely cultivated in the country, being produced in three growing seasons and grown by farmers with different technological profiles, at different scales and production systems (Brusamarello et al. 2017). In this scenario, numerous challenges limit production potential, one of the main causes being biotic stresses (Basavaraja et al. 2020).

The common bean is host to a wide range of diseases caused by different pathogens that affect, to a

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greater or lesser extent, all cultivars recommended by genetic improvement programs (Assefa et al. 2019). Numerous studies made it possible to deepen knowledge on the variability of pathogens (Padder et al. 2017; Nietsche et al. 2002), inheritance of disease resistance (Souza et al. 2016; Costa et al. 2017), inoculation methods (Bigirimana and Höfte 2001; Rezende et al. 2018), standardization of disease response rating scales (Pastor-Corrales et al. 1992; Van-Schoonhoven and Pastor-Corrales 1987), in addition to identifying numerous resistance loci to different pathogen lines throughout genomes (QTL and genes with greater effect) using molecular markers (Keller et al. 2015; Bassi et al. 2017).

In Brazil, at least 15 epidemiologically important diseases can potentially cause severe damage (Wendland et al. 2018). Among the diseases that affect common bean crop, anthracnose (*Colletotrichum lindemuthianum*) and angular leaf spot (*Pseudocercospora griseola Sacc*.) stand out due to the significant production losses in major producing regions worldwide (Singh and Schwartz 2010). These diseases are caused by pathogens that exhibit pathotype diversity and a monogenic and/or quantitative inheritance pattern of resistance (Padder et al. 2017; Nay et al. 2019b), which hinder the development of genetically resistant cultivars. Depending on the level of susceptibility of the cultivar, these diseases can cause losses of 80–100% (Singh and Schwartz, 2010).

Genomic advances incorporated into breeding programs have enabled the identification of molecular tools for more effective marker-assisted selection (MAS). The strategy of identifying markers linked to disease resistance loci in the common bean has been widely used and once validated, many of the markers show significant MAS application potential (Burt et al. 2015; Perseguini et al. 2016; Zuiderveen et al. 2016; Wu et al. 2017; Lobaton et al. 2018; Gil et al. 2019; Nay et al. 2019a; Fritsche-Neto et al. 2019). In regards to the potential impacts, the implementation of molecular tools that aid the selection of resistant genotypes in breeding programs is a strong strategy in the early selection of target alleles, increasing efficiency and reducing time and costs in the selection of superior genotypes for breeding programs (Alvares et al. 2019).

For anthracnose, currently, 14 genes are characterized and officially accepted by the Genetics Committee of the Bean Improvement Cooperative - BIC, of which four have allelic series: *Co-1* (*Co-1*, *Co-1*^{2}, *Co-1*^{3}, *Co-1*^{4} and *Co-1*^{5}); *Co-2*; *Co-3* (*Co-3*, *Co-3*^{2}, *Co-3*^{3}, *Co-3*^{4} and *Co-3*^{5}); *Co-4* (*Co-4*, *Co-4*^{2} and *Co-4*^{3}); *Co-5* (*Co-5* and *Co-5*^{2}); *Co-6*; *co-8*; *Co-11*; *Co-12*; *Co-13*; *Co-14; Co-15; Co-16;* and *Co-17* [Chen et al. 2017; http://www.bic.uprm.edu]. For angular leaf spot, three resistance loci were mapped and named according to the BIC Genetics Committee, as *Phg-1* (AND 277), *Phg-2* (Mexico 54) and *Phg-3* (Ouro Negro). The *Phg-1* locus, of Andean origin, was mapped in the subtelomeric region of chromosome Pv01 in the AND 277 cultivar (Meziadi et al. 2016; Gonçalves-Vidigal et al. 2011) and has been used by breeding programs in Brazil. More recently, two QTLs ALS 4.1 (G5686) and ALS 10.1 (G5686 and CAL143) were renamed as *Phg-4* and *Phg-5* locus, respectively (Souza et al. 2016).

The main objective of this study was to evaluate and validate several SNP markers previously identified as linked to anthracnose resistance loci (*Co-4*^{2} and *Co-u*) and angular leaf spot resistance loci (*Phg-1, Phg-2* and *Phg-5*). Using a common bean diverse panel that included important sources of resistance for Brazilian agriculture and a segregating population (BRS Cometa × SEL 1308), we characterized these genotypes for the presence of resistance alleles to these diseases and validated potential SNP markers to be incorporated into MAS routine of Embrapa common bean breeding program.

Material and methods

Genetic material

We used 376 diverse common bean genotypes from several Brazilian and international breeding programs that show stable resistance responses to anthracnose and angular leaf spot diseases, including cultivars, elite parent plants and sources of resistance (donor parent plants). Information on the institution of origin, gene *pool*, grain type and reaction to anthracnose and angular leaf spot are presented in Table S1. Phenotypic information regarding the reaction to the two diseases was obtained for 240 of the 376 lines in this study, of which 139 were phenotyped for anthracnose and 101 for angular leaf spot (Table S1).

Ten seeds from each genotype were deposited on sheets of germitest paper, moistened with autoclaved

distilled water and then placed in a germinator (Mangelsdorf) at a constant temperature of 25 °C and 27% humidity for seven days. Two leaf discs obtained from the primary leaves through a puncher were individually collected and transferred to 96 sterile deep well plates. The plates were stored in a freezer at − 80 °C for 24 h and then submitted to lyophilization for six hours using a freeze dryer (Liotop® model L101). Finally, the plates were sent to Intertek Agritech (Sweden) for genotyping. This genotyping service performs KASP marker analysis (Rasheed et al. 2016).

Molecular analysis with SNP markers

Nine SNPs linked to disease resistance genes in common bean were selected from a portfolio of molecular markers made available by the "High Throughput Genotyping (HTPG)" project (Bohar et al. 2020) [https://cegsb.icrisat.org/marker-panels-2/].

Three of them are linked to anthracnose resistance genes (two with the *Co-4*^{2} from *Co-4* locus and one with the *Co-u* locus) (Cieslak et al. 2015; Oblessuc et al. 2015; Burt et al. 2015; Zuiderveen et al. 2016) and six are linked to angular leaf spot (one with the *Phg-1* gene, three with *Phg-2* and two with *Phg-5*) (Gonçalves-Vidigal et al. 2011; Lobaton et al. 2018; Nay et al. 2019a, Gil et al. 2019), as described in Table 1. Genotyping was performed at the genotyping provider Intertek AgriTech, through an agreement with HTPG (Bohar et al. 2020).

The snpPV0071 marker, linked to the *Phg-2* locus (Nay et al. 2019a), was the only one that displayed a monomorphic profile for the susceptible allele (G:G) and was discarded from the analysis. This marker is specific to tag the resistance *Phg-2* locus at G10474 line, not genotyped in this study.

Additionally, the snpP8282v3-817 (GRAF1) linked to the *Co-4* resistance locus (Cieslak et al. 2015) was evaluated through experiments with a TaqMan® hydrolysis probe (ThermoFisher) (Shen et al. 2009; Applied Biosystems 2021) conducted at the Biotechnology Laboratory at Embrapa Rice and Beans, as described below (DNA extraction and genotyping of target alleles).

A simple linear regression analysis was performed using R software, version 4.1.2 (R Core Team 2021), at significance level of $p<0.05$, based on the genotypes of the SNP markers associated with phenotypic values.

Validation of snpPV0070 and snpP8282v3-817 (*Co-4*^{2}), and snpPV0025 (*Phg-2*) markers

From the nine SNP markers, genotyped by Intertek Agritech, four SNPs that showed an association between the marker and disease resistance were submitted to an additional validation step as described

Table 1 SNP markers associated with common bean disease resistance genes and their respective identification, position in the genome and indication of genotypes carrying the target alleles

SNP	Allele	Chromosome	Pathogen	Gene *pool*	Resistant parent	Susceptible parent	Reference[a]
snpPV0051	*Phg-1*	Pv01	ALS	A	AND 277	–	GonçalvesVidigal et al. (2011)
snpPV0025	*Phg-2*	Pv08	ALS	M	G10474 e México 54	Sprite e VAX1	Lobaton et al. (2018)
snpPV0033	*Phg-2*	Pv08	ALS	M	G10474	Sprite e VAX1	Lobaton et al. (2018)
snpPV0071	*Phg-2*	Pv08	ALS	M	G10474	–	Nay et al. (2019a, b)[a]
snpPV0027	*Phg-5*	Pv10	ALS	A	G5686	Sprite	Lobaton et al. (2018)
snpPV0079	*Phg-5*	Pv10	ALS	A	G5686	–	Nay et al. (2019a, b)[a]
snpPV0068	*Co-4*^{2}	Pv08	AN	M	SEL 1308 e B09197	Black Magic e Nautica	Oblessuc et al. (2015). Burt et al. (2015)
snpPV0070	*Co-4*^{2}	Pv08	AN	M	SEL 1308 e B09197	Black Magic e Nautica	Oblessuc et al. (2015), Burt et al. (2015)
P8282v3-817	*Co-4*^{2}	Pv08	AN	M	SEL 1308	BRS Cometa	Cieslak et al. (2015)
snpPV0046	*Co-u*	Pv02	AN	M	Montcalm G	–	Zuiderveen et al. (2016)

SNP single nucleotide polymorphism, *R* resistant, *S* susceptible, *ALS* angular leaf spot, *AN* anthracnose, *M* Mesoamerican, *A* Andean

[a]Full references are in the references section

below. These markers were converted into TaqMan® (ThermoFisher) probes (Shen et al. 2009; Applied Biosystems 2021). In order to design the probes, the sequences containing target SNPs were aligned to the common bean reference genome (Schmutz et al. 2014) using the BLAST command, and a search for repetitive elements was conducted using RepeatMasker, with both analyses available on the Phytozome platform [Phytozome v12.1: Home (doe.gov)]. Probes were designed using the online Custom TaqMan Assay Design Tool (Thermo Fisher) available at [https://www.thermofisher.com/order/custom-genomic-products/tools/genotyping/].

Segregating population and contrasting lines

The TaqMan® SNP markers linked to the anthracnose resistance allele *Co-4*^{2} were validated using an F_2 population of 149 plants, derived from the cross between BRS Cometa ♀ (female parent, susceptible to *C. lindemuthianum* pathotype 73) and SEL 1308 ♂ (male parent, harboring the allele *Co-4*^{2}, resistant to pathotype 73) (Table S2), which was phenotyped for the reaction to *C. lindemuthianum* pathotype 73. The TaqMan® SNP marker linked with angular leaf spot (*Phg-2*) was validated in a set of 30 contrasting common bean genotypes for the angular leaf spot reaction (Table S3), evaluated under field conditions in different tests performed by the Embrapa common bean breeding program.

Inoculation of *Colletotrichum lindemuthianum* and disease evaluation

The *C. lindemuthianum* isolate CL1869 (pathotype 73) was used to inoculate 149 F_2 plants from the cross between BRS Cometa x SEL 1308. F_2 seeds, as well as ten seeds from each parent plant and the susceptible control (Rosinha G2) were sown in polystyrene seedling trays. Plants were inoculated seven days after sowing, at stage V2 (fully expanded primary leaves) (Pastor-Corrales et al. 1992). The spore solution (1.2×10^6 spores mL-^{1}) was applied to the abaxial and adaxial surfaces of the primary leaves with the aid of a hand sprayer (De Vilbiss, No. 15). After inoculation, the plants were incubated in a humidity chamber for 48 h, at 20 ± 2 °C, with relative humidity of around 95%, controlled by a misting system, and a 12-hour light/dark photoperiod. Next, misting was interrupted, and the inoculated plants were kept in a controlled environment under the same temperature and photoperiod described above, where they remained until the disease symptoms were evaluated.

Symptoms were assessed seven days after inoculation, based on a grading scale proposed by Pastor-Corrales and Tu (1989), in which grade 1 represents no symptoms and 9 dead plants due to fungal disease. Plants with grades between 1 and 3 were considered resistant and the others susceptible (Table S2).

DNA extraction and genotyping of markers for target alleles

Genomic DNA from 149 F_2 plants (BRS Cometa × SEL 1308) (Table S2) and from the set of 30 contrasting genotypes related to angular leaf spot reaction (Table S3) was extracted using the CTAB method, according to the protocol proposed by Doyle and Doyle (1990), modified by Ferreira and Grattapaglia (1998). DNA concentration was estimated using a Qubit® (Thermo Scientific®, Waltham, USA) fluorometer, and integrity visualized by electrophoresis on 1.0% agarose gel stained with ethidium bromide.

TaqMan® SNP genotyping assays were amplified with the Taqman® GTXpress™ (Thermo Fisher Scientific, Waltham, MA, USA) reagent, according to the manufacturer's guidelines. Amplification was conducted using the QuantStudio 7 Flex Real-Time PCR system (Applied Biosystems) under the following conditions: 60 °C for 30 s, 95 °C for 20 s, followed by 50 cycles of 95 °C for 3 s and 60 °C for 30 s, and a final extension of 60 °C for 30 s. This was followed by allele analysis using the Genotyping Analysis Module, V.3.7.

Genetic-statistical analysis

The phenotypic and genotypic data for anthracnose reaction in the F_2 generation (BRS Cometa × SEL 1308) were submitted to the chi-square test ($\chi 2$) to test the 3R_:1rr and 1RR:2Rr:1rr segregation hypotheses (R: resistant; rr: susceptible), respectively, adopting a 5% significance level. Linkage analysis between the SNP marker and the *Co-4*^{2} allele was performed using the OneMap package (Margarido et al. 2007) and the estimated recombination

frequency converted into genetic distance (cM). All analyses were conducted using R software, version 4.1.2 (R Core Team 2021).

The selection efficiency (SE) for codominant markers was estimated according to the methodology described by Liu (1998), using the following estimator:

$SE(\%) = \left(1 - 4rf^2\right) \times 100$, where "rf" is the recombination frequency.

Results and discussion

Eight of the nine SNPs that were selected out of the HTPG project data and genotyped at Intertek (88.8%) were polymorphic and were considered suitable for genotyping analysis.

SNP markers linked to the *Phg* resistance alleles

Of the seven markers that exhibit a technically adequate genotyping profile, snpPV0033 did not amplify the target allele in the sources carrying the *Phg-2* allele.

The *Phg-1* allele was amplified by the snpPV0051 marker (A:G, where the underline allele is linked to resistance) in only 103 (27.4%) of the 376 lines evaluated. This SNP marker is located in a repetitive region of the common bean genome, identified using the RepeatMasker tool (Table 2), which may explain why the fragment was not amplified well. Of the 103 genotyped lines, the "A" allele linked to *Phg-1* was amplified in 11 lines (Table S4), including AND 277, the source of the *Phg-1* (Souza et al. 2016) and resistant to the 11 most important *P. griseola* pathotypes in Brazil, such as 63–23, 63–31, 63–47 and 63–63 (Damasceno-Silva et al. 2015). Pathotype 63–63 is considered the most aggressive, causing susceptibility symptoms in all differential cultivars from the Andean and Mesoamerican common bean gene *pool* (Nay et al. 2019b). The angular leaf spot resistant line CAL 143 was derived from the cross between G12229 and AND 277 (Nay et al. 2019b) and contains the allele "A" at the snpPV00051 for the *Phg-1* resistance locus in its genome (Table S4). Furthermore, the *Phg-1* and *Co-1⁴* alleles were reported to be strongly linked, at a distance of 0.0 cM (Gonçalves-Vidigal et al. 2011), jointly conferring resistance to the *P. griseola* pathotype 63–23 and *C. lindemuthianum* pathotypes 65, 73 and 2047 (Gonçalves-Vidigal et al. 2011). Due to the inconsistent amplification of snpPV0051 marker in the evaluated lines, it was not validated in a reduced set of contrasting lines for resistance to angular leaf spot. However, the snpPV0051 marker has potential for use in the assisted selection of populations from crosses with known sources of the *Phg-1* allele.

The snpPV0025 marker (G:T) linked to *Phg-2* allele, located on the Pv08 chromosome, exhibited the "G" allele in the Mexico 54 and Cornell 49–242 breeding lines (Table S5), which are known sources of *Phg-2* (Souza et al. 2016; Nay et al. 2019b). *Phg-2* has a wide diversity of functional haplotypes for a resistance gene in common bean (Nay et al. 2019b),

Table 2 Genotyping summary of 10 SNP markers associated with common bean disease resistance genes

SNP	Locus/Allele	Polymorphism	Allele 1	Allele 2	Amplified?	NA	%NA	Monomorphic?	RepeatMasker?
snpPV0051	*Phg-1*	A:G	A:A (R)	G:G	Yes	273/376	72.60		Yes
snpPV0025	*Phg-2*	G:T	G:G (R)	T:T (S)	Yes	0/376	0.00		No
snpPV0033	*Phg-2*	T:C	T:T (R)	C:C (S)	Yes	0/376	0.00		No
snpPV0071	*Phg-2*	T:G	T:T (R)	G:G (S)	Yes	0/376	0.00	Yes	No
snpPV0027	*Phg-5*	T:C	T:T (R)	C:C (S)	Yes	1/376	0.26		No
snpPV0079	*Phg-5*	A:G	A:A (R)	G:G (S)	Yes	2/376	0.53		No
snpPV0068	*Co-4²*	G:C	G:G (R)	C:C (S)	Yes	0/376	0.00		No
snpPV0070	*Co-4²*	G:T	G:G (R)	T:T (S)	Yes	25/376	6.60		No
snpP8282v3-817	*Co-4²*	A:G	A:A (R)	G:G (S)	Yes	1/181	0.55		No
snpPV0046	*Co-u*	A:G	A:A (R)	G:G (S)	Yes	2/376	0.53		No

SNP single nucleotide polymorphism, *R* resistant, *S* susceptible, *NA* no amplification

The underline alleles are linked to resistance

confers resistance to *P. griseola* pathotypes 63–19 and 63–39, and was found to be the most relevant allele for angular leaf spot resistance in Brazil (Bassi et al. 2017). It was also detected in the PT 65 line, which has shown a high level of resistance to angular leaf spot under controlled inoculation and in the field (Pereira et al. 2019; Pereira et al. 2016). The line MAIII 16.159 from the recurrent selection program for angular leaf spot resistance conducted at the Universidade Federal de Lavras (Pereira et al. 2019) and 11 lines from Embrapa's breeding program also displayed the "G" allele of the snpPV0025 marker, suggesting the presence of the resistant *Phg-2* allele (Table S5). In addition, studies showed that *Phg-2* was responsible for the resistance found in breeding lines evaluated under field and greenhouse conditions, and effective against *P. griseola* isolates from Colombia, Uganda and Brazil (Sartorato et al. 2000; Nay et al. 2019b). *Phg-2* different resistance haplotypes that confer a broad spectrum of resistance to different *P. griseola* pathotypes from the Andean and Mesoamerican gene *pool* were described by Nay et al. (2019a).

Due to its importance for breeding programs, the use of *Phg-2* allele sources has been frequently adopted in crossing blocks, in addition to studies that seek to develop markers strongly linked to *Phg-2* (Gil et al. 2019; Miller et al. 2018; Sartorato et al. 2000). For example, the improved MAB 348, MAB 349, MAB 351, MAB 352, MAB 353, MAB 354 and MAB 484 lines, with high angular leaf spot resistance, contain the G10474 line, source of *Phg-2*, as one of their parents (Gil et al. 2019). The results of this study indicate that the *Phg-2* allele is present in elite germplasms developed by Embrapa, Universidade Federal de Lavras—UFLA, Instituto Agronômico de Campinas—IAC, Instituto de Desenvolvimento Rural do Paraná—IDR/IAPAR, Agropecuária Terra Alta—TAA and International Center for Tropical Agriculture—CIAT, and it has been detected in 72 lines/cultivars (Table S5). However, as pointed out by Gi et al. (2019), care should be taken when using the snpPV0025 (ALS_08_62193174) for MAS, since it has been used to tag and introgress the *Phg-2* allele from Mesoamerican MAB sources into Andean breeding lines, and do not tag specific Mesoamerican alleles. Nay et al. (2019a) shed greater light on this by identifying pathotype-specific haplotypes at the *Phg-2* gene and offering new molecular markers to be tested and used in MAS.

The markers snpPV0027 (T:C) and snpPV0079 (A:G), both linked to the *Phg-5* allele, contained the "T" and "A" alleles, respectively, only in the G5686 line identified as a *Phg-5* source (Keller et al. 2015). The *Phg-5* gene from the line G5686 originates from the Andean gene *pool* and confers resistance to several *P. griseola* pathotypes from the Andean and Mesoamerican origins. Mahuku et al. (2009) studied the reaction of the G5686 line against 15 *P. griseola* pathotypes and reported resistance for 53 and 72% of the Andean and Mesoamerican isolates tested, respectively. However, for some races reported as the most frequent and wide distributed in Brazil, such as 63–23, 63–31, 63–47 and 63–63, the line G5686 carrying the *Phg-5* resistance allele was characterized as moderately susceptible or susceptible (Mahuku et al. 2009), limiting their use as source of resistance by the common bean breeding programs in Brazil.

In the common bean—*P. griseola* pathosystem, combining the *Phg-1* (Andean) and *Phg-2* (Mesoamerican) alleles would be important to develop plants resistant to the pathotypes 63–19, 63–23 and 63–39 (Bassi et al. 2017). The molecular characterization of Embrapa's breeding germplasm allowed the identification of elite lines containing pyramided alleles, such as CNFC 16636 (*Phg-1* + *Phg-2*) (Table S4) and the differential varieties for anthracnose G2333, G2858 and PI 207262 (*Co-u* + *Co-4*^{2}) (Table S6). This result demonstrates the need to obtain lines that simultaneously combine alleles that confer resistance to anthracnose and angular leaf spot, in addition to the other agronomic characteristics demanded by the market.

SNP markers linked to the *Co* resistance alleles

The *Co-4* locus is located close to a telomeric region of the chromosome Pv08, characterized by containing about 18 copies of the *COK-4* gene and described as being associated with anthracnose resistance in the common bean (Oblessuc et al. 2015). In the present study, four SNP markers linked with the anthracnose-resistant locus were analyzed. In case of the markers snpPV0068 (G:C) and snpPV0070 (G:T), the "G" alleles (of both SNPs) were identified in the G-2333, SEL 1308, PI 207262, K-10, K-13, and CNFC 5547 lines (Table S6), which are

known sources of the resistant allele *Co-4*^{2} (Kelly and Vallejo 2004; Vieira et al. 2018). Another 22 black seeded lines also shown this same allele (Table S6). Some of these lines, already characterized in terms of resistance/susceptibility, are part of the differential varieties of anthracnose pathotypes or are originated from anthracnose resistance breeding programs (Table S6). The presence of "G" alleles in the two SNPs also coincided with the polymorphisms identified in the snpP8282v3-817 marker (GRAF1, A:G) (Cieslak et al. 2015) in detecting the *Co-4*^{2} allele in the lines K-10, K-13 and CNFC 5547 (Table S6). However, there was no "A" allele amplification of the snpP8282v3-817 marker in the 22 black seeded lines amplified by snpPV0070 (Table S6). This result indicates that snpPV0070 is not specific for the *Co-4*^{2} allele, but capable of detecting resistance alleles for the *Co-4* locus. An alternative to snpPV0070 are the markers snpPV0068 and snpP8282v3-817, both amplified in lines that are known to carry the *Co-4*^{2} allele (Table S6). Portilla et al (2021) when evaluating the *Co-4* marker snpPV0069 (closely linked to the snpPV0068 and snpPV0070), developed as tagging the resistance from G2333 genotype (Lobaton et al. 2018) and available at HTPG portfolio, did not identify any significant effect of association, which was suggestive of a race specific resistance gene interaction. The *Co-4*^{2} is a dominant allele of the *Co-4* locus and has been used by breeding programs worldwide (Kelly and Vallejo 2004; Vieira et al. 2018). This is because its broad spectrum of resistance to several *C. lindemuthianum* pathotypes (Balardin and Kelly 1998; Silvério et al. 2002).

For the snpPV0046 (A:G, *Co-u* locus), associated with the *Co-u* allele, the "A" allele is linked to the resistance to anthracnose (Zuiderveen et al. 2016; Oblessuc et al. 2014; Geffroy et al. 2008). In this study, the "A" allele was identified in 51 of the 376 lines genetically characterized with snpPV0046, including the BAT 93 breeding line (Table S7), which contains the parental genotype PI 207262 (Geffroy et al. 2008) and is a source of the *Co-u* allele (Geffroy et al. 2008). In the present study, the "A" allele was also amplified in PI 207262, suggesting that *Co-u* may come from this parent or from a mutation that occurred during the development of BAT 93. Of the 51 lines that contain the "A" allele, 13 are from the black seeded group, 4 from the Carioca group and the remainder belong to different commercial classes, such as "rajado", jalo, white, and calima (Table S7), which are detected in Andean and Mesoamerican gene *pools*. Of these, 12 are resistant cultivars that belong to differentiating varieties for anthracnose pathotypes (Table S7). The *Co-u* locus is located in the Pv02, very close to *I* locus, conferring resistance to important common bean viruses, such as the *Bean common mosaic virus*, potyviruses and comovirus (Meziadi et al. 2016; Geffroy et al. 2008). At the molecular level, these two loci may have a common origin, since most resistance genes in plants encode nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins, responsible for recognizing several pathogens (Geffroy et al. 2009). Recent information confirms this hypothesis, since the functional annotation of resistance genes has revealed common protein motifs, such as: Leucine Zippers-LZ, Leucine Rich Repeat-LRR and protein kinase domains (Protein Kinase—PK) (Fritsche-Neto et al. 2019; Nogueira et al. 2019; Queiroz et al. 2019; Nay et al. 2019a; Banoo et al. 2020; Gonçalves-Vidigal et al. 2020; Costa et al. 2021). Thus, validating the snpPV0046 marker for assisted selection is particularly important for programs aimed at incorporating multiple resistance into elite germplasms.

In addition, the introgression of resistance alleles from different gene *pools* (Andean and Mesoamerican) in the same line by marker-assisted selection is an important strategy for developing common bean cultivars with broad and long-term resistance (Miller et al. 2018; Vieira et al. 2018; Miklas et al. 2006; Kelly and Miklas 1998; Guzmán et al. 1995). In Brazil, an example of an important combination of anthracnose resistance alleles is that of the Mesoamerican allele *Co-4*^{2} and the Andean allele *Co-1*^{4} in SEL1308 and K13 breeding lines (Vieira et al. 2018; Souza et al. 2014).

Simple linear regression analysis of SNP marker effects

Of the eight SNP markers analyzed, five (snpPV0046, snpPV0068, snpPV0070, snpP8282v3-817 and snpPV0025) showed potential for indirect selection of common bean genotypes containing the *Co-4*^{2} and *Phg-2* alleles and were therefore submitted to simple linear regression analysis. Only for the marker snpPV0046 the regression model was significant (Table S8), and it does not explain much

of the variability (low R^2 of 3%). Although marker effects were not significant in the sample set of lines containing information regarding the reaction to anthracnose and angular leaf spot (snpPV0046 = 133; snpPV0068 = 134; snpPV0070 = 115; snpP8282v3-817 = 46 and snpPV0025 = 78), all lines carrying the target SNPs showed resistance, suggesting that these alleles can be monitored by the linked markers in the lines of interest, paretal screening and even during the population breeding. The absence of significance in the regression test is certainly because many lines, even though resistant, did not exhibit the target allele of this study, suggesting the presence of other anthracnose and angular leaf spot resistance alleles. When the regression analysis was applied in a subset of genotypes contrasting for the resistance/susceptibility to the anthracnose and angular leaf spot diseases (Table S9), the *Co-4²* and *Phg-2* marker effects were significant (Table 3) and the slope values were negative for all markers (Table 3), revealing an association between the SNP markers and resistance alleles for anthracnose and angular leaf spot. The introgression of resistance alleles into elite bean germplasms assisted by molecular markers is a promising strategy in breeding programs, given that it reduces time and costs in the initial selection stages (Sakiyama et al. 2014). Figure 1 illustrates the importance of the resistance genes *Co-u* ("A") and *Phg-2* ("G"), and the resistance allele *Co-4²* ("G") in reducing mean phenotypic values in the lines that contain them.

Additionally, gene annotation revealed that snpPV0046 (*Co-u*), snpPV0068 (*Co-4²*), snpPV0070 (*Co-4²*) and snpP8282v3-817 (*Co-4²*) are located in gene regions that encode defense proteins in plants (Table 4). The snpPV0046 marker is found in the gene that encodes the Mitogen-Activated Protein Kinase (MAPK), which interacts with salicylic acid, a plant hormone known to play a role in plant-acquired resistance against pathogen infection (Jagodzik et al. 2018). The snpPV0068 marker is located in the MYB Transcription Factor coding region, playing an essential role in the control of cellular processes in response to biotic and abiotic stresses (Ambawat et al. 2013), such as resistance to *Pseudomonas syringae pv. tomato* (*Pst* DC3000) in *Arabidopsis* (Zhang

Table 3 Summary of the regression analysis framework between SNP markers and resistance reaction to anthracnose or angular leaf spot of common bean elite genotypes

Source of variation	Df	SS	MS	F-value	*p*-value	R^2	Inclination[b]
snpPV0046 (*Co-u*)—Anthracnose							
A versus G[a]	1	283.9	283.9	255.7	2.2E−16	0.86	− 2.54
Residual	42	46.6	1.1				
snpPV0068 (*Co-4²*)—Anthracnose							
G versus C	1	158.5	158.5	175.7	2.97E−12	0.88	− 2.95
Residual	23	20.7	0.9				
snpPV0070 (*Co-4²*)—Anthracnose							
G versus T	1	167.9	167.9	93.0	2.14E−10	0.77	− 2.41
Residual	28	50.6	1.8				
snpP8282v3-817 (*Co-4²*)—Anthracnose							
A versus G	1	112.2	112.2	78.2	2.45E−07	0.83	− 3.03
Residual	15	21.5	1.4				
snpPV0025 (*Phg-2*)— Angular Leaf Spot							
G versus T	1	66.2	66.2	72.2	4.52E−08	0.78	− 1.76
Residual	20	18.3	0.9				

Df degree of freedom, *SS* sum of squares, *MS* mean squares

The underline alleles are linked to resistance

[a]Contrast considered in the regression analysis between marker alleles and the severity of anthracnose or angular leaf spot

[b]Angular coefficient of the linear regression equation. The negative sign on the slope indicates that the allele is associated with resistance

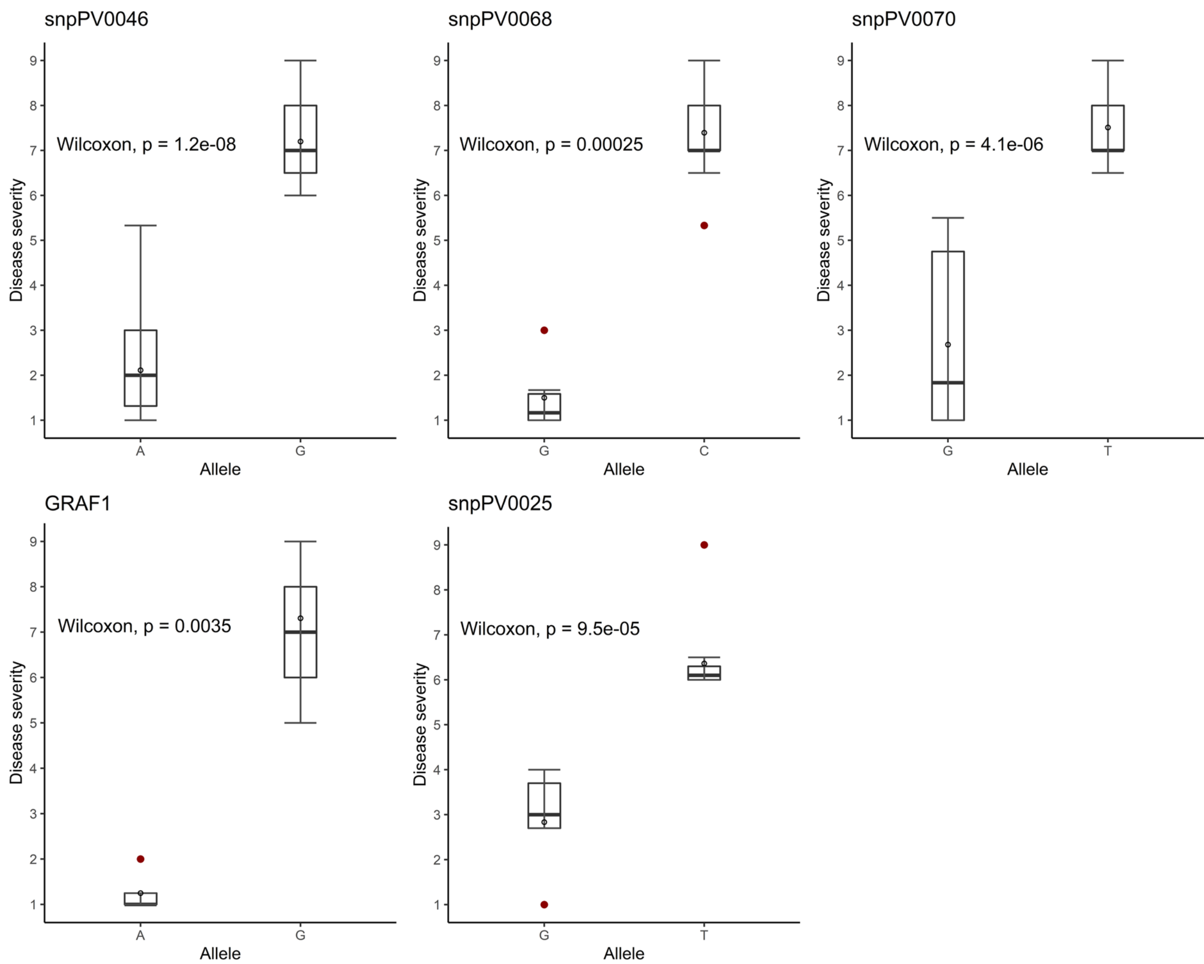

Fig. 1 Difference in phenotypic variance between common bean genotypes containing the 'A' (*Co-u*—snpPV0046), 'G' (*Co-4*^{2}—snpPV0068, snpPV0070 and GRAF1-snpP8282v3-817) and 'G' alleles (*Phg-2*—snpPV0025) for resistance to anthracnose (*Co*) and angular leaf spot (*Phg*) diseases. The non-parametric Wilcoxon test (1945) was performed considering 44, 30, 21, 17 and 22 lines for markers snpPV0046, snpPV0068, snpPV0070, snpP8282v3-817 and snpPV0025, respectively

Table 4 Gene annotation performed on the Phytozome platform for the five SNP regions associated with common bean disease resistance genes selected for analysis, validation and marker-assisted selection

SNP marker	Locus/Allele	Primary transcript	Description
snpPV0025	*Phg-2*	Phvul.008G280700.2	PTHR11132:SF38—GB
snpPV0046	*Co-u*	Phvul.002G328300.1	Mitogen-Activated Protein Kinase 16-Related
snpPV0068	*Co-4*^{2}	Phvul.008G028000.1	MYB Transcription Factor
snpPV0070	*Co-4*^{2}	Phvul.008G028400.1	Dominio Serine Threonine Kinase/homólogo ao gene *COK-4*
snpP8282v3-817	*Co-4*^{2}	Phvul.008G028200	Dominio Serine Threonine Kinase

SNP single nucleotide polymorphism

et al. 2019). The snpPV0070 and snpP8282v3-817 markers are located in the Phvul.008G028400 and Phvul.008G028200 transcripts, respectively, homologous to the *COK-4* locus (STK domain) (Table 4), previously described as an anthracnose resistance source in common bean (Melotto and Kelly 2001).

Validation of the snpPV0070, snpP8282v3-817 and snpPV0025 markers

TaqMan® hydrolysis probes were developed for the snpPV0070 and snpP8282v3-817 markers linked to the *Co-4²* allele. 149 F_2 plants from the cross between BRS Cometa and SEL1308 were genotyped with these two probes and phenotyped for reaction to *C. lindemuthianum* pathotype 73 (Table S2). Of these, 110 were characterized as resistant and 39 as susceptible, in line with the expected ratio of 3R:1S ($\chi 2=0.11$; $p=0.74$) (Table 5). Previous studies support the hypothesis that resistance to anthracnose in SEL 1308 is controlled by a single major gene, with complete dominance (Young et al. 1998; Oblessuc et al. 2015). Markers snpPV0070 and snpP8282v3-817 maintained 1RR:2Rr:1rr ratio (Table 5), segregating as expected for codominant markers. Linkage analysis revealed that the snpPV0070 and snpP8282v3-817 used to genotype the F_2 population (BRS Cometa x SEL 1308) are strongly linked to the *Co-4²* allele, with a recombination frequency of 0.026 (2.6 cM) and 0.019 (1.9 cM), respectively (Table 5). Markers snpPV0070 and snpP8282v3-817 showed selection efficiency (SE) of 99.7% and 99.8%, respectively, indicating the high potential value of molecular markers in strong linkage disequilibrium in MAS routine of breeding programs.

The TaqMan® probe developed for snpPV0025 (G:T) linked to the *Phg-2* allele was evaluated in a set of contrasting elite lines for resistance to angular leaf spot (Table S3). This target SNP was able to detect the *Phg-2* allele, despite its amplification in varieties that are susceptible to angular leaf spot (Table S3). This is in accordance with previously reported that snpPV0025 would only effectively tag *Phg-2* in Andean genetic backgrounds. Additional SNP markers have been identified as associated to with *Phg-2* and should be tested (Gil et al. 2019; Nay et al. 2019a). On the other hand, the complementary analysis performed with RAPD_SEO4 (Sartorato et al. 1999) and STS_g796 (Miller et al. 2018) markers resulted in specific markers for the

Table 5 Genotypic and phenotypic segregation of individuals from the F_2 population derived from the crossing between BRS Cometa × SEL 1308 (*Co-4²*), evaluated for reaction to *Colletotrichum lindemuthianum* pathotype 73, the causal agent of anthracnose in the common bean

Genotype/Phenotype	Observed ratio	Expected ratio	Hypothesis	χ^2	*P*-value	rf	Distance[a]	SE
snpPV0070								
T:T	40	37	1:2:1	0.72	0.70	0.026	2.6 cM	99.7%
G:T	76	75						
G:G	33	37						
P8282v3-817								
G:G	39	37	1:2:1	0.65	0.72	0.019	1.9 cM	99.8%
A:G	77	75						
A:A	33	37						
Class								
Resistant (1–3)	110	112	3:1	0.11	0.74	–	–	–
Susceptible (4–9)	39	37						
Total	149	149	–	–	–	–	–	–

The underline alleles are linked to resistance

rf recombination fraction, *SE* selection efficiency

[a]Distance in centiMorgans; *P*-value associated to the null hypothesis not rejected (1:2:1 for molecular markers data and 3:1 for phenotype data); χ^2, chi-square; snpPV0070: "G:G" dominant homozygous for *Co-4²*, "G:T" heterozygous for *Co-4²* and "T:T" recessive homozygous for *Co-4²*; snpP8282v3-817: "A:A" dominant homozygous for *Co-4²*, "A:G" heterozygous for *Co-4²* and "G:G" recessive homozygous for *Co-4²*

Phg-2 locus, amplifying in the resistance sources Mexico 54 and MAR-2 (Table S3). These two markers have been frequently used in studies aimed at *Phg-2* gene introgression (Sanglard et al. 2016; Miller et al. 2018). In addition, SEO4 also amplified in lines DM 108, CNFC 17142, CNFC 17395 and CNFC 18710; while g796 amplified in lines CNFC 15086 and CNFC 17153 (Table S3). Among the parental lines used by the Embrapa breeding program in crossing blocks, DM108 stands out, originating from the cross between BRS Rudá and MAR-2 (Sanglard et al. 2016). The MAR-2 line contains the resistant *Phg-2* allele in its genome; however, the allelic variation of this locus in this variety is still not clear (Nay et al. 2019a). Thus, the presence of the *Phg-2* allele in the elite lines from Embrapa common bean breeding program may be related to the previous use of the DM108 line as parental line in the crossing blocks. The *Phg-2* allele is particularly important in angular leaf spot resistance, since it confers resistance to the most prevalent pathotypes in Brazilian common bean growing areas (Bassi et al. 2017).

Conclusion

Seven of the nine SNP markers provided by the HTPG project showed potential for routine use in MAS at Embrapa common bean breeding program (snpPV0025—*Phg-2*; snpPV0027—*Phg-5*; snpPV0046—*Co-u*; snpPV0068—*Co-4*^{2}; snpPV0070—*Co-4*^{2}; snpP8282v3-817—*Co-4*^{2}; snpPV0079—*Phg-5*) and other breeding programs interested in these resistance alleles.

The markers snpPV0025 and snpPV0070 linked to *Phg-2* and *Co-4* loci, respectively, are indicated to monitor the presence of target alleles in crosses involving well-characterized parental lines, such as Mexico 54 (*Phg-2*) and SEL1308 (*Co-4*^{2}).

The genotyping systems based on hydrolysis probes developed in this study (TaqMan® SNP) for the snpPV0070 and snpP8282v3-817 markers (*Co-4*^{2}), and snpPV0025 marker (*Phg-2*) enabled the specific amplification of target alleles and are therefore suitable for use in MAS.

In addition, snpPV0070 and snpP8282v3-817 markers showed high selection efficiency (99.7 and 99.8%, respectively) for the allele *Co-4*^{2} that confers anthracnose resistance and may be used to considerably improve efficiency in identifying superior genotypes in the common bean breeding programs.

Acknowledgements This paper is a cooperative contribution on validation of SNP markers linked to disease resistance genes for their effective use in the routine of Embrapa (Brazilian Agricultural Research Corporation) common bean breeding program. The main goal was to characterize and select elite parent lines according the presence of disease resistance alleles, as well as validate SNP markers for MAS of superior genotypes from populations derived from these parent lines. It reports an elegant example of cross validation and effective use of SNP markers for MAS in an applied breeding program of a very social important orphan crop (common bean or dry bean).

Author contributions LG-M, RV, LM and TS planned the study; LG-M, LR and JM-J were responsible for the laboratory analysis; APM developed and phenotyped the F_2 population; BR provided the sequence and expertise of the SNP markers; LG-M, HP and TS was responsible for the data analysis; LG-M and RV drafted the manuscript; The final version of the manuscript was revised by all authors.

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Data availability Relevant data are included in this paper and its associated Online Resources.

Declarations

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References

Alvares RC, Stonehouse R, Souza TLPO, Miklas P, Bett KE, Melo LM, Rodrigues LA, Souza LL, Pereira HS (2019) Generation and validation of genetic markers for the selection of carioca dry bean genotypes with the slow-darkening seed coat trait. Euphytica 215:141. https://doi.org/10.1007/s10681-019-2461-y

Ambawat S, Sharma P, Yadav NR, Yadav RC (2013) MYB transcription factor genes as regulators for plant responses: an overview. Physiol Mol Biol Plants 19:307–321. https://doi.org/10.1007/s12298-013-0179-1

Applied Biosystems (2021) TaqMan® SNP Genotyping Assays USER GUIDE. https://assets.thermofisher.com/TFSAssets/LSG/manuals/MAN0009593_TaqManSNP_UG.pdf. Accessed 29 June 2021

Assefa T, Mahama A, Brown AV, Cannon EKS, Rubyogo JC, Rao IM, Blair MW, Cannon SB (2019) A review of breeding objectives, genomic resources, and marker-assisted methods in common bean (*Phaseolus vulgaris* L.). Mol Breed 39:20. https://doi.org/10.1007/s11032-018-0920-0

Balardin RS, Kelly JD (1998) Interaction between *Colletotrichum lindemuthianum* races and gene *pool* diversity in *Phaseolus vulgaris*. J Am Soc Hort Sci 123:1038–1047

Banoo A, Nabi A, Rasool RS, Mahiya-Farooq Shah MD, Ahmad M, Sofi PA, Aasiya-Nabi Itoo H, Sharma PN, Padder BA (2020) North-western himalayan common beans: population structure and mapping of quantitative anthracnose resistance through genome wide association study. Front Plant Sci 11:571618. https://doi.org/10.3389/fpls.2020.571618

Basavaraja T, Pratap A, Dubey V, Gurumurthy S, Gupta S, Singh NP (2020) Molecular and conventional breeding strategies for improving biotic stress resistance in common bean. In: Gosal SS, Wani SH. (eds) Accelerated plant breeding 3: 389–421. https://doi.org/10.1007/978-3-030-47306-8_13

Bassi D, Briñez B, Rosa JS, Oblessuc PR, Almeida CP, Nucci SM, Silva LCD, Chiorato AF, Vianello RP, Camargo LEA, Blair MW, Lasry L (2017) Linkage and mapping of quantitative trait loci associated with angular leaf spot and powdery mildew resistance in common beans. Genet Mol Biol 40:109–122. https://doi.org/10.1590/1678-4685-GMB-2015-0314

Bigirimana J, Höfte M (2001) Bean anthracnose: inoculation methods and influence of plant stage on resistance of *Phaseolus vulgaris* cultivars. J Phytopathol 149:403–408. https://doi.org/10.1111/j.1439-0434.2001.tb03870.x

Bohar R, Chitkineni A, Varshney RK (2020) Genetic molecular markers to accelerate genetic gains in crops. Biotechniques 69:158–160. https://doi.org/10.2144/btn-2020-0066

Brusamarello AP, Oliveira PH, Sebim DE, Baretta DR (2017) Performance of bean (*Phaseolus vulgaris* L.) genotypes in the second-season under high and low technology management in Parana, Brazil. Acta Agron 66:436–441. https://doi.org/10.15446/acag.v66n3.55555

Burt AJ, William HM, Perry G, Khanal R, Pauls KP, Kelly JD, Navabi A (2015) Candidate gene identification with SNP marker-based fine mapping of anthracnose resistance gene *Co-4* in common bean. PLoS One 10:e0139450. https://doi.org/10.1371/journal.pone.0139450

Chen M, Wu J, Wang L, Mantri N, Zhang X, Zhu Z, Wang S (2017) Mapping and genetic structure analysis of the anthracnose resistance locus Co-1HY in the common bean (*Phaseolus vulgaris* L.). PLoS ONE 12:e0169954. https://doi.org/10.1371/journal.pone.0169954

Cieslak JF, Coelho GRC, Souza TLPO, Vianello RP (2015) SNP discovery in genomic regions flanking the common bean anthracnose resistance locus *Co-4*. Annu Rep Bean Improv Coop 58:39–40

Costa LC, Nalin RS, Dias MA, Ferreira ME, Song Q, Pastor-Corrales MA, Hurtado-Gonzales OP, Souza EA (2021) Diferent loci control resistance to diferent isolates of the same race of *Colletotrichum lindemuthianum* in common bean. Theor Appl Genet 134:543–556. https://doi.org/10.1007/s00122-020-03713-x

Costa LC, Nalin RS, Ramalho MAP, de Souza EA (2017) Are duplicated genes responsible for anthracnose resistance in common bean? PLoS One 12:e0173789. https://doi.org/10.1371/journal.pone.0173789

Damasceno-Silva KJ, Souza EA, Freire CNS, Ishikawa FH (2015) Anastomosis groups and molecular variation in *Pseudocercospora griseola*. Gen Mol Res 14:7436–7445. https://doi.org/10.4238/2015.July.3.19

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

Embrapa Arroz e Feijão (2021) Dados conjunturais da produção de feijão-comum (*Phaseolus vulgaris* L.) e caupi (*Vigna unguiculata* (L.) Walp) no Brasil (1985 a 2019): área, produção e rendimento. Santo Antônio de Goiás: Embrapa Arroz e Feijão. http://www.cnpaf.embrapa.br/socioeconomia/index.htm. Accessed 27 June 2021

Ferreira ME, Grattapaglia D (1998) Introdução ao uso de marcadores moleculares em análise genética. Embrapa-Cenargen, Brasília

Fritsche-Neto R, Souza TLPO, Pereira HS, Faria LC, Melo LC, Novaes E, Brum IJB, Jean-Luc Jannink (2019) Association mapping in common bean revealed regions associated with anthracnose and angular leaf spot resistance. Sci Agric 76:321–327. https://doi.org/10.1590/1678-992X-2017-030

Geffroy V, Macadré C, David P, Pedrosa-Harand A, Sévignac M, Dauga C, Langin T (2009) Molecular analysis of a large subtelomeric nucleotide-binding-site-leucine-rich-repeat framily in two representative genotypes of the major gene *pools* of *Phaseolus vulgaris*. Genetics 181:405–419. https://doi.org/10.1534/genetics.108.093583

Geffroy V, Sévignae M, Billant P, Dron M, Langin T (2008) Resistance to *Colletotrichum lindemuthianum* in *Phaseolus vulgaris*: a case study for mapping two independent genes. Theor Appl Genet 116:407–415. https://doi.org/10.1007/s00122-007-0678-y

Gil J, Solarte D, Lobaton JD, Mayor V, Barrera S, Jara C, Beebe S, Raatz B (2019) Fine-mapping of angular leaf spot resistance gene *Phg-2* in common bean and development of molecular breeding tools. Theor Appl Genet 132:2003–2016. https://doi.org/10.1007/s00122-019-03334-z

Gonçalves-Vidigal MC, Cruz AS, Garcia A, Kami J, Vidigal Filho PS, Sousa LL, MeClean P, Gepts P, Pastor-Corrales MA (2011) Linkalge 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. Theor Appl Genet 122:893–903. https://doi.org/10.1007/s00122-010-1496-1

Gonçalves-Vidigal MC, Gilio TAS, Valentini G, Vaz-Bisneta M, Vidigal Filho OS, Song Q, Oblessuc PR, Melotto M (2020) New Andean source of resistance to anthracnose

and angular leaf spot: Fine-mapping of disease-resistance genes in California Dark Red Kidney common bean cultivar. PLoS ONE 15:e0235215. https://doi.org/10.1371/journal

Guzmán P, Gilbertson RL, Nodari R, Johnson WC, Temple SR, Mandala D, Mkandawire ABC, Gepts P (1995) Characterization of variability in the fungus *Phaeoisariopsis griseola* suggests coevolution with the common bean (Phaseolus vulgaris). Phytopathology 85:600–607

Jagodzik P, Tajdel-Zielinska M, Ciesla A, Marczak M, Ludwikow A (2018) Mitogen-activated protein kinase cascades in plant hormone signaling. Front Plant Sci 9:1387. https://doi.org/10.3389/fpls.2018.01387

Keller B, Manzanares C, Jara C, Lobaton JD, Studer B, Raatz B (2015) Fine-mapping of a major QTL controlling angular leaf spot resistance in common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 128:813–826. https://doi.org/10.1007/s00122-015-2472-6

Kelly JD, Miklas PN (1998) The role of RAPD markers in breeding for disease resistance in common bean. Mol Breed 4:1–11

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

Kotue TC, Marlyne JM, Wirba LY (2018) Nutritional properties and nutrients chemical analysis of common beans seed. MOJ Biol Med 3:41–47. https://doi.org/10.15406/mojbm.2018.03.00074

Liu BH (1998) Statistical genomics: linkage, mapping and QTL analysis. CRC Press, Boca Raton, p 648

Lobaton JD, Miller T, Gil J, Ariza D, Hoz JF, Soler A, Beebe S, Duitama J, Gepts P, Raatz B (2018) Resequencing of common bean identifies regions of inter-gene *pool* introgression and provides comprehensive resources for molecular breeding. Plant Genome 11:170068. https://doi.org/10.3835/plantgenome2017.08.0068

Mahuku GS, Iglesias ÁM, Jara C (2009) Genetics of angular leaf spot in the Andean common bean accession G5686 and identification of markers linked to the resistance genes. Euphytica 167:381–396. https://doi.org/10.1007/s10681-009-9897-4

Margarido G, Souza A, Garcia A (2007) OneMap: software for genetic mapping in outcrossing species. Hereditas 144:78–79. https://doi.org/10.1111/j.2007.0018-0661.02000.x

Melotto M, Kelly JD (2001) Fine mapping of the *Co-4* locus of common bean reveals a resistance gene candidate, *COK-4*, that encodes for a protein kinase. Theor Appl Genet 103:508–517

Meziadi C, Richard MMS, Derquennes A, Thareau V, Blanchet S, Gratias A, Pflieger S, Geffroy V (2016) Development of molecular markers linked to disease resistance genes in common bean based on whole genome sequence. Plant Sci 242:351–357. https://doi.org/10.1016/j.plantsci.2015.09.006

Miklas PN, Kelly JD, Beebe SE, Blair MW (2006) Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. Euphytica 147:105–131. https://doi.org/10.1007/s10681-006-4600-5

Miller T, Gepts P, Kimmo S, Arunga E, Chilagane LA, Nchimbi-Msolla S, Namusoke A, Namayanja A, Tedla YR (2018) Alternative markers linked to the *Phg-2* angular leaf spot resistance locus in common bean using the Phaseolus genes marker database. Afr J Biotechnol 17:818–828. https://doi.org/10.5897/AJB2018.16493

Nay MM, Mukankusi CM, Studer B, Raatz B (2019) Haplotypes at the *Phg-2* Locus are determining pathotype-specificity of Angular Leaf Spot resistance in common bean. Front Plant Sci 10:1126. https://doi.org/10.3389/fpls.2019.01126

Nay MM, Souza TLPO, Raatz B, Mukankusi CM, Gonçalves-Vidigal MC, Abreu AFB, Melo LC, Pastor-Corrales MA (2019) A review of angular leaf spot resistance in common bean. Crop Sci 59:1376–1391. https://doi.org/10.2135/cropsci2018.09.0596

Nietsche S, Borém A, Alzate-Marin AL, Costa M, Rocha RC, Caixeta ET, Barros EG, Moreira MA (2002) Variabilidade genética da patogenicidade de *Phaeoisariopsis griseola* no Brasil. Summa Phytopathol 28:331–335

Nogueira GB, Santos LV, Queiroz CB, Corrêa TLR, Menicucci RP, Bazzolli DMS, Araújo EF, Queiroz MV (2019) The histidine kinase slnCl1 of *Colletotrichum lindemuthianum* as a pathogenicity factor against *Phaseolus vulgaris* L. Microbiol Res 219:110–122. https://doi.org/10.1016/j.micres.2018.10.005

Oblessuc PR, Baroni RM, Pereira GS, Chiorato AF, Carbonell SAM, Briñez B, Costa e Silva L, Garcia AAF, Camargo LEA, Kelly JD, Benchimol-Reis LL (2014) Quantitative analysis of race-specific resistance to *Colletotrichum lindemuthianum* in common bean. Mol Breed 34:1313–1329. https://doi.org/10.1007/s11032-014-0118-z

Oblessuc PR, Francisco C, Melotto M (2015) 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. Theor Appl Genet 128:1193–1208. https://doi.org/10.1007/s00122-015-2500-6

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 (1992) Recomendaciones y acuerdos delprimer taller de antracnosis en América Latina. In: Pastor-Corrales MA (ed) La antracnosis del frijol común, *Phaseolus vulgaris*, en América Latina. CIAT, Cali, pp 240–250

Pastor-Corrales MA, Tu JC (1989) Anthracnose. In: Schwartz HF, Pastor-Corrales MA (eds) Bean production problems in the tropics. CIAT, Cali, pp 77–104

Pereira R, Abreu AFB, Nalin RS, Souza EA (2019) Phenotyping for angular leaf spot severity and its implication in breeding common bean for resistance. Sci Agric 76:415–423. https://doi.org/10.1590/1678-992X-2017-0394

Pereira R, Souza EA, Costa LC, Abreu AFB (2016) Reaction of common bean lines to *Pseudocercospora griseola* in different environmental conditions. Annu Rep Bean Improv Coop 59:121–122

Perseguini JMKC, Oblessuc PR, Rosa JRBF, Gomes KA, Chiorato AF, Carbonell SAM, Garcia AAF, Vianello RP, Benchimol-Reis LL (2016) Genome-wide association studies of anthracnose and angular leaf spot resistance in common bean (*Phaseolus vulgaris* L.). PLoS ONE 11:e0150506. https://doi.org/10.1371/journal.pone.0150506

Portilla AE, Mayor-Duran VM, Buendia HF, Blair MW, Cichy K, Raatz B (2021) Climbing bean breeding for disease resistance and grain quality traits. Legum Sci. https://doi.org/10.1002/leg3.122

Queiroz CB, Correia HLN, Santana MF, Batista DS, Vidigal PMP, Brommonschenkel SH, Queiroz MV (2019) The repertoire of effector candidates in *Colletotrichum lindemuthianum* reveals important information about *Colletotrichum* genus lifestyle. Appl Microbiol Biotechnol 103:2295–2309. https://doi.org/10.1007/s00253-019-09639-9

R Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rasheed A, Wen W, Gao FM, Zhai S, Jin H, Liu JD, Guo Qi, Zhang Y, Dreisigacker S, Xianchun X, Zhonghu H (2016) Development and validation of KASP assays for functional genes underpinning key economic traits in wheat. Theor Appl Genet 129:1843–1860. https://doi.org/10.1007/s00122-016-2743-x

Rezende Y, Tesfaye K, Mukankusi C, Arunga E, Gepts P (2018) Simple and rapid detached leaf technique for screening common beans (*Phaseolus vulgarise* L.) in vitro against angular leaf spot (*Pseudocercospora griseola*) disease. Afr J Biotechnol 17:1076–1081. https://doi.org/10.5897/AJB2018.16584

Sakiyama NS, Ramos HCC, Caixeta ET, Pereira MG (2014) Plant breeding with marker-assisted selection in Brazil. Crop Breed Appl Biotechnol 14:54–60

Sanglard DA, Machado MAM, Batista FER, Rocha FS, Souza TLPO, Barros EG (2016) Pyramided lines of "Carioca" common bean and their reaction to *Pseudocercospora griseola*. Annu Rep Bean Improv Coop 59:197–198

Sartorato A, Nietsche S, Barros EG, Moreira MA (1999) Inheritance of angular leaf spot resistance and RAPD markers linked to disease resistance gene in common beans. Annu Rep Bean Improv Coop 42:21–22

Sartorato A, Nietsche S, Barros EG, Moreira MA (2000) RAPD and SCAR markers linked to resistance gene to angular leaf spot in common beans. Fitopatol Bras 25:637–642

Schmutz J, Mcclean PE, Mamidi S, Wu GA, Cannon SB, Grimwood J et al (2014) A reference genome for common bean and genome-wide analysis of dual domestications. Nat Genet 46:707–713. https://doi.org/10.1038/ng.3008

Shen GQ, Abdullah KG, Wang QK (2009) The TaqMan Method for SNP Genotyping. In: Komar A (ed) Single nucleotide polymorphisms. Methods in molecular biology™ (methods and protocols). Humana Press, Totowa

Silvério L, Vidigal-Filho MC, Vidigal Filho PS, Barelli MAA, Thomazella C, Nunes WMC (2002) Genetic resistance to *Colletotrichum lindemuthianum* race 2047 in G 2333. Annu Rep Bean Improv Coop 45:74–75

Singh SP, Schwartz HF (2010) Breeding common bean for resistance to diseases: a review. Crop Sci 50:2199–2223

Souza TLPO, Gonçalves-Vidigal MC, Raatz B, Mukankusi CM, Abreu AFB, Melo LC, Pastor-Corrales MA (2016) Major loci controlling resistance to the angular leaf spot of common bean. Annu Rep Bean Improv Coop 58:xv–xviii

Souza TLPO, Wendland A, Rodrigues MS, Almeida LCS, Correia FR, Rodrigues LA, Pereira HS, Faria LC, Melo LC (2014) Anthracnose resistance sources to be explored by the common bean breeding programs in Brazil. Annu Rep Bean Improv Coop 57:217–218

Van-Schoonhoven A, Pastor-Corrales MA (1987) Standard System for the Evaluation of Bean Germplasm. CIAT, Cali, Colombia, 1987

Vieira AF, Almeida LCS, Rodrigues LA, Costa JGC, Melo LC, Pereira HS, Sanglard DA, Souza TLPO (2018) Selection of resistance sources to common bean anthracnose by field phenotyping and DNA marker-assisted screening. Gen Mol Res 17:gmr18066. https://doi.org/10.4238/gmr18066

Wendland A, Lobo Júnior M, Faria JC (2018) Manual de identificação das principais doenças do feijoeiro-comum. Embrapa, Brasília

Wu J, Zhu J, Wang L, Wang S (2017) Genome-wide association study identifies NBS-LRR-encoding genes related with anthracnose and common bacterial blight in the common bean. Front Plant Sci 8:1398. https://doi.org/10.3389/fpls.2017.01398

Young RA, Melotto M, Nodari RO, Kelly JD (1998) Marker-assisted dissection of the oligogenic anthracnose resistance in the common bean cultivar, 'G 2333.' Theor Appl Genet 96:87–94

Zhang Y-L, Zhang C-L, Wang G-L, Wang Y-X, Qi C-H, Zhao Q, You C-X, Li Y-Y, Hao Y-J (2019) The R2R3 MYB transcription factor MdMYB30 modulates plant resistance against pathogens by regulating cuticular wax biosynthesis. BMC Plant Biol 19:362. https://doi.org/10.1186/s12870-019-1918-4

Zuiderveen GH, Padder BA, Kamfwa K, Song Q, Kelly JD (2016) Genome-wide association study of anthracnose resistance in Andean Beans (*Phaseolus vulgaris*). PLoS ONE 11:e0156391. https://doi.org/10.1371/journal.pone.0156391

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20 CONCLUSÃO GERAL

A seleção assistida por marcadores moleculares através de ensaios não destrutivos reduz o tempo e os custos com a seleção de alelos-alvo nas gerações iniciais. A seleção assistida com marcadores codominantes permite a identificação de progênies combinando os alelos-alvo em homozigose. Os marcadores P8282v3-817, ANAAJK6, ANCFFDU e PvbHLHp12804 possuem segregação mendeliana 1:2:1. Além da seleção precoce dos alelos-alvo, foi possível identificar progênies com grão carioca dentro dos padrões comerciais.

A resistência à antracnose presente na cultivar andina BRSMG Realce é controlada por um único gene de efeito maior (R^2=54,6%) no cromossomo 4, que foi previamente nomeado como *Co-Realce*. A região do *Co-Realce* inclui genes de resistência previamente descritos como associados a interação patógeno-hospedeiro. O *Co-Realce* é diferente dos genes R já mapeados no Pv04 (*Co-3*, *Co-15* e *Co-16*). Os snp12782 (1,182,123 pb), snp3308 (505,696 pb) e snp1327 (477,285 pb) são indicados para monitorar a introgressão do alelo *Co-Realce*, com 99,0% de eficiência de seleção. Propõe-se que o *Co-Realce* seja nomeado oficialmente de acordo com as normas estabelecidas pelo Comitê de Genética da BIC (Bean Improvement Cooperative).

Sete marcadores oriundos do projeto HTPG (High Throughput Genotyping) apresentaram potencial para serem incorporados à seleção assistida: snpPV0025-*Phg-2*, snpPV0027-*Phg-5*, snpPV0046-*Co-u*, snpPV0068-*Co-4*^{2}, snpPV0070-*Co-4*^{2}, snpP8282v3-817-*Co-4*^{2} e snpPV0079-*Phg-5*. Os marcadores snpPV0025 e snpPV0079 são indicados para monitorar a presença dos alelos-alvo *Phg-2* e *Phg-5*, respectivamente. O sistema de genotipagem baseado em ensaios de hidrólise do tipo TaqMan® para os marcadores snpPV0070, snpP8282v3-817 e snpPV0025 são específicos para os alelos-alvo e, portanto, adequados para o uso na seleção assistida. Os marcadores snpPV0070 e snpP8282v3-817 possuem elevada eficiência de seleção (99,7 e 99,8%, respectivamente) para o alelo *Co-4*^{2} que confere resistência à antracnose e, portanto, são úteis para seleção precoce de genótipos superiores nos programas de melhoramento de feijão comum. A validação de marcadores previamente identificados como ligados aos alelos-alvo é essencial para aumentar a eficiência da seleção de genótipos com combinações alélicas superiores às já existentes.