REBERTH RENATO DA SILVA

IDENTIFICATION AND VALIDATION OF GENOMIC REGIONS ASSOCIATED WITH AGRONOMIC TRAITS AND DISEASE IN COMMON BEAN

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras, como parte da exigência do Programa de Pós-Graduação em Genética e Melhoramento de Plantas, área de concentração em Genética Quantitativa Aplicada no Melhoramento de Plantas, para a obtenção do título de Doutor.

Profa. Dra. Elaine Aparecida de Souza
Orientadora

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IDENTIFICAÇÃO E VALIDAÇÃO DE REGIÕES GENÔMICAS ASSOCIADAS ÀS CARACTERÍSTICAS AGRONÔMICAS E DOENÇA NO FEIJÃO COMUM

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APROVADA em 20 de dezembro de 2023.

Prof. Dr. Juan Manuel Osorno	NDSU
Dra. Rosana Pereira Vianello	EMBRAPA
Profa. Dra. Luciana Lasry Benchimol-Reis	IAC
Profa. Dra. Lucimara Cruz de Souza	UFLA

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Profa. Dra. Elaine Aparecida de Souza
Orientadora

LAVRAS - MG
2023

I am grateful for the wisdom, health, and gift of life bestowed upon me by God.

OFEREÇO

I would like to express my heartfelt gratitude to my family for their unwavering support and care throughout my journey. I am deeply grateful for the constant presence and the valuable inspiration that you have provided, which has motivated me to tackle obstacles with determination and perseverance. Thank you for being there for me.

DEDICO

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“Imagination is more important than knowledge. For knowledge is limited to all we now know and understand, while imagination embraces the entire world, and all there ever will be to know and understand.”

Albert Einstein

RESUMO

Para aumentar a competitividade e a eficiência dos sistemas produtivos do feijoeiro, é importante identificar e validar regiões genômicas associadas a características agronômicas de interesse. Destacam-se, entre essas características, a arquitetura ereta da planta, o número de dias para o florescimento e a resistência à doenças, especialmente à antracnose. Essa estratégia assegura a aplicação eficaz desses marcadores no contexto do melhoramento genético assistido por marcadores moleculares, promovendo avanços significativos na produtividade e qualidade dos grãos. Objetivou-se a partir desse estudo 1) Realizar o mapeamento de associação para arquitetura de planta e número de dias para o início do florescimento. 2) Validar o marcador SNP previamente identificado como ligado ao alelo de resistência ao isolado Lv238 da raça 65 de *Colletotrichum lindemuthianum*. Para atingir o primeiro objetivo, foi utilizado a abordagem de mapeamento associativo, utilizando um Painel de diversidade da UFLA com 121 linhagens genotipadas com o BARCBEAN6K_Illumina SNP Chip com 5398 SNPs e fenotipadas em campo em duas safras. Quanto a validação, para marcador SNP ss715646893 (1.165.722pb) foram utilizados uma população F_2 do cruzamento entre as cultivares BRS Estilo x BRS Valente e um painel de diversidade com 49 acessos a partir da síntese e genotipagem com a sonda TaqMan™. A resistência ao isolado Lv238 é controlada por um gene (R^2 = 72%), mapeado em uma região do cromossomo Pv04 que possui 17 proteínas de receptor de quinase com repetições (LRR- RLKs). O resultado do mapeamento de associação identificou 4 SNPs para arquitetura ereta de plantas e outros 2 SNPs para o número de dias para o início do florescimento. Esses SNPs foram identificados nos cromossomos Pv08 e Pv10. Em relação à validação, o sistema de genotipagem baseado em ensaios de hidrólise do tipo TaqMan™ para o marcador SNP ss715646893 foi específico para o alelo-alvo Co_L. O marcador SNP ss715646893 apresentou frequência de recombinação de 11,40% e com eficiência de seleção de 94,80% na população F_2. Os resultados obtidos evidenciam a importância da identificação e validação de marcadores moleculares associados à alelos-alvo para caracteres de importância agronômica em feijão-comum. Essa estratégia é fundamental para a escolha de genitores e de genótipos que apresentem alelos de interesse. Há expectativa de aumento na eficiência de seleção e no ganho genético por unidade de tempo nos programas de melhoramento genético do feijoeiro.

Palavras-chave: *Phaseolus vulgaris* L.; Arquitetura ereta de plantas; *Colletotrichum lindemuthianum*; Melhoramento molecular.

ABSTRACT

To increase the competitiveness and efficiency of common bean production systems, it is important to identify and validate genomic regions associated with agronomically important traits. Among these traits, upright plant architecture, the number of days to flowering, and resistance to diseases, especially anthracnose, stand out. This strategy ensures the effective application of these markers in the context of marker-assisted selection genetic breeding, promoting significant advances in yield and grain quality. The objective of this study was to: 1) Perform association mapping for plant architecture and the number of days to the beginning of flowering. 2) Validate the previously identified SNP marker linked to the resistance allele to the Lv238 isolate of the race 65 of *Colletotrichum lindemuthianum*. To achieve the first objective, the association mapping approach was used, using a UFLA diversity panel with 121 lines genotyped with the BARCBEAN6K_Illumina SNP Chip with 5398 SNPs and phenotyped in the field in two seasons. For validation, for the SNP marker ss715646893 (1,165,722pb), an F_2 population from the cross between the cultivars BRS Estilo x BRS Valente and a diversity panel with 49 lines were used from synthesis and genotyping with the TaqMan™ probe. Resistance to the Lv238 isolate is controlled by a gene (R^2 = 72%), mapped in a region of chromosome Pv04 that has 17 repeat Kinase receptor proteins (LRR-RLK). The result of the association mapping identified 4 SNPs for upright plant architecture and another 2 SNPs for the number of days to the beginning of flowering. These SNPs were identified on chromosomes Pv08 and Pv10. Regarding validation, the genotyping system based on TaqMan™ type hydrolysis assays for the SNP marker ss715646893 was specific for the target allele Co_L. The SNP marker ss715646893 presented a recombination frequency of 11.40% and a selection efficiency of 94.80% in the F_2 population. The results obtained highlight the importance of identifying and validating molecular markers associated with target alleles for agronomic traits in common bean. This strategy is essential for the selection of parents and genotypes that present alleles of interest. There is an expectation of increased selection efficiency and genetic gain per unit of time in common bean breeding programs.

Keywords: *Phaseolus vulgaris* L.; Upright architecture; *Colletotrichum lindemuthianum;* Molecular breeding.

SUMÁRIO

FIRST PART

1. GENERAL INTRODUCTION

Among the main challenges for common bean production are upright architecture, early maturity, and resistance to diseases, especially anthracnose. Both family farmers and highly technified producers recognize early maturity and upright architecture as crucial traits that impact production systems (BRUSAMARELLO et al., 2017; LEMOS et al., 2020). Cultivars with an upright architecture offer benefits by facilitating management during the vegetative phase and harvest, resulting in a more efficient mechanized harvest, and reducing the risks associated with adverse weather conditions during harvest (PIRES et al., 2014). Furthermore, the upright architecture contributes to grain quality and protection against pathogens (HUANG; KEMP, 1989; PEREIRA et al., 2019; KADER; BALASUBRAMANIAN; CHATTERTON, 2018). Anthracnose, caused by *Colletotrichum lindemuthianum*, is one of the most destructive crop diseases, resulting in up to 100% production losses (SHAFI et al., 2022).

In this context, knowledge about the inheritance and the genomic regions that govern these traits can contribute to the development and validation of molecular markers closely associated with these traits, intended to be incorporated as a routine in marker-assisted selection (MAS) (GOMES-MESSIAS et al., 2022). Advances in common bean genomics have driven the constant improvement of assisted selection tools (VALDISSER et al., 2017). The common bean genome is estimated to be approximately ~587 million base pairs (Mpb), distributed among 11 chromosomes. Nowadays, there are two reference genomes available, both for germplasm of Andean origin (SCHMUTZ et al., 2014) and for Mesoamerican origin (VLASSOVA et al., 2016). This availability has facilitated resequencing approaches, as the information generated is easily aligned and compared, revealing discrepancies across the entire genome (VALDISSER et al., 2017; WU et al., 2019). This not only favors studies that seek to identify candidate genes but also assists in sequence annotation.

For common bean, a set of several widely distributed SNPs has already been developed (SONG et al., 2015; CICHY et al., 2015; MULLER et al., 2015; VALDISSER et al., 2016; 2017) and has been used in recent research (VIDIGAL FILHO et al., 2020; ALMEIDA et al., 2021; COSTA et al., 2021). The increase in the use of the Whole Genome Sequencing (WGS) approach has made it possible to assess base-level variations across the genome (SCHMUTZ et al., 2014; VLASSOVA et al., 2016; SOUZA et al., 2023). Souza et

al. (2023) performed a resequencing of the entire genome of 40 common bean genotypes aimed to identify SNPs associated with resistance genes annotated in common bean. The study identified over 6 million SNPs, with an average of 38228 SNPs per chromosome.

These strategies make it possible to conduct Genome-Wide Association studies (GWAs), providing greater mapping resolution for the identification of Quantitative trait loci (QTLs) (SCHMUTZ et al., 2014). In the context of GWAs, gametic phase disequilibrium occurs due to other factors such as selection, genetic drift, population structure, and kinship. In this way, the large volume of genomic data, combined with several bioinformatics resources in constant development, has made it possible to accelerate and expand the understanding of inheritance patterns related to several traits. This information can be converted into valuable tools for the detailed analysis of genomic regions harboring resistance gene clusters, such as the *B4*, *Co-4*, *Co-2*, *Co-x,* and *I* clusters (MEZIADI et al., 2016; RICHARD et al., 2017).

Genomic studies have an important role in the understanding of resistance gene clusters (MEZIADI et al., 2016; RICHARD et al., 2017). More than 25 resistance loci for anthracnose have been previously described, encompassing multiple alleles (SHAFI et al., 2022). The interaction between pathogen and host is highly complex, considering not only the pathogenic variability among the more than 298 races of *C. lindemuthianum* but also the variability within the same race (NUNES et al., 2022; PAULINO et al., 2022). From the point of view of common bean breeding, this complexity gains relevance, since cultivars can demonstrate resistance to some isolates and, simultaneously, be susceptible to others belonging to the same race (DAVIDE; SOUZA, 2009; ISHIKAWA; RAMALHO; SOUZA, 2011).

The inheritance of resistance of common bean cultivars to six different isolates belonging to race 65 of *C. lindemuthianum* was investigated by Costa et al. (2017). The distinct reaction pattern of common bean cultivars when inoculated with the different isolates of race 65 and the 15R:1S segregations observed in the F_2 generation of several crosses indicate the possibility of duplicated genes conferring specific resistance to each of the isolates used. This result highlights that the specificity of the pathogen-host interaction also occurs at the isolate level within the race. Therefore, 12 genes were identified associated with resistance to these six isolates, revealing a significant diversity of alleles originating from different genes involved in anthracnose resistance. This scenario makes obtaining cultivars

with durable resistance challenging (COSTA et al., 2017). Therefore, it is necessary to identify and validate these genes to isolates, aiming for their use in MAS.

The validation of molecular markers constitutes a subsequent step in the work of identifying genomic regions through GWAs. The validation process must be conducted in a different context to verify the efficiency of the selection (VIEIRA et al., 2018). This strategy must be implemented continuously, especially for the main resistance genes (GOMES-MESSIAS et al., 2022; VIEIRA et al., 2018). Nowadays, studies have focused on high-resolution mapping, aiming to reduce the distance between markers and alleles of interest but the resolution is a challenge yet. These markers are then validated in different genotypes, thus increasing the efficiency of allelic pyramiding programs (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).

The present work had two objectives:1) Carrying out association mapping for plant architecture and number of days to flowering. This study of GWAs for these traits, using a diversity panel from the active germplasm bank that presents lines developed for this type of trait, with the recurrent selection strategy. 2) Validate the SNP marker previously identified as linked to the resistance allele to the Lv238 isolate of *C. lindemuthianum* race 65.

2. THEORETICAL REFERENCE

2.1. Common bean crop

Common bean (*Phaseolus vulgaris* L.) is a fundamental element for food, nutritional and cultural security in the cuisine of several countries, especially in developing countries in South America, Central America, and southwest Africa (UEBERSAX et al., 2023). Brazil is the largest producer and consumer of common bean in the world, responsible for approximately 75% of production in MERCOSUR countries and 10% of global production (FAOSTAT, 2023). Common bean production in the country is led by the states of Paraná, Minas Gerais, Mato Grosso, Goiás, Bahia, and São Paulo (CONAB, 2023). In the 2021/2022 season, Brazil had a production of 2.55 million tons of common bean, cultivated in an area of 1.62 million hectares, resulting in an average yield of 1571 $kg.ha^{-1}$ (EMBRAPA ARROZ e FEIJÃO, 2023).

In Brazil, the most consumed commercial types are carioca, black, red, pink, purple, and jalo (VIEIRA et al., 2005; SOUZA et al., 2013). Carioca beans are the most produced and consumed in the country and represent 70% of production (CAPANEMA et al., 2019; EMBRAPA ARROZ e FEIJÃO, 2023), but in some regions, there is a preference for other commercial types. For example, black beans are widely consumed in Rio Grande do Sul, Santa Catarina, Rio de Janeiro, and southern Espírito Santo (BARBOSA; GONZAGA, 2012; FARIA et al., 2014). Red beans are popular in the Zona da Mata region of Minas Gerais.

There was a considerable reduction in the cultivated area between 1985 and 2022 (EMBRAPA ARROZ e FEIJÃO, 2023). However, production continued to grow, with 2.55 million tons produced in the 2021/2022 season. This is due to the increase in yield, which went from 514 $kg.ha^{-1}$ in 1985 to 1571 $kg.ha^{-1}$ in 2022 (EMBRAPA ARROZ e FEIJÃO, 2023). The common bean crop is a source of income for small and large producers, and contributes socially, generating jobs, due to the cultivation in three seasons throughout the year (wet, dry, and autumn/winter) and requires high manpower throughout the crop cycle (GUIMARÃES; DE SOUZA, 2019; HOLTZ et al., 2018). On the national scene, the highest grain yields were obtained by the Distrito Federal and the state of Goiás, with grain yields of 2712 $kg.ha^{-1}$ and 2449 $kg.ha^{-1}$, respectively, in the third season (CONAB, 2023).

A large part of Brazilian production is carried out by family farmers, mainly focused on subsistence, with low use of external inputs, which results in a low average yield, of

around 1571 kg.ha^{-1} (EMBRAPA ARROZ e FEIJÃO, 2023). In the other segment are rural entrepreneurs who adopt high levels of technology, such as irrigation and efficient control of pests and diseases (GUIMARÃES; DE SOUZA, 2019; HOLTZ et al., 2018). In these areas, the average yield can exceed 3000 kg.ha $^{-1}$ (BORÉM; CARNEIRO, 2015; HOLTZ et al., 2018).

For the sustainability of common bean breeding programs in the country and to guarantee significant increases in the yield of this legume, it is necessary to invest in new research that contributes to the understanding of the genomic regions associated with traits of agronomic interest in common bean, aiming at the selection effective cultivation of cultivars with good agronomic performance and durable resistance to the main phytopathogens present in the national territory. Among the diseases that affect common bean crops, anthracnose stands out, as it promotes large losses, especially when environmental conditions are favorable for the development of the disease (SINGH; SCHWARTZ, 2010; SHAFI et al., 2022).

2.2. Traits of agronomic interest in common bean

2.2.1. General aspects of anthracnose in common bean

Anthracnose is one of the most severe and common bean diseases, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara and has a significant impact on grain yield (SHAFI et al., 2022; BISNETA et al., 2021). When susceptible cultivars are exposed to favorable conditions, such as moderate temperatures and high humidity, the damage to yield can be significant, reaching 100% in severe situations (PADDER et al., 2017; VIDIGAL FILHO et al., 2020). Furthermore, if the disease reaches advanced levels, it can damage the grains, reducing their final quality and making them unfit for consumption (MURUBE; CAMPA; FERREIRA, 2019; PADDER et al., 2017).

The pathogenic variability of *C. lindemuthianum*, a pathogen, is the main obstacle in obtaining durable resistance in common bean cultivars (FERREIRA; CAMPA; KELLY, 2013; PADDER et al., 2017; PEREIRA et al., 2010; PINTO et al., 2012). According to Nunes et al. (2021), approximately 298 races of *C. lindemuthianum* have been identified worldwide. In Brazil, 89 races were identified (NUNES et al., 2021; PAULINO et al., 2021).

In studies carried out in bean-producing regions in Brazil, races 65, 73, 81, and 89 were the most common (FERREIRA et al., 2008; ISHIKAWA et al., 2005; MAHUKU;

RIASCOS, 2004; PINTO et al., 2012; RIBEIRO et al., 2016; SILVA; SOUZA; ISHIKAWA, 2007). However, the most recent studies by Nunes et al. (2021) and Paulino et al. (2021) have shown that races 65, 73, and 81 are the most frequent. Race 65 has been described as stable and widely distributed in most bean-producing regions. However, according to Ferreira, Campa and Kelly (2013) and Ishikawa et al. (2012), variation within races has been reported in *C. lindemuthianum* races 73, 81, and 89. Furthermore, Santos et al. (2008) also confirm the presence of variation within these races.

In 1996, Kelly and Young proposed a system to designate resistance alleles of *C. lindemuthianum* found in common bean cultivars. They suggested using the symbol "*Co*" followed by a number or letter. So far, 25 resistance alleles have been identified and validated, which come from different genes (Table 1). Multiple alleles have been reported at the *Co-1*, *Co-3*, *Co-4*, and *Co-5* loci. However, except for the *co-8* allele, identified in cultivar AB136 (ALZATE-MARIN et al., 1997), which is reported to be conferred by a recessive allele, in all other cases, the dominant allele is responsible for resistance. Recent research indicates the presence of another 11 genes and a *C. lindemuthianum* resistance allele (Table 1). However, these have not yet been validated by the Bean Improvement Genetics Committee Cooperative (BIC).

According to recent studies, the presence of qualitative resistance does not preclude the existence of quantitative resistance for the genetic control of resistance to anthracnose (RAHMANZADEH et al., 2022; SHAFI et al., 2022). Unlike qualitative resistance, which is governed by a few main effect genes, specific to pathogen races, quantitative resistance is driven by many secondary effect genes, not specific to pathogen races. This results in durable resistance and is known as partial resistance because of the continuous distribution of phenotypic classes ranging from maximum resistance to maximum susceptibility. In recent years, several QTLs that confer partial resistance in the *P. vulgaris – C. lindemuthianum* pathogen system have been identified (Table 2) (RAHMANZADEH et al., 2022; SHAFI et al., 2022).

In a recent study, Shafi et al. (2022) compiled the QTLs identified in other works and designed them into a consensus map to facilitate use in breeding programs. The phenotypic variation explained by these QTLs ranged from 3.97 – 46.8%, with a mean of 16.54%. The meta-analysis identified 11 meta-QTLs (MQTLs) on six common bean chromosomes and 10 hotspot QTLs (Table 2). Nine of the 11 MQTLs were validated using marker associations (MTAs) reported in GWAS studies. Functional annotation of the MQTL regions revealed

1251 defense-related genes, including R genes and proteins containing NBS-LRR domains and kinases (SHAFI et al., 2022).

Table 1. Common bean anthracnose resistance sources, resistance gene or allele, Phaseolus vulgaris (Pv) chromosome (chr), molecular marker, position allele was mapped, and references.

Resistance source	Gene	PV chr	Molecular Marker	Position	Reference
AND 277	*Co-1*^{4}	Pv01	ss715645251	49,583,965	Alzate-Marin et al. (2003); Gonçalves-Vidigal et al. (2011); Lima et al. (2023)
Jalo EEP558	*Co-x*	Pv01	P05	49,546,670	Geffroy et al. (2008)
	Co-1^4	Pv01	M5	490924749	Richard et al. (2014)
Kaboon	*Co-1*^{2}	Pv01	ss715645251	49583965	Melotto & Kelly (2000); Zuiderveen et al. (2016)
Jaguar	*Co-1*	Pv01	IND01_502219	49510484	Zuiderveen et al. (2016)
California Dark Red Kidney	*CoPv01*^{CDRK}	Pv01	ss7156645248	49828427	Lovatto el al. (2023)
Paloma	*Co-Pa*	Pv01	SS82	49444405	Lima Castro et al. (2017)
Hongyundou	*Co-1HY*	Pv01	TF1	49570786	Chen et al. (2017)
Pitanga	*Co-14*	Pv01	g1224	48999518	Gonçalves-Vidigal et al. (2016)
Xana	*CoPv02c*^{X}	Pv02	IND_2_403966	40950265	Campa et al. (2014)
BAT 93	*Co-u*	Pv02	close to *I* gene	NA[b]	Geffroy et al. (2008)
SEL1308	*Co-17*	Pv03	NDSU_IND_3_004	551937	Trabanco et al. (2015)
Jalo Listras Pretas	*Co-13*	Pv03	$OV20_{680}$	NA[b]	Lacanallo & Gonçalves-Vidigal (2015)
Mexico 222	*Co-3*	Pv04	PV-ctt001	458859	Bannerot et al. (1965); Rodríguez-Suárez et al. (2008)
A495	*Co-3*^{3} *(Co-9)*	Pv04	SB12	289696	Mendez de Vigo et al. (2005)
BAT 93	*Co-3*^{3}	Pv04	SNP04_1022546	1286490	Young et al. (1998); Geffroy et al. (1999)
	Co-3c^X	Pv04	SW12	589909	Young et al. (1998); Geffroy et al. (1999)
JaloEEP531	*Co-y / Co-z*	Pv04	D1174	NA[b]	Geffroy et al. (1999)
Ouro Negro	*Co-3*^{4}	Pv04	g2303	3634313	Gonçalves-Vidigal et al. (2013)
Corinthiano	*Co-15*	Pv04	g2685	9432376	Sousa et al. (2015)
Crioulo 159	*Co-16*	Pv04	g2467	1537169	Coimbra-Gonçalves et al. (2016)

Resistance source	Gene	PV chr	Molecular Marker	Position	Reference
Beija Flor	*Co-Bf*	Pv04	NA[b]	3,592	Marcon et al. (2020); Xavier et al. (2020)
G2333	*Co-3*^{5}	Pv04	SNP04_1022546	1286490	Vallejo and Kelly (2009); Sousa et al. (2014)
Mexico 227	*Co-3*^{2}	Pv04	PV-ctt001	458859	Fouilloux (1979)
Mexico 222	*Co-3*	Pv04	PV-ctt001	458859	Bannerot et al. (1965); Rodríguez-Suárez et al. (2008)
PI 207262	*Co-3*^{3}	Pv04	SNP04_1022546	1286490	Alzate-Marin et al. (2007)
BRSNG Realce	*Co-Realce*	Pv04	snp3308	505696	Gomes-Messias et al. (2022)
Xana	*Co-3c*^{X}	Pv04	SW12	589909	Campa et al. (2014)
SEL 1360	*Co-5*^{2}	Pv07	g12333$_{250}$	6984298	Young and Kelly (1997)
MSU7-1	*Co-5*^{2}	Pv07	g12333$_{250}$	6984298	Sousa et al. (2014)
TU	*Co-5*	Pv07	g12333$_{250}$	6984298	Fouilloux (1976); Young & Kelly (1997); Campa et al. (2009)
AB 136	*Co-6*	Pv07	SZ04	9624142	Alzate-Marin al. (2000); Gonçalves-Vidigal et al. (2001); Campa et at. (2017)
G2333	*Co-5*^{2}	Pv07	g12333$_{250}$	6984298	Vallejo and Kelly (2009); Sousa et al. (2014)
	Co-4^{2}	Pv08	PvSNPCOK-4	2281755	Young et al. (1998); Alzate-Marin et al. (2001)
PI 207262	*Co-4*^{3}	Pv08	PvSNPCOK-4	2281755	Alzate-Marin et al. (2007)
TO	*Co-4*	Pv08	PvSNPCOK-4	2281755	Fouilloux (1976); Kelly and Vallejo (2004)
SEL1308	*Co-4*^{2}	Pv08	PvSNPCOK-4	2281755	Young et al. (1998)
Cornell 49242	*Co-2*	Pv11	SCAreoli	50846058	Mastenbroek (1960); Geffroy et al. (1998); Kelly & Vallejo (2004)
Michelite	*Co-11*	UM[a]	NA[b]	NA[b]	Gonçalves-Vidigal et al. (2007)
Jalo Vermelho	*Co-12*	UM	NA[b]	NA[b]	Gonçalves-Vidigal et al. (2008a)

Understanding the inheritance and genomic regions that promote resistance to anthracnose through association studies, biparental mapping, RILs, and MAGIC population is crucial for common bean breeding (PAULINO et al., 2021; NUNES et al., 2021). By identifying major effect genes and alleles, as well as minor effect QTLs, it is possible to validate molecular markers related to them, making them useful in marker-assisted selection breeding programs and basic studies of fine mapping and cloning of genomic regions associated with resistance to anthracnose in common bean (PAULINO et al., 2021; NUNES et al., 2021; SHAFI et al., 2022). Thus, the combined use of molecular markers linked to both main and secondary effect regions can contribute durable resistance to *C. lindemunthianum* in common bean (PAULINO et al., 2021; NUNES et al., 2021).

Table 2. Details of 11 Meta-QTLs (MQTLs) identified for anthracnose disease resistance in common bean.

MQTL name	Chr.	Flanking markers	Physical interval (bp)	No. of QTLs involved	*QTLs involved
MQTL1.1	1	ss715647678–ss715648193	2691475–3444171	4	Geffroy, (2008), Gilio, (2020)
MQTL1.2	1	ss715639332–ss715647941	7026852–8046958	2	Zuiderveen et al. (2016), Geffroy, (2000)
MQTL1.3	1	ss715642648–ss715639492	17159280–39410152	3	González, 2015 González, 2015, Gonçalves - Vidigal 2020
MQTL3.1	3	ss1399950353–ss715646941	2121717–2620445	2	Oblessuc et al. (2014), Geffroy, (2000)
MQTL4.1	4	ss715648687–ss715644944	328788–1638581	4	Méndez-Vigo et al. (2005), Costa et al. (2021), Coimbra-Gonçalves et al. (2016)
MQTL4.2	4	ss715647356–ss715642594	45349844-45414255	10	González et al. (2015), Geffroy, (2000), Chen et al. (2017), Boersma, (2013), Coimbra-Gonçalves et al. (2016)
MQTL4.3	4	ss715648140–ss715646129	41929814–42755828	5	Coimbra-Gonçalves et al. (2016), Sousa et al. (2015), Geffroy, (2000), Geffroy, (2008)
MQTL5.1	5	ss715650037–ss715650116	1150129–2501072	2	Almeida et al. (2021), González et al. (2015)
MQTL7.1	7	ss715648393–ss715645685	61520–606814	4	Geffroy, (2000), González et al. (2015), Geffroy, (2000)
MQTL7.2	7	ss715646464–ss715648885	4144345–4742127	2	Mungalu, (2020), Geffroy, (2000)
MQTL8.1	8	ss715646678–ss715646686	392355–569881	5	Oblessuc et al. (2014), González et al.(2015)

2.2.2. Common bean plant architecture

The type of plant that is in high demand both among family farmers and among those with a high technological level is the upright architecture (LEMOS et al., 2020). Plants with this trait facilitate operations in the crop during the vegetative phase and at harvest (PIRES et al., 2014). In addition, the cultivation of plants with an upright architecture provides better grain quality and there is evidence that it mitigates the damage caused by some important pathogens for the common bean crop (HUANG; KEMP, 1989; PEREIRA et al., 2017; KADER; BALASUBRAMANIAN; CHATTERTON, 2018). Breeding programs in Brazil have been successful in obtaining new cultivars with good upright plant architecture (FARIA et al., 2008; MELO et al., 2010; SILVA et al., 2013; RAMALHO et al., 2016; PEREIRA et al., 2017; WENDLAND et al., 2018). However, despite their upright architecture being valued by farmers, the appearance of the grains of these cultivars is not desired by farmers and, above all, by consumers. Few cultivars, such as IPR Águia and IAC Polaco, have shown good performance for both of these traits.

Obtaining cultivars characterized by upright architecture and light grains with slow-darkening requires the crossing of parents exhibiting strong contrasts in these two traits. It is essential to selectively choose lines that display favorable phenotypes for both traits (LEMOS et al., 2020). Although this procedure is commonly used, success has not yet been achieved in obtaining lines that associate upright architecture and light grains with slow-darkening. The main reason for this is that the number of genes involved in the inheritance of these traits is not small, although they present relatively high heritability (NIENHUIS; SINGH, 1986; SANTOS; VENCOVSKY, 1986; SILVA et al., 2013). Plant architecture, for example, is influenced by several traits, such as hypocotyl diameter, internode length, number of branches, and presence of “stay green”, among others (TEIXEIRA et al., 1999). The control of the expression of these traits involves several genes that may be related to the same metabolic pathways and, therefore, interact with each other.

Previous studies have shown that upright plant architecture is a trait that is controlled by a small number of genes and has high heritability estimates ranging from 0.7 to 0.85 (SANTOS et al., 1986; TEIXEIRA et al., 1999; KUZBAKOVA et al., 2022). Although the inheritance of the trait involves several modifying genes, these genes are linked to other traits. In the case of upright plant architecture, it has been reported that the inheritance is

predominantly due to genes with additive effects (SILVA et al., 2013). There are several factors that determine the upright architecture of plants, including the number of nodes, the distance between them, plant height, and stem diameter (KUZBAKOVA et al., 2022). Taller plants with greater height at the insertion of the first pod facilitate mechanical harvesting (KUZBAKOVA et al., 2022). Additionally, their long and sturdy stems allow for an upright plant architecture (KUZBAKOVA et al., 2022).

According to CIAT (1978), it is possible to classify the common bean plant into four types (I to IV), which have different characteristics. Type I is characterized by a determinate growth habit, with terminal reproductive buds on the main stem and branches, and the absence of node production on the main stem after the onset of flowering. Type I cultivars are generally more precocious and flower and mature in a shorter period than other cultivars.

Type II plants, on the other hand, have an indeterminate growth habit, with vegetative terminal buds on the main stem and branches, and production of nodes after the beginning of flowering. These plants have erect branches that are born at the lower nodes of the main stem, are erect, and have a relatively compact canopy. The number of lateral stems is slightly higher than in type I, and the cultivar usually has more than 12 nodes on the main stem.

Type III plants also have terminal vegetative buds on the main stem and branches, but show stronger branching, with a variable number of prostrate branches arising from lower nodes. The plant is prostrate and can spread, with branch development being extremely variable and with some tendency to climb under certain conditions, but generally with poor climbing ability. Flowering usually starts at the bottom of the plant.

Finally, type IV plants are characterized by a high climbing capacity, with an indeterminate habit and the production of vegetative terminal buds on the main stem and branches. These plants have a strong production of nodes on the main stem after the beginning of flowering, poorly developed branches, and a strong tendency to climb on supports. Flowering occurs from the bottom to the top of the plant, and the flowering period is the longest, allowing the occurrence of plants with flowers opening and also with pods forming (CIAT, 1978).

As mentioned, type II plants have many advantages, as they allow the use of technologies that facilitate cultural practices, in addition to enabling mechanized harvesting due to their erect size. Thus, common bean breeding programs aimed at obtaining cultivars with upright architecture select plants of this type. Vieira Júnior (2015) proposed an ideotype

for selecting plants with upright architecture, including this growth habit and a diameter of at least 1 cm in the main stem to avoid lodging. In addition, a vigorous central stem with many distributed lateral branches is necessary to allow good aeration and to avoid pathogens, maximum emission of veins for greater production of flowers and pods, and facilitate mechanized harvesting (VIEIRA JÚNIOR, 2015). According to this author, the insertion height of the first pods must be sufficient to avoid contact with the soil and the pods must have a structure that minimizes the germination of grains in the pods, especially in wet conditions.

An approach widely used by breeding programs to assess the architecture of common bean plants is the use of the rating scale, which consists of attributing visual grades to the plot as a whole. Ramalho et al. (1998) proposed a rating scale that takes into account the number of stems and branches, as well as the presence and size of veins. However, this strategy becomes inefficient and time-consuming when it is necessary to evaluate individual plants, which is a routine activity in breeding programs.

Understanding the inheritance of plant architecture in common bean is important for breeding programs. However, these traits are polygenic and are associated with other relevant traits, making the selection process for plant type quite challenging. To address this issue, Moura et al. (2013) evaluated common bean lines and investigated the relationships between traits related to plant architecture and the score assigned to the plots. The results of the path analysis performed by the authors suggest that the hypocotyl diameter is one of the traits most strongly related to the architectural note, in addition to being easily measurable and having high accuracy (MOURA et al., 2013). In a later study, Silva et al. (2013) reported that additive effect genes are involved in controlling architectural note and hypocotyl diameter. Additionally, the authors observed that the evaluation of the architecture based on the diameter of the hypocotyl presents greater heritability and a lower coefficient of experimental variation when compared to the use of the grade scale (REZENDE et al., 2018). These results indicate the effectiveness of using hypocotyl diameter as an efficient and accurate alternative for evaluating the architecture of common bean plants.

2.3. Associative mapping and gametic phase disequilibrium

The identification of genomic regions associated with agronomic traits of interest in the common bean is essential for the development of molecular markers related to these regions. Understanding how plant genomes work is an important challenge of this century

(RUPRECHT et al., 2017). Several efforts are being made to understand how genes control and express phenotypic traits through the construction of genetic maps and the identification of loci related to these traits (AGRAWAL et al., 2020; BHATIA, 2020). The process of building genetic maps ensures a better characterization of the genes associated with the traits of interest, as it is important to know their position in the genome and, consequently, their relationship with other genes (CARNEIRO; VIEIRA, 2002).

The QTL identification and validation process is a strategy to increase the efficiency of breeding programs for some traits of agronomic interest. There are two ways to perform mapping: traditional QTL mapping and associative mapping or linkage disequilibrium mapping (also known as genomic-wide association studies - GWAS). Both have been used to map common bean agronomic trait genes (COSTA et al., 2021; ALMEIDA et al., 2021). For autogamous species, traditional mapping must be done based on some types of populations such as F_2 Populations, Backcrosses, or Recombinant Inbred Lines (RILs) (FERREIRA; GRATTAPAGLIA, 1998, CARNEIRO; VIEIRA, 2002; COCKRAM; MACKAY, 2018).

To overcome these challenges of traditional genetic mapping, GWAS analysis was proposed, which is more economical and faster than traditional linkage mapping, as it uses diversity panels, composed of collections of germplasm or natural populations, instead of segregating populations (MYLES et al., 2009). These selected panels have recombination events accumulated over countless generations, reducing linkage disequilibrium (LD) and providing greater resolution and accuracy in locating the genes of interest (GONZÁLEZ et al., 2017; KEMPER et al., 2012). To perform the GWAS, a mapping population, phenotypic data, and a large number of markers, usually, SNPs, are required so that the functional alleles are in LD with at least one of the markers. The GWAS allows the detection of valid genetic associations for the entire population, expanding the allelic variation detected in traditional bi-parental mapping. AM analyzes several alleles at a time in individuals from a diverse population, unlike the traditional method of bi-parental mapping, which analyzes only two alleles per locus, in each crossing (RAFALSKI, 2010). This allows the detection of genetic associations in the entire population, expanding the variety of detected alleles compared to the traditional method (FLINT-GARCIA et al., 2003).

AM and LD are distinct concepts. While AM is reflected in the significant association between a molecular marker and a specific phenotype, LD refers to the non-random association between two alleles, markers, or QTLs within a population (GUPTA et al., 2005; MYLES et al., 2009; RAFALSKI, 2010). The LD is important in carrying out AM studies, as

it determines the number and density of the markers (FLINT-GARCIA et al., 2003). The LD decay is necessary to improve the mapping resolution and can be achieved utilizing successive rounds of recombination (intercrossing) (MACKAY; POWELL, 2007). Thus, the construction of genetic maps is based on the analysis of LD between genotyped markers distributed throughout the genome. Some factors that promote LD are genetic drift, population structure, bottleneck, selection, and epistasis (ROSSI et al., 2009).

NGS technology allows for the sequencing of large quantities of nucleotide bases in a short period and at a low cost per base sequenced. This technology has made it possible to obtain many markers from large sets of genotypes. Due to the high number of SNPs obtained through NGS, an effort is being made to gather them into genotyping panels (chips). These chips are composed of SNPs with a high frequency of polymorphism, validated, and well-distributed throughout the genome. The first genotyping panel for common bean was reported by Blair et al. (2013) and contained 763 SNPs. Later, Song et al. (2015) developed the BARCBean6K_3 BeadChip (Illumina) with 5398 validated SNPs, which is now being used in several studies.

Since the emergence of NGS technology and the reduction of sequencing costs, many GWAS studies have been conducted with common bean, using large numbers of SNPs sequenced in broad diversity panels to assess a variety of agronomic traits such as growth habit (ALMEIDA et al., 2020; CICHY et al., 2015), nitrogen fixation (KAMFWA et al., 2015), flowering time (MOGHADDAM et al., 2016), phenology and traits of production components (DELFINI et al., 2016; KAMFWA et al., 2015; MOGHADDAM et al., 2016; OLADZAD et al., 2019; WU et al., 2020), the commercial and nutritional aspect of grains (CAPRONI et al., 2020; CICHY et al., 2015; DELFINI et al., 2021; DIAZ et al., 2021; ERDOGMUS et al., 2020; GARCÍA-FERNANDEZ et al., 2021; SADOHARA, 2021; SADOHARA et al., 2022), tolerance to abiotic stress (HOYOS-VILLEGAS et al., 2017; WU et al., 2021; WU et al., 2022; SOLTANI et al., 2017).

Other works related to disease resistance, including resistance to root rot caused by *Pythium ultimum* (DRAMADRI et al., 2020), to root rot caused by *Rhizoctonia solani* (OLADZAD et al., 2019), root rot caused by *Fusarium solani* (HUSTER et al., 2021; PAULINO et al., 2021; ZITNICK-ANDERSON et al., 2020), common bacterial blight (SIMONS et al., 2021), haloed bacterial blight (MONTEIRO et al., 2021; TOCK et al. al., 2017), white mold (CAMPA, GARCIA-FERNÁNDEZ, FERREIRA, 2020), anthracnose (COSTA et al., 2021; BANOO et al., 2020; ZUIDERVEEN et al., 2016; WU et al., 2017)

angular leaf spot (ALMEIDA et al., 2021; VIDIGAL FILHO et al., 2020; NAY et al., 2019; FRITSCHE-NETO et al., 2019; PERSEGUINI et al., 2016).

The publication of the bean reference genome (SCHMUTZ et al., 2014), which belonged to the Andean line G19833 and had 587Mb, allowed the mapping and comparison of the positions of most SCAR, SSR, and SNP markers. In 2016, the Mesoamerican line was also sequenced, with a genome of 549.6 Mb (VLASOVA et al., 2016). In this way, it was possible to obtain information about the physical position of the markers in the genome and the flanking sequences of these markers, which allowed the development of markers strictly linked to the genes of interest. Furthermore, due to the availability of the reference genome and the wide range of SNPs, it was possible to develop high-throughput genotyping chips for the construction of linkage maps (SONG et al., 2015). The annotation of the bean genome also allowed the search for candidate genes of the mapped alleles. However, it is necessary to validate these genomic regions to apply this knowledge to breeding programs through MAS.

2.4. Validation of SNPs

Validation of SNPs is of great importance for breeding programs that utilize MAS (SHAFI et al., 2022). The availability of new genomic resources for common bean, such as multiple *P. vulgaris* genome reference sets including G19833 (SCHMUTZ et al., 2014), BAT 93 (VLASOVA et al., 2016), OAC-REX (Perry et al., 2013), and UI-1111 (UI111 v1.1, DOE-JGI and USDA-NIFA, http://phytozome.jgi.doe.gov/), in addition to the diversity panels composed of numerous re-sequenced genotypes resulting in a large number of SNPs (CICHY et al., 2015; MOGHADDAM et al., 2016; WALLACE et al., 2018; LOBATON et al., 2018; OLADZAD et al., 2019), along with assay chips containing 6,000-12,000 SNPs (SONG et al., 2015; MIKLAS et al., 2020), have significantly enhanced the ability to identify markers strongly associated with target genes.

To take full advantage of genomic association studies in common bean, it is crucial to increase the density of markers to achieve higher genome resolution, providing a more comprehensive understanding of the genetic of the complex traits in common bean. These markers should adequately represent the LD blocks (haplotypes) of the common bean genome, which typically have an average size of 130 Kb (HUANG et al., 2012; VALDISSER et al., 2017). Consequently, once SNPs strongly linked to target agronomic traits are identified, it is essential to validate them before integrating them into the MAS process (SHAFI et al., 2022). This validation can be accomplished by evaluating populations derived

from parents with contrasting trait values or elite lines that involve the parent in which the marker was initially identified. By employing such validation strategies, we can ensure the accuracy and reliability of the identified markers, ultimately enhancing the efficacy of breeding programs in common bean research.

Different methods can be used to validate SNP markers (GAEDIGK et al., 2015). One such approach is the use of TaqMan®, a versatile system compatible with different real-time PCR platforms. The TaqMan SNP Genotyping System Assay employs custom assays, enabling the analysis of numerous SNPs with a limited number of samples or a smaller set of SNPs with a larger number of samples (Applied Biosystems™) (FRANCISCO et al., 2005). Another technology that relies on the detection of SNPs through TaqMan® probes is Fluidigm™ platform which allows the simultaneous genotyping of 48 to 96 SNPs using the same set of samples, offering a high-throughput solution for SNP genotyping, facilitating efficient and cost-effective analysis of a substantial number of genetic markers (FEDICK, 2013). Other technologies are suitable for analyzing a limited number of SNPs and varying sample sizes include: 1) SNPshot^{R} method that allows the analysis of up to 10 SNPs simultaneously using a fluorescent detection system, capillary electrophoresis, and platform detection on several ABI automatic analyzers (Applied Biosystems); 2) SNPstream®, which is also an automated multiplex genotyping system capable of analyzing 12 to 48 SNPs in 384 samples (Beckman Coutler); 3) KASP^{TM} (*Kompetitive Allele Specific* PCR) based on PCR, where the SNPs are individually analyzed and allelic polymorphism is detected using real-time PCR (LGG) equipment (JAGTAP et al., 2020). Additionally, GT-seq (Genotyping-in-Thousands by Sequencing) is a cost-effective method used to genotype panels of relatively small SNPs (ranging from 50 to 500) in a few thousand individuals, such as germplasm banks (FEDICK, 2013). GT-seq offers the advantage of specifically targeting the SNPs of interest, making it a flexible and affordable option for genotyping.

Heid et al. (1996) published the pioneering study, which introduced the use of TaqMan probes, titled "Real-Time Quantitative PCR", upon the concepts of real-time PCR previously described by Higuchi et al. (1993) and leveraging the advancements in fluorogenic probes. These probes consist of a FAM reporter dye (6-carboxyfluorescein) and a TAMRA quencher (6-carboxy-tetramethylrhodamine), and they have become widely utilized as fluorescent reagents in quantitative real-time PCR assays due to their exceptional specificity and efficiency. During the amplification reaction, following DNA denaturation, the TaqMan probe, specifically designed for the target sequence, anneals to the complementary DNA

sequence with the assistance of primers. As the extension phase begins, the 5'->3' exonuclease activity of Taq DNA polymerase cleaves the probe, separating the reporter from the quencher and resulting in the emission of fluorescence.

To enhance the efficiency of QTL detection, it is important to improve the efficiency of field trial evaluation (FERREIRA et al., 2018; VELAZCO et al., 2020). Adequate investments should also be made in data processing and robust statistical analysis (SANTOS et al., 2014; VELAZCO et al., 2020). Given the inherent challenges, in practice, MAS for quantitative traits often focuses on the QTLs of main effect that explain a significant portion of the traits of interest, rather than considering all QTLs (OLIVEIRA et al., 2013). The utilization of molecular markers is a valuable technique for early selection of resistant genotypes, enabling the incorporation of multiple resistances into elite germplasm (GOMES-MESSIAS et al., 2022). The identification of markers linked to resistance loci in common bean has been extensively employed and validated, with many markers holding potential for implementation in MAS (Gil et al., 2019; FRITSCHE-NETO et al., 2019; GOMES-MESSIAS et al., 2022). Genotyping based on the TaqMan® system for spnPV 0070 (*Co-4*^{2}) and snpP 8282v3-817 (*Co-4*^{2}) and snpPV 0025 (*Phg-2*) markers facilitated the specific amplification of target alleles, exhibiting selective efficiencies of 99.7% and 99.8%, respectively (GOMES-MESSIAS et al., 2022).

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SECOND PART - Papers

PAPER 1 - IDENTIFICATION OF GENOMIC REGIONS ASSOCIATED WITH AGRONOMIC TRAITS IN COMMON BEAN

Theoretical and Applied Genetic Journal Standards

(PRELIMINARY VERSION)

ABSTRACT

Plant architecture and precocity represent important traits that confer increased competitiveness to production systems. The adoption of an upright architecture not only facilitates mechanized harvesting but also mitigates the prevalence of certain diseases. This study aimed to undertake association mapping for plant architecture and the number of days to the flowering, utilizing the UFLA Diversity Panel comprising 121 lines genotyped with the BARCBEAN6K_Illumina SNP chip encompassing 5398 SNPs. The assessment encompassed plant architecture at stage R5 (ARC1), plant architecture at stage R8 (ARC2), and the number of days to the flowering (DF). Notably, four significant SNPs were discerned for ARC1, three SNPs for ARC2, and three SNPs for DF. Furthermore, diverse genomic regions were linked to distinct evaluation times, with chromosomes Pv02, Pv05, Pv07, and Pv08 associated with upright architecture, and chromosomes Pv02, Pv08, and Pv10 associated with DF. At different stages of development, different genomic regions were expressed for upright plant architecture. These findings underscore the efficacy of Genome-Wide Association Studies (GWAS) in enhancing our comprehension of the genetic architecture governing traits such as ARC1, ARC2, and DF in common bean. The identification of these Quantitative Trait Nucleotides QTNs aids in understanding of the inheritance of upright plant architecture and precocity.

Keywords: *Phaseolus vulgaris* L., Upright architecture, precocity, association mapping.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important crop in economic, social, and nutritional terms, particularly in developing countries (UEBERSAX et al., 2023). Brazil is one of the largest producers and consumers of common bean worldwide, contributing approximately 75% of the total production of Mercosur countries and 10% of global production (FAOSTAT, 2023). Both family farmers and highly mechanized farmers consider both early maturity and upright architecture as important traits, as they significantly impact the efficiency of their production systems (BRUSAMARELLO et al., 2017; LEMOS et al., 2020).

Cultivars that have an upright architecture offer various benefits that play a crucial role in enhancing yield and operational efficiency in common bean cultivation (KELLY, 2001). This particular trait makes crop operations easier during the vegetative phase and at harvest, leading to more effective mechanized harvesting (ECKERT et al., 2011; SOLTANI et al., 2016). Additionally, the risk of damage caused by rainfall that coincides with the harvest is reduced (ECKERT et al., 2011; PIRES et al., 2014). Furthermore, growing plants with an upright architecture results in better grain quality and can help reduce damage from certain pathogens that commonly affect common bean crops (HUANG; KEMP, 1989; PEREIRA et al., 2017; KADER; BALASUBRAMANIAN; CHATTERTON, 2018). In Brazil, breeding programs have successfully recommended new cultivars with an upright plant architecture due to the benefits it provides (FARIA et al., 2008; MELO et al., 2010; SILVA et al., 2013; RAMALHO et al., 2016; PEREIRA et al., 2017; WENDLAND et al., 2018). However, there is still a lack of knowledge about the specific genomic regions that control this trait.

Previous quantitative genetic studies indicate that upright architecture is oligogenic with high heritability estimates of 0.7-0.85. (SANTOS et al., 1986; TEIXEIRA et al., 1999). In the work of Resende et al. (2018) six quantitative trait loci (QTL) for upright plant architecture were identified, explaining 14 to 33.3% of the variance. Although the inheritance of the oligogenic trait involves several modifying genes, these genes are linked to other traits. Several factors determine the upright architecture of plants, including the number of nodes, the distance between them, plant height, and stem diameter (KUZBAKOVA et al., 2022). Taller plants with greater height at the insertion of the first pod facilitate mechanical harvesting (KUZBAKOVA et al., 2022). Additionally, their long and sturdy stems allow for an upright plant architecture (KUZBAKOVA et al., 2022).

The number of days to flowering (DF) of lower magnitude is related to early maturity, which guarantees greater dynamism in production systems. This helps with crop rotation, drought tolerance, and reduces irrigation costs (GONZÁLEZ et al., 2021). The inheritance of DF is considered oligogenic, with heritability ranging from 0.70 to 0.86, indicating a high magnitude (NASCIMENTO et al., 2018; MOGHADDAM et al., 2016; RAGGI et al., 2019). Despite its oligogenic nature, several modifying genes are associated with the inheritance of this trait. Reports suggest the predominance of additive effects in the expression of this trait. Flowering is regulated by a complex network of genes that integrate several metabolic pathways related to photoperiod, vernalization, phytohormone production, and other routes responsible for flowering time (MOGHADDAM et al., 2016). Several QTL have been described for DF variation (BLAIR et al., 2006; MOGHADDAM et al., 2016; BHAKTA et al., 2017; WALLACH et al., 2018; GONZÁLEZ et al., 2021).

The DF, plant architecture at stage R5 (ARC1), and plant architecture at stage R8 (ARC2) play fundamental roles in promoting competitiveness in the production system (BRUSAMARELLO et al., 2017; LEMOS et al., 2020). As mentioned previously, few studies have identified genomic regions associated with these traits, within the context of the active germplasm bank panel (DELFINI et al., 2021; NASCIMENTO et al., 2018; RAGGI et al., 2019; RESENDE et al., 2018; SOLTANI et al., 2021). Therefore, this work's main objective is to identify the genomic regions correlated with DF, ARC1, and ARC2 using lines from the UFLA active germplasm bank.

MATERIAL AND METHODS

UFLA common bean diversity panel

In this study, a panel consisting of 121 common bean lines from the Germplasm Bank of the Universidade Federal de Lavras (UFLA, Lavras, Brazil) was evaluated, including commercial lines obtained by different Brazilian institutions, lines from ten cycles of the recurrent selection programs targeting angular leaf spot resistance, seed yield, and upright architecture. Most of these lines are of the Carioca-type, but it also includes other market classes such as black, red, and others (Table S1).

Genotyping using the BARCBean6K_3 Illumina beadchip

The seeds of these 121 common bean lines were sown in polystyrene trays containing Topstrato® substrate. The experiment was carried out at a greenhouse, in a randomized block design with three replications, and four seedlings per plot. Newly emerged trifoliate leaves from each of the four seedlings and three samples were collected and total genomic DNA was extracted using a modified CTAB (hexadecyltrimethylammonium bromide) extraction protocol (Doyle and Doyle, 1987). DNA concentration was measured using a Nanodrop spectrophotometer and the quality was checked on a 1.5% agarose gel. DNA samples from each of the plants were then genotyped with the BARCBEAN6K_3 Illumina BeadChip containing 5,398 SNPs (SONG et al., 2015) at the USDA-ARS Beltsville Agricultural Research Center's Soybean Genomics and Breeding Laboratory, Beltsville, MD, USA following the Infinium HD Assay Ultra protocol (Illumina, Inc. San Diego, CA, USA). The Illumina BeadArray Reader was used to measure the fluorescence signal intensity. Automatic allele recall was performed using Illumina GenomeStudio v. 1.9.4 and allele call data were visually inspected for each locus.

Phenotypic evaluation of panel in the field

The panel was tested in the field at “Centro de Desenvolvimento Tecnológico e Científico da Universidade Federal de Lavras” in the wet season of 2022/2023. The design used was a randomized complete block design, with 2-rows plots of 1 m, and two replications. Agronomic management of experiments was according to common bean crop in the region. Irrigation was provided by sprinklers in the lack of rain.

The DF, that is, the number of days from sowing until the moment when 50% of plants presented at least one open flower was determined. The ARC1 and ARC2 was evaluated using a score descriptive scale, ranging from 1 to 9 (Table 2), proposed by Collicchio et al. (1997) at flowering stage (R5) and R8, respectively.

Table 1 - Score scale used to evaluate the plant architecture, proposed by Collicchio et al. (1997).

Note	Description
1	Habit I or II, upright architecture plant with one stem and high insertion of the first pods
2	Habit I or II, upright architecture plant, with a short guide
3	Habit I or II, upright architecture plant, with some branches
4	Habit I or II, upright architecture plant, with some long guides
5	Habit II or III, upright architecture plant, with many branches and a tendency to prostrate
6	Habit II or III, semi-upright architecture plant, slightly prostrate
7	Habit III, semi-upright architecture plant, slightly prostrate
8	Habit III, prostrate
9	Habit III, plant with long internodes, very prostrate

Analysis of phenotypic data

Descriptive statistics and analysis of variance (ANOVA), as well as genetic parameters, were estimated using the R software (R Core Team, version 4.0.4). The normality of the ANOVA residuals was verified using the Shapiro-Wilk test (SHAPIRO and WILK, 1965). After obtaining the adjusted average of the scores for each block, the Best Unbiased Linear Estimator (BLUE) was applied using the R software (R Core Team, version 4.0.4).

Genome-Wide Association Analyses

After filtering for low-quality SNPs, monomorphic ones, and those with the least frequent allele frequency (MAF > 0.05), a total of 3117 polymorphic markers were used for GWAS analysis. Genome-wide efficient mixed-model association (GEMMA) (ZHOU and STEPHENS, 2014) was used to perform the association mapping. Principal component analysis (PRICE et al., 2006) was used to estimate population structure. Population-relatedness was calculated using the GEMMA algorithm for centered-relatedness. Bootstrapping was performed 10,000 times on the empirical distribution of *P*-values, and the SNPs in the <0.05 and 0.5% of the distribution were considered highly significant and significant, respectively (OLADZAD et al., 2019). Two models were tested using GEMMA

within each phenotypic distribution. The MM (mixed model) includes population structure and relationship (2PCA + kinship matrix), and the EMMA (efficient mixed-model analysis) model only accounted for relatedness. The model with the lower mean square deviation (MAMIDI et al., 2011) was used for further analysis, such as EMMA for ARC1 and MM for DF and ARC2. The phenotypic variation explained by the most significant markers and the cumulative effect was estimated by the likelihood-ratio-based R^2 (SUN et al., 2010) using the genABEL package available in software R (AULCHENKO et al., 2007; R Core Team, 2015). Manhattan plots were developed using the mhplot() function available in software R (ZHAO, 2007) to visualize the distribution of SNP *P*-values in the genome. The population structure analysis was conducted using STRUCTURE (Pritchard et al. 2000). A Q matrix was developed to describe the percent subpopulation for each common bean line in analysis. The ideal number of subpopulations was found by examining the optimal Delta K value (Evanno et al. 2005) in STRUCTURE Harvester (Earl and vonHoldt 2012).

Identification of candidate genes

The identification of putative candidate genes associated with significant SNPs derived from the GWAS analysis was performed according to the physical position of the SNPs. Candidate genes were inferred using Jbrowse Phytozome v13.0 (GOODSTEIN et al., 2012) and *Phaseolus vulgaris* genome v1.1 UI111 the common bean reference genome (Middle American), available at (https://phytozome-next.jgi.doe.gov/). A genomic region was delimited considering a 200-Kb window centered for each SNP. Subsequently, functional annotation for these genes was recorded to infer their possible role in conferring in the DF, ARC1 and ARC2.

RESULTS

Phenotypic evaluation for ARC1, ARC2, and DF

The residues of the phenotypic data of the 121 lines in the UFLA diversity panel were normally distributed, as expected for traits controlled by more than one gene. The score for ARC1 and ARC2 ranged from 1 to 9 and for DF it ranged from 32 to 43 days. All traits evaluated were statistically different ($p < 0.05$). The BRSMG Uai cultivar, an upright architectural pattern, presented a mean score of 1.5, and the BRS Radiante cultivar, an early maturity pattern, presented a mean score of 32 days (Figures 1, 2, and 3). Broad-sense

heritability (H^2) was calculated using the analysis of variance (ANOVA), being 0.84 for ARC1, 0.67 for ARC2, and 0.80 for DF.

Figure 1 - Frequency distribution of the average scores of the individual analysis for the number of days to flowering (DF) of the 121 common bean lines evaluated during the wet season of 2022/2023.

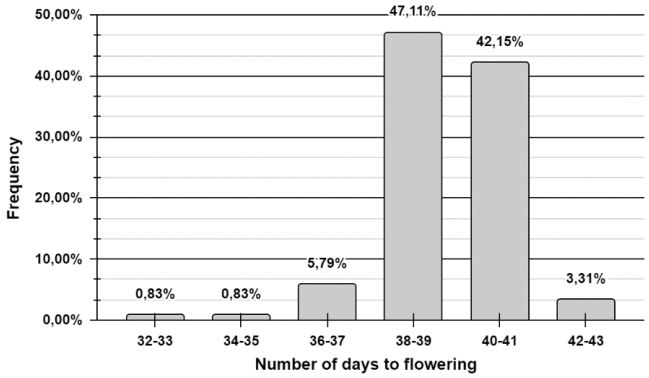


Figure 2 - Frequency distribution of average scores from the individual analysis of plant architecture during the flowering period R5 (ARC1) of the 121 common bean lines evaluated during the wet season of 2022/2023.

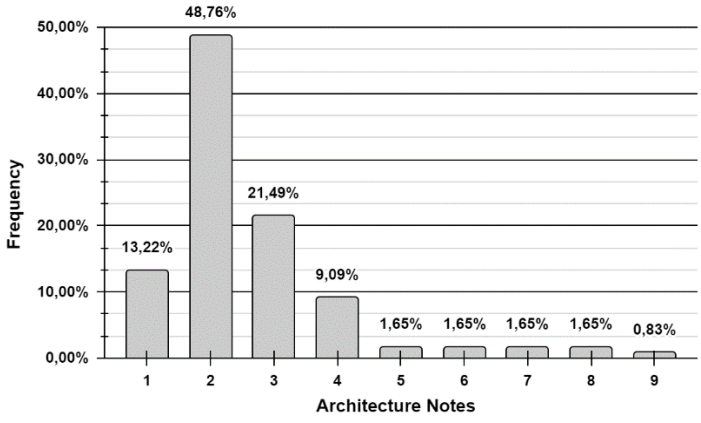
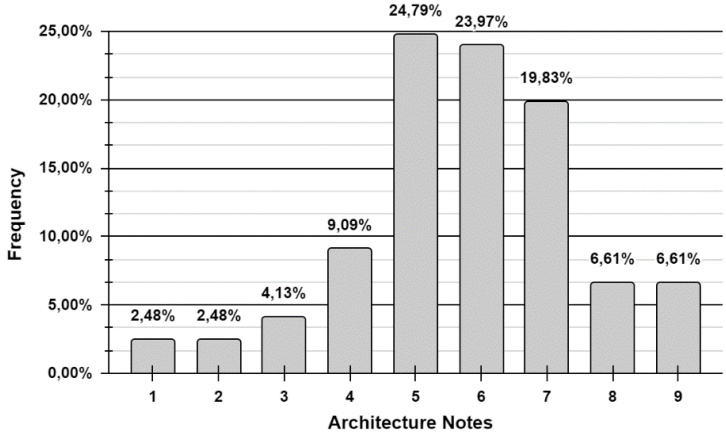


Figure 3 - Frequency distribution of the average scores of the individual analysis of plant architecture at the R8 (ARC2) stage of the 121 common bean lines evaluated during the wet season of 2022/2023.



GWAS for ARC1, ARC2, and DF.

Among the initial 5398 SNP markers, after applying the filters based on read depth, quality, heterozygosity, and minor allele frequency, 121 genotypes were used to perform GWAS with a total of 3117 SNPs. Several models were tested for each distribution of phenotypic data: FarmCPU, EMMA, and MM. The MM model presented the lowest mean square deviation value (0.14) for ARC1 and DF, and also for ARC2 with lowest mean square deviation value (0.15). The results using the linear mixed model are presented in Figures 1, 2 and 3.

The association analysis was evaluated for the traits and identified associated regions for each one. For ARC1, four regions were identified: one SNP on the Pv07 chromosome, which explained 2.73% of the phenotypic variation, and three SNPs on the Pv08 chromosome, explaining 2.62%, 3.65%, and 4.75% of the phenotypic variation, respectively (Table 2). For ARC2, three genomic regions were identified on chromosomes Pv02, Pv05, and Pv08, with SNPs explaining 3.16%, 4.36%, and 3.76% of the phenotypic variation, respectively (Table 2). For DF, three regions were also identified on chromosomes Pv02, Pv08, and Pv10, explaining 7.65%, 6.35%, and 8.43% of the phenotypic variation. These genomic regions have been previously reported, and these results help to validate these regions. The results were visualized using Manhattan plots and QQ plots (Figure 4). Regarding the linkage disequilibrium (DL), it was observed that the decay was medium, around 400K as expected for an autogamous species. The total number of subpopulations considered was k = 3 (Fig. S2).

Figure 4 - Manhattan plots and its respective quantile-quantile plots of (a) plant architecture in R5 (b) number of days to flowering (c) plant architecture in R8 data for UFLA common bean diversity panel genotyped with 3117 SNPs during the wet season 2022/2023. Markers red-colored passed the cutoff value of 0.05.

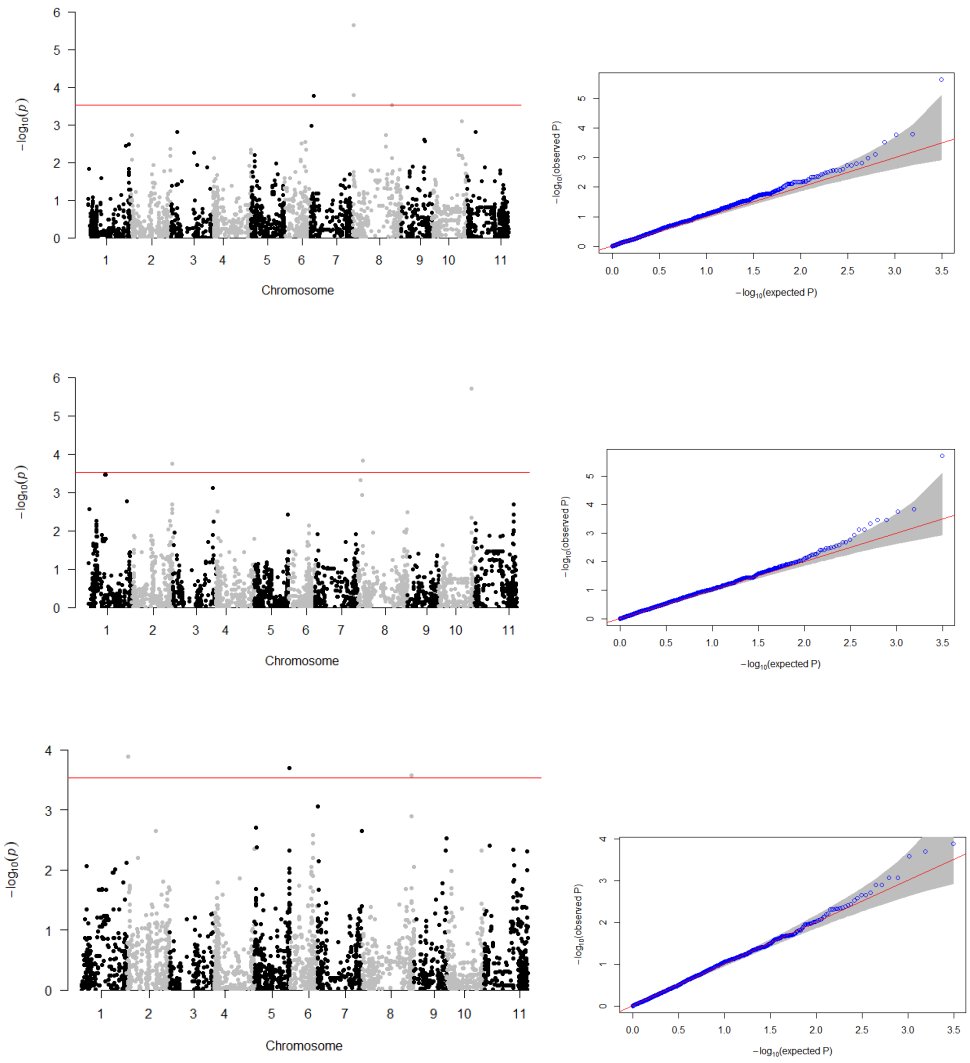


Table 2 - SNPs markers (V1.0 assembly), obtained from association mapping using the UFLA common bean diversity panel, evaluated phenotypically for ARC1, ARC2 and DF during the wet season of 2022/2023.

Trait	SNP	SNP allele	chromosome	Physical position (bp)	p-value	R^2
ARC1	ss715646462	C	Pv07	4196826	1.70E-04	2.73
	ss715647117	G	Pv08	1711667	1.63E-04	2.62
	ss715647119	A	Pv08	1732812	2.27E-06	3.65
	ss715640265	G	Pv08	48288822	2.96E-04	4.75
ARC2	ss715639434	T	Pv02	1837704	1.30E-04	3.16
	ss715639375	A	Pv05	40201016	2.03E-04	4.36
	ss715646531	C	Pv08	56747675	2.63E-04	3.76
DF	ss715648453	T	Pv02	48375925	1.76E-04	7.65
	ss715647905	T	Pv08	6217643	1.46E-04	6.35
	ss715648597	T	Pv10	39369158	1.94E-06	8.43

Identification of candidate genes for ARC1, ARC2, and DF

SNPs were located considering a 200-Kb window centered for each SNP to candidate genes with the hypothetical annotation of their proteins. This procedure was performed using the common bean reference genome, by the *Jbrowse genome* located in Phytozome v13.0 for version 1.1 of UI111 the common bean reference genome (Middle American), available at (https://phytozome-next.jgi.doe.gov/). In all, 10 significant SNPs were found, four SNPs for the ARC1 trait, three for the ARC2 trait, and another three for the DF trait.

The SNP (ss715646462) was found to be associated with the PV07:4.19Mb region in ARC1. This region is located 53Kb upstream from the gene model *PvUI111.07G050100*, which is annotated as Cysteine-rich receptor-like protein kinase (Table 3). Another three SNPs (ss715647117), (ss715647119), and (ss715640265) was located at the chromosome Pv08, 1.71Mb, 1.73Mb, and 48.28Mb region in ARC1, respectively (Table 3). The region of the first SNP is located 15Kb upstream from the gene model *PvUI111.08G021400*, which is annotated as Translation initiation factor 2B, delta subunit (eIF-2Bdelta/GCD2). The region of the second SNP is located 11Kb upstream from the gene model *PvUI111.08G021600*, which is annotated as Leucine-Rich Repeat-Containing protein. The last SNP this region is located 34Kb upstream from the gene model *PvUI111.08G174900*,

which is annotated as Phosphoribosylaminoimidazolesuccinocarboxamide synthase / SAICARs.

The SNP (ss715639434) has been identified in association with the Pv02:1.83Mb region in ARC2 (Table 3). This region is situated 35Kb upstream from the gene model *PvUI111.02G016200*, annotated as KOG1457 - RNA binding protein (contains RRM repeats). Another SNP (ss715639375) has been linked to Pv05:40.20Mb and is located 65Kb upstream from the gene model *PvUI111.05G134700*, annotated as ATP-Dependent Helicase-Like protein (Table 3). Additionally, the SNP (ss715646531) has been associated with the Pv08:56.74Mb region in ARC2, positioned 45Kb upstream from the gene model *PvUI111.08G235700*, annotated as PPR repeat family (PPR_2).

Regarding DF analysis, the SNP (ss715648453) was associated with the PV02:48.3Mb region, which is located 17Kb upstream from the gene model *PvUI111.02G303000*. This gene is annotated as pectinesterase (Table 3). Another SNP (ss715647905) was associated with the PV08:6.21Mb region, which is located 0.5Kb upstream from the gene model *PvUI111.08G068900*. This gene is annotated as beta-amylase. Additionally, the SNP (ss715648597) was identified in the PV10:39.36Mb region, which contains the model gene *PvUI111.10G098200*. This gene is located within 13Kb interval, which is annotated as small subunit ribosomal protein S33 (MRPS33) (Table 3).

Table 3 - Predicted function of detected genes based on candidate regions and physical position of significant SNPs.

Trait	SNP	chromosome	Gene	Gene annotation and predicted function based on protein domain
ARC1	ss715646462	Pv07	*PvUI111.07G050100*	Cysteine-rich receptor-like protein kinase
	ss715647117	Pv08	*PvUI111.08G021400*	Translation initiation factor 2B, delta subunit (eIF-2Bdelta/GCD2)
	ss715647119	Pv08	*PvUI111.08G021600*	Leucine-Rich Repeat-Containing protein
	ss715640265	Pv08	*PvUI111.08G174900*	Phosphoribosylaminoimidazolesuccinocarboxamide synthase / SAICARs
ARC2	ss715639434	Pv02	*PvUI111.02G016200*	KOG1457 - RNA binding protein (contains RRM repeats)
	ss715639375	Pv05	*PvUI111.05G134700*	ATP-Dependent Helicase-Like protein
	ss715646531	Pv08	*PvUI111.08G235700*	PPR repeat family (PPR_2)
DF	ss715648453	Pv02	*PvUI111.02G303000*	Pectinesterase 53-related
	ss715647905	Pv08	*PvUI111.08G068900*	Beta-amylase 7
	ss715648597	Pv10	*PvUI111.10G098200*	small subunit ribosomal protein S33 (MRPS33)

DISCUSSION

Using GWAS, many QTNs associated with agronomic and morphological traits in common bean have already been identified (KAMFWA et al., 2015; MOGHADDAM et al., 2016; SOLTANI et al., 2016; NASCIMENTO et al., 2018; WU et al., 2020). However, few panels have accessions of Mesoamerican origin and are adapted to Brazilian conditions (DELFINI et al., 2021; RESENDE et al., 2018; VALDISSER et al., 2020). Thus, the diversity panel used in this study stands out as a comprehensive representation of the genotypes obtained through breeding efforts for this crop in Brazil, which are important because they contain sources of disease resistance and genes for agronomic traits.

Within this panel, there are genotypes from UFLA's active germplasm bank, along with the main cultivars that have been recommended over the years by this program. Furthermore, it incorporates elite lines derived from different recurrent selection programs. These lines exhibit remarkable diversity among themselves, the result of multiple crossings designed to accumulate favorable alleles. Additionally, cultivars from other common bean breeding programs in Brazil, such as EMBRAPA, IAPAR, and UFV, were included in this panel.

Population structure has a significant role as one of the dependent variables in the model, being essential to control spurious associations. The total number of subpopulations considered was k = 3 (Fig. S2). The magnitude of the LD extension was significantly higher than what was expected for GWAs. This phenomenon is, in part, a result of the autogamous nature of the common bean and the reduced rates of recombination events, as documented in previous studies (PERSEGUINI et al., 2016). Another factor contributing to this observation is the fact that the panel includes genotypes that, to some extent, share some degree of relationship due to common origins in recurrent selection programs. This genetic similarity, even if these are divergent for some traits, contributes to the wide extension of LD. Thus, despite expectations, the historical and evolutionary recombination events in the panel under analysis have been little explored (ZHU et al., 2008). Recombination events in cultures with homozygous genetic backgrounds are ineffective in causing LD decay, resulting in extensive (large) and slow LD decay. The slow decay of LD and the large extent of LD observed in this study corroborate previous reports in common bean (PERSEGUINI et al., 2016; COSTA et al., 2021; LIU et al., 2022).

LD is a critical factor influencing GWAS resolution (MOGHADDAM et al., 2016). When LD is high, the resolution of GWAS is low, making it more difficult to identify the gene or genetic marker associated with a trait or phenotype (HUANG and HAN, 2014). LD can lead to an association between a SNP and multiple genes, which makes it difficult to determine which gene is responsible for the trait. On the other hand, when the LD is low, the resolution of GWAS is high. This means that it is possible to more accurately identify the gene or genetic marker associated with a trait. However, in this situation, multiple genes are candidates for being associated with a particular SNP. Therefore, it is important to consider the interference of LD on the results of a GWAS (MOGHADDAM et al., 2016). The choice between high or low resolution depends on the specific objectives of the study, the availability of genetic data, and an understanding of the complexity of the genetic traits under analysis. Considering biological interpretation, high LD no interferes with the indication of regions associated with these traits.

DF is an important trait for common bean breeding. The heritability estimate was high magnitude (0.80) and similar to other studies in the literature (NASCIMENTO et al., 2018; MOGHADDAM et al., 2016; RAGGI et al., 2019). Therefore, most of the phenotypic variation in DF is due to genetic factors. However, there is also evidence that DF is controlled by several genes located in different regions of the genome (NASCIMENTO et al., 2018; MOGHADDAM et al., 2020; RAGGI et al., 2019). There are reports that additive effects predominate in the expression of this trait. According to Mendes et al. (2008), there was a good fit of the additive-dominant model, however, dominance is less important in controlling this trait (BARILLI et al., 1999), which can enable gains from the selection. Thus, the inheritance of this trait is oligogenic and presents some modifying genes (MENDES et al., 2008; NASCIMENTO et al., 2018; MOGHADDAM et al., 2016; RAGGI et al., 2019). Flowering is controlled by a complex network of genes that together integrate different metabolic routes associated with photoperiod, vernalization, gibberellin production, and other routes that are responsible for flowering time (MOGHADDAM et al., 2016).

In this work, three candidate genes for DF were found. The first gene related to DF is the gene *PvUI111.02G303000* encoding pectinesterase. In some species, the pectinesterase gene is overexpressed during the flowering period, since the auxin signal alters the expression of this gene and causes it to act in the modification of cell walls through the demethylesterification of cell wall pectin, which initiates the remodeling of the cell wall leading to rapid enlargement and movement of the petals, which is a dynamic movement

(SHIRASAWA et al., 2022). Another gene is the *PvUI111.08G068900* gene that encodes beta-amylase. When the night temperatures are high, the synthesis and accumulation of starch in wheat grains is limited by beta amylase. This causes an imbalance in the ratio of starch, protein and lipids in various parts of the grain. This change then affects the mobilization of resources and can impact the growth, flowering, and germination process (IMPA et al., 2020). Another gene is *PvUI111.10G098200,* which encodes small subunit ribosomal protein S33 (MRPS33). The function of nucleolar proteins is still unknown. HSU et al. (2021) found that SAHY, a nucleolar protein, plays a crucial role in pre-rRNA processing and normal plant growth together with auxin transport and signaling. There are no reports that this enzyme has a direct association with flowering.

Plant architecture is an important trait for many crops, especially for common bean. Plants with an upright architecture are more resistant to winds, and fungal diseases such as white mold and mainly are more practical for mechanized harvesting (KUZBAKOVA et al., 2022; PIRES et al., 2014). Understanding the genetic and environmental factors that are involved with plant architecture is important for plant breeding. Previous studies have shown that the heritability of plant architecture is of high magnitude, ranging from 0.70 to 0.85 (SANTOS et al., 1986; TEIXEIRA et al., 1999; KUZBAKOVA et al., 2022). In this work, the heritability estimate was 0.84 for ARC1 and 0.67 ARC2. This trait involves several genes and several morphological traits, such as the number of nodes, the distance between nodes, the type of growth, the presence of tendrils, and the height of insertion of the first pod, which are associated with its expression (KUZBAKOVA et al., 2022).

Environmental factors such as day length, soil type, fertilization, and rainfall patterns affect plant architecture expression. In this sense, the genotypes should be evaluated in different seasons of common bean grown, mainly the wet season in Brazil where there are unfavorable environmental conditions such as higher precipitation and normally the plants can be prostated. Therefore, it is important to carry out selection in this season for plant architecture. In this study, the lines have been evaluated two times (R5 and R8 stage) in the wet season. Therefore, this situation allowed us to identify genomic regions associated with this trait. Previous studies have reported the nature of the additive effect in controlling this trait and the possibility of success in the selection process.

In this work, four candidate genes related to ARC1 were found. The *PvUI111.07G050100* gene encodes cysteine-rich receptor-like protein kinase. The cysteine-rich receptor-like protein kinase CRK28 modulates Arabidopsis growth

and development and influences abscisic acid responses (PELAGIO-FLORES et al., 2020). *PvUI111.08G021400* gene encodes translation initiation factor 2B, delta subunit (eIF-2Bdelta/GCD2). There are no reports that this enzyme has a direct association with the plant architecture. Another gene is *PvUI111.08G021600* which encodes a leucine-rich repeat-containing protein. It is a type of protein involved in a wide variety of cellular processes, including cell signaling, immunity, and development (ALVAREZ-DIAZ et al., 2022). Leucine-rich repeat (LRR) proteins are characterized by the presence of repeated sequences of amino acids, including leucine, which is a hydrophobic amino acid. LRR proteins can interact with other proteins, lipids, and carbohydrates and play a role in many different cellular functions. The last associated gene with the ARC1 is *PvUI111.08G174900*, which encodes Phosphoribosyl-aminoimidazole-succinocarboxamide synthase (SAICARs). This is a key enzyme in the de novo purine biosynthesis pathway in plants. It catalyzes the seventh step in this pathway, the conversion of 5'-phosphoribosyl-5-aminoimidazole-4-carboxylic acid (CAIR) and L-aspartate to 5-aminoimidazole-4-succinocarboxamide ribotide (SAICAR) and ADP (SOUCKOVA et al., 2022). There are no reports that this enzyme has a direct association with the plant architecture.

Three distinct genes were identified on different chromosomes in the context of ARC2. The *PvUI111.02G016200* gene is responsible for encoding a RNA binding protein (contains RRM repeats). These proteins play a crucial role in regulating gene expression, specifically influencing processes related to cell wall biosynthesis and cellulose deposition, ultimately impacting plant growth and form. These RRM-containing proteins are involved in auxin transport, a key hormone for stem elongation and gravitropism in Arabidopsis, whose mutations in PIN genes affect auxin distribution and consequently influence plant architecture (BANNETT, 2015). The *PvUI111.05G134700* gene encodes ATP-Dependent Helicase-Like Protein. There are no reports that this enzyme has a direct association with upright plant architecture. On the other hand, the *PvUI111.08G235700* gene encodes PPR repeat family. Some PPR proteins have been shown to play a role in determining plant architecture, including the growth and orientation of stems and branches. This is likely because they regulate the expression of genes involved in cell wall biosynthesis, hormone signaling, and other developmental processes. In Arabidopsis was identified a PPR protein called TCP15 that is involved in the regulation of branch angle, whose loss-of-function mutations in TCP15 resulted in plants with more upright branches (MANASSERO et al., 2013).

During each phase of plant architecture assessment, a distinct trend is observed. In the R5 stage, the majority of plants exhibit an upright architecture with a prevalence of scores below 3. However, when the evaluation is conducted at the end of the growth cycle, the majority score above 3. This phenomenon is particularly prominent during the wet season due to prevailing environmental conditions. In this context, the genomic regions governing upright architecture do not align across the various stages under evaluation. Consequently, assessing plant size should be conducted during the most advanced stages and under challenging conditions.

Furthermore, it is essential that these studies subsequently go through a validation process of the identified markers, so that they can be routinely incorporated into plant breeding programs with marker-assisted selection. This validation is essential to guarantee the effectiveness and reliability of these markers in genomic selection, contributing to increasing the efficiency of selecting traits of agronomic interest.

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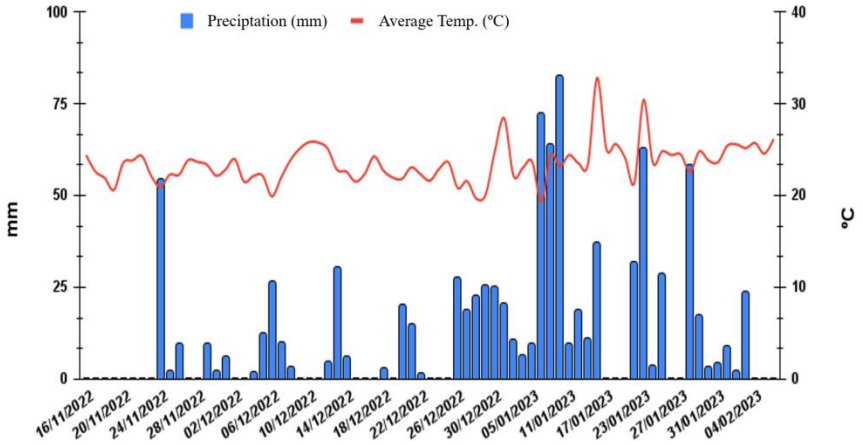
Supplementary Material

Figure S1 - Temperature and precipitation during the wet season of the 2022/2023 in the experimental area.



Source: INMET (2023).

Table S1 – Phenotypic means of common bean lines from the active germplasm bank of the Universidade Federal de Lavras-UFLA obtained in the wet season of the 2022/2023 for the number of days to flowering (DF), plant architecture at stage R5 (ARC1), plant architecture at stage R8 (ARC2).

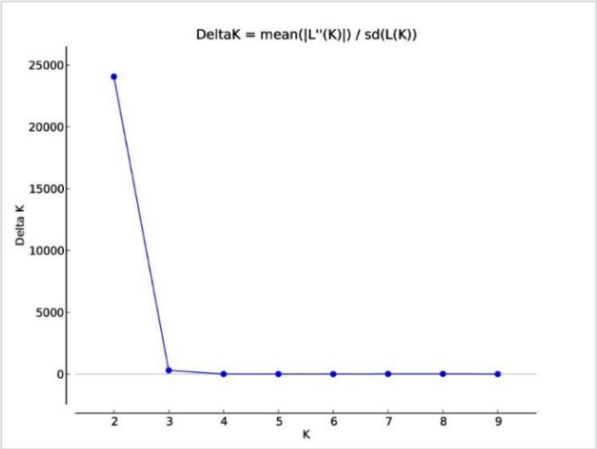
Treat	Line	DF	ARC1	ARC2
1	Corinthiano	38	7.5	9.0
2	MAVll-127	39	1.5	7.5
3	VC37	40	3.0	7.0
4	MAIII-16.159	40	3.5	6.5
5	MAVIII-78	41	2.5	4.5
6	MAVIII-89	38	2.0	8.0
7	CARIOCA	38	3.5	6.5
8	CNFCMG-11-06	38	2.5	6.5
9	BRS AMETISTA	39	2.5	7.0
10	CXII-1	39	3.0	6.0
11	CNFCMG 11-08	39	2.0	7.5
12	MBC1 17/5	38	2.5	8.0
13	CXII-23	38	2.5	6.5
14	MAIX 14	38	2.5	7.0
15	CXII-13	40	2.5	5.5

Treat	Line	DF	ARC1	ARC2
16	MBCO-22/3	40	2.5	5.5
17	CXII-8	38	2.5	6.5
18	E09/10-28	38	2.5	7.0
19	MAVI-60	39	2.5	7.0
20	CARIOCA MG	40	2.0	6.5
21	MAVI-39	39	2.5	5.5
22	MAIII-17.185	40	4.5	6.5
23	MAIV-8.102	38	2.0	5.5
24	CXII-15	39	2.0	5.0
25	mai-8.9	42	2.5	6.5
26	MAIV-18.259	40	4.0	5.0
27	MAIV-15.524	40	1.5	6.0
28	VC 27	40	3.0	5.5
29	VC 25	41	3.0	5.5
30	MADREPEROLA	38	4.0	7.5
31	VC 24	41	2.0	5.5
32	RPCVIII 13	38	1.5	4.5
33	MAIX 10	39	3.0	5.5
34	MBCI 32/14	39	4.0	7.0
35	MB 89	39	2.5	7.0
36	CXI 18	40	2.0	5.0
37	OURO NEGRO	36	9.0	9.0
38	RPCVIII 7	39	3.0	4.0
39	PEROLA	39	3.0	6.0
40	MAJESTOSO	38	4.0	6.0
41	VR 18	38	7.0	9.0
42	VR 15	40	8.0	9.0
43	CNFRX 15 275	40	2.5	5.0
44	VR 16	38	8.0	8.5
45	VR 17	38	6.0	7.5
46	CNFJ 15288	38	5.0	5.0
47	RADIANTE	32	4.0	9.0
48	BRS TIMBÓ	42	3.0	3.5
49	CNFP MG 11-18	39	2.5	5.0
50	CNFP MG 11-08	40	2.0	4.0
51	CNFPMG -1106	40	2.5	4.5
52	CNFPMG -11-21	40	1.5	6.0
53	BRS CAMPEIRO	37	1.5	7.5
54	BRS VALENTE	39	2.5	5.0
55	VP 30	38	2.0	7.5
56	VP 31	37	2.0	9.0

Treat	Line	DF	ARC1	ARC2
57	BRS ESTEIO	39	2.0	5.5
58	CNFP11978	40	2.5	3.0
59	BRS ESPLENDOR	40	2.0	6.0
60	VC20	40	2.0	5.0
61	VC 21	40	4.0	6.0
62	VC 19	39	3.0	6.0
63	CV-6	38	2.5	5.0
64	EMB9	40	2.0	6.5
65	VC 18	37	3.0	5.0
66	BRS NOTAVEL	37	1.0	8.0
67	VC 17	39	3.5	4.5
68	IPR UIRAPURU	40	1.5	7.0
69	BRS COMETA	40	3.0	3.0
70	BRS SUBLIME	40	2.5	5.5
71	CXIII 4.484	37	3.0	5.5
72	CXIII 1.23	40	2.0	6.5
73	CXIII 85.62	39	2.5	6.0
74	CXIII 1.1	39	3.0	6.0
75	CXIII 1.7A	39	2.0	7.0
76	CXIII1.7B	38	1.5	6.5
77	RPXI -1	38	1.5	4.5
78	RPXI -6	39	1.5	3.5
79	RPXI-26	40	1.5	1.5
80	RPXI-7	40	2.0	2.0
81	RPXI-38	39	2.0	3.0
82	RPXI-43	38	1.5	8.0
83	RPXI-14	40	1.5	5.0
84	MAXII- 22	40	2.5	7.0
85	MAX-1	39	3.0	7.0
86	MAX-2	42	3.5	8.0
87	MAX-7	39	2.0	7.0
88	MAII-23	39	2.5	6.0
89	MAX-5	39	2.5	5.5
90	MAX-9	38	2.0	8.0
91	MAXII-7	40	2.5	2.5
92	MAX-8	38	3.0	7.5
93	MAXII-20	39	3.0	7.5
94	MAXII-5	40	2.5	5.5
95	MAX-11	40	2.5	7.0
96	MAX-4	39	2.5	7.0
97	VP-33	40	2.5	5.5

Treat	Line	DF	ARC1	ARC2
98	VP-34	40	1.5	5.5
99	VR-20	34	2.5	3.5
100	CNFP 10794	40	3.0	4.5
101	CNFP 11979	41	3.5	4.0
102	CNFP 15194	40	2.0	2.5
103	CNFP 15677	40	2.0	7.0
104	CNFP 15680	40	1.0	4.5
105	VC36	40	3.5	5.5
106	VC35	39	4.0	5.5
107	VC38	40	4.0	6.5
108	BRS Horizonte	40	2.0	5.0
109	BRSPitanga	40	2.0	6.5
110	BRS Bentivi	40	2.5	4.5
111	BRS União	41	3.5	6.5
112	BRSUai	40	1.5	1.5
113	Roxo90	38	2.5	8.5
114	IPR Colibbri	36	6.0	6.5
115	ESAL503	40	4.5	7.5
116	ESAL535	42	3.0	5.5
117	ESAL664	40	2.5	6.0
118	ESAL517	39	4.5	9.0
119	ESAL561	40	2.0	6.0
120	ESAL502	38	5.0	9.0
121	ESAL652	38	2.5	6.5

Figure S2: Population structure K values for STRUCTURE analysis to methodology of Evano et al (2005). The analysis was based on 3117 polymorphic SNPs and 121 lines from UFLA diversity panel.



PAPER 2 - VALIDATION OF SNP MARKER FOR Lv238 of *Colletotrichum lindemuthianum* RACE 65 IN COMMON BEAN

The Plant Genome Magazine Standards

(PRELIMINARY VERSION)

ABSTRACT

Achieving cultivars with durable resistance to *Colletotrichum lindemuthianum* stands as a significant challenge in common bean genetic breeding, given the wide pathogenic variability exhibited by this fungus. Remarkably, even isolates classified within the same race display considerable variability. The primary objective of this study was to validate the previously identified SNP marker ss714656893 located on the Pv04 chromosome, which was shown to account for 72% of the phenotypic variation in earlier research. To achieve this, an F_2 population was derived from the cross between BRS Estilo and BRS Valente. Phenotypic assessments were conducted using the Lv238 isolate, while genotyping utilized the TaqMan^{TM} probe specifically designed for the aforementioned marker. Additionally, a panel comprising 49 lines was employed in the study. Our findings unveiled a robust selection efficiency of 94.80% for data obtained from the F_2 population and 83.83% for lines with distinct genetic backgrounds. The validation of SNP markers constitutes an important step in their application within marker-assisted selection. This strategic approach has an important role in advancing the development of cultivars with durable resistance, particularly against the challenges posed by *Colletotrichum lindemuthianum*.

Keywords: *Phaseolus vulgaris* L., *Colletotrichum lindemuthianum*, Molecular breeding.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important crop because it is a fundamental element for the food, economic, and cultural security of several countries, especially in developing countries in South America, Central America, and Southwest Africa (UEBERSAX et al., 2023). Among the various diseases that affect the crop, anthracnose, caused by the hemibiotrophic fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara, is one of those that most affect the productive potential and quality of the grains of this crop (NABI et al., 2022; BISNETA et al., 2021). When susceptible cultivars are exposed to favorable conditions, such as moderate temperatures and high humidity, the damage to yield can be significant, reaching 100% in severe situations (PADDER et al., 2017; VIDIGAL FILHO et al., 2020). The most effective and safe way to control this disease is by means of genetic resistance. However, the wide pathogenic variability is one of the main obstacles to obtaining durable genetic resistance in common bean cultivars (FERREIRA; CAMPA; KELLY, 2013; PADDER et al., 2017; PEREIRA et al., 2010; PINTO et al., 2012). Around 298 races of *C. lindemuthianum* have been described in the world, with more than 1,500 isolates (NUNES et al., 2021; PADDER et al., 2017; PAULINO et al., 2021).

Resistance to anthracnose in common beans involves both qualitative and quantitative aspects. The Bean Improvement Cooperative (BIC) has approved 14 resistance genes to *C. lindemuthianum*, identified in common bean cultivars. These genes, named according to Kelly and Young (1996) nomenclature, include *Co-1*, *Co-2*, *Co-3*, *Co-4*, *Co-5*, *Co-6*, *Co-11*, *Co-12*, *Co-13*, *Co-14*, *Co-15*, *Co-16*, *Co-17*, and others. Additional genes and a resistance allele have been reported but await validation by the BIC genetics committee, as *Co-x*, *Co-w*, *Co-u* (Geffroy et al., 2008), *Co-y*, *Co-z* (GEFFROY et al., 1999) and *Co-v* (GEFFROY, 1997) found in the cultivar Jalo EEP558, *Co-AC* (NANAMI et al., 2017), *Co-Pa* (LIMA CASTRO et al., 2017), *CoPv01* ^{CDRK} (GONÇALVES-VIDIGAL et al., 2020), and the *Co-1*^{HY} *allele* characterized in the Hongyundou cultivar (CHEN et al., 2017). Recent studies highlight that qualitative resistance doesn't exclude the presence of quantitative resistance. Shafi et al. (2022) identified QTLs, with phenotypic variation ranging from 3.97% to 46.8%, averaging 16.54%. Their meta-analysis identified 11 meta-QTLs and 10 hotspot QTLs on six common bean chromosomes, revealing 1,251 defense-related genes in these regions, including R genes and proteins with NBS-LRR domains and kinases. Race 65 is cosmopolitan, and Mesoamerican dispersed in the north, center, and south of America (PADDER et al., 2017). In Brazil, there is a great diversity of anthracnose pathogen races, with a total of 89 detected,

making the country the holder of the greatest diversity of this pathogen in the world (PAULINO et al., 2022). Several breeding programs have already targeted the resistance to anthracnose (PINTO et al., 2012; RIBEIRO et al., 2016). However, a high genetic and pathogenic variability of isolates from race 65 has been reported (RODRÍGUEZ-SUÁREZ et al., 2005; ISHIKAWA et al., 2008, 2011; DAVIDE and SOUZA, 2009).

In order to better distinguish *C. lindemuthianum* race 65 isolates, Ishikawa, Ramalho, Souza (2011) suggested a set of differentiators made up of commercial common bean lines from Brazil. The inheritance of resistance of common bean cultivars to six isolates belonging to race 65 of *C. lindemuthianum* was recently investigated by Costa et al. (2017). The distinct reaction pattern of common bean cultivars when inoculated with the different isolates of race 65 and the 15R:1S segregations observed in the F_2 generation of several crosses indicate the possibility of duplicated genes conferring specific resistance to each of the isolates used. This result highlights that the specificity of the pathogen-host interaction also occurs at the isolate level within the race. Therefore, 12 genes were identified associated with resistance to these six isolates, revealing a significant diversity of alleles originating from different genes involved in anthracnose resistance. This scenario makes obtaining cultivars with durable resistance challenging (COSTA et al., 2017). Therefore, it is necessary to identify and validate these genes to isolates, aiming for their use in marked-assisted selection (MAS).

In this context, Costa et al. (2021), attempting to identify SNPs associated with resistance to isolates Lv238 and Lv134 of race 65, carried out QTL mapping using the F_2 generation of the BRS Estilo x BRSMG Ouro Vermelho population, in addition to association mapping with a diversity panel composed of 189 lines. The genotyping of the biparental population was performed with the BARCBEAN_6K_3 BeadChip, which had 999 SNPs, while the UDP was genotyped with the BARCBEAN_6K_3 BeadChip, which had 5398 SNPs. The same SNPs were associated with both approaches, and the region that confers resistance to the Lv238 isolate was identified between 1.1345 Mb and 1.165 Mb on the Pv04 chromosome (COSTA et al., 2021). This information can be converted into valuable tools for the detailed analysis of genomic regions harboring resistance gene clusters, such as the *B4* clusters (MEZIADI et al., 2016; RICHARD et al., 2017). The SNP ss714656893 explained 72% of the phenotypic variation (COSTA et al., 2021). The extension of the region is significant in terms of base pairs, due to the large extension of the linkage blocks in the F_2 population, and due to the high linkage disequilibrium in the GWAS panel, resulting from the high degree of kinship between some lines (COSTA et al., 2021). However, the exact position

could not be identified due to low resolution and the absence of closer markers linked to this gene (COSTA et al., 2021).

In this sense, the identified SNPs require a validation process before their regular incorporation into MAS. Advances in genomics allow the discovery of more efficient markers for MAS. Several studies have been dedicated to these markers (PERSEGUINI et al., 2016; ZUIDERVEEN et al. 2016; WU et al., 2017; LOBATON et al. 2018; GIL et al. 2019; NAY et al., 2019; FRITSCHE-NETO et al., 2019; GOMES MESSIAS et al., 2022). MAS is a powerful strategy, and to optimize it, it is crucial to use several markers in the selection of genotypes with desired traits in the initial phases of breeding programs, especially to discard genotypes that do not present the desired alleles (ALVARES et al., 2019; GOMES MESSIAS et al., 2022).

As previously mentioned, several SNPs have been found to be associated with anthracnose resistance. However, only a few of them have undergone a validation process. Therefore, the objective of this study was to evaluate and validate the SNP (ss714656893), which was previously identified as associated with the Co_L resistance gene, as reported by Costa et al. (2017; 2021). We used an F_2 population derived from the cross between BRS Estilo and BRS Valente cultivars and an additional diverse panel comprising 49 common bean genotypes. We conducted phenotyping and genotyping of these populations with the presence of resistance alleles to the Lv238 isolate of race 65, thereby validating the SNP marker for its potential use in common bean breeding programs, using the MAS.

MATERIAL AND METHODS

Genotypes

We used 259 common bean genotypes, including 210 F_2 generation plants resulting from crossing the BRS Estilo and BRS Valente cultivars. Additionally, we included 10 cultivars, 31 elite lines, and 8 sources of anthracnose resistance from diverse origins in Brazil and international breeding programs. Phenotypic and genotypic information were collected from these 259 genotypes, which were then assessed for resistance to the Lv238 isolate of race 65 of *C. lindemuthianum*.

Molecular analysis with the SNP marker

The resistance inheritance of isolate Lv238 was studied by Costa et al. (2017) by crossing several parents. The study revealed that the resistance trait is controlled by two duplicated genes, with a segregation of 15 Resistant: 1 Susceptible in the F_2 generation. However, when cultivars BRSMG Ouro Vermelho and BRS Estilo were crossed, it was observed that they differ in only one gene based on the segregation pattern.

The most significant SNP marker from GWAS, with an R^2 value of 72%, was identified as ss715646893 (Table 1). This marker is located at 1.1657 Mb in Pv04 and has the allele (T) for resistance. The detection of this marker's association with resistance was achieved through GWAS and biparental crossing. The SNP was assessed through an experiment with the TaqMan® hydrolysis probe (ThermoFisher) (SHEN et al., 2009; APPLIED BIOSYSTEMS, 2021), conducted at the "Laboratório de Biotecnologia da EMBRAPA Arroz e Feijão", as described below (DNA extraction and genotyping of target alleles).

Table 1 SNP markers associated with resistance to isolate Lv238 of *C. lindemuthianum* race 65, with their respective identification, position, coefficient of determination (R^2) in GWAS by Costa et al. (2021) and interaction with genotypes that carry the target gene.

SNP	Allele	Chr.	Position	R^2 (%)	Resistant	Susceptible
ss715646889	T	Pv04	1134467	21	BRS Estilo	BRSMG Ouro Vermelho
ss715646891	T	Pv04	1147939	52	BRS Estilo	BRSMG Ouro Vermelho
ss715646892	T	Pv04	1155846	56	BRS Estilo	BRSMG Ouro Vermelho
ss715646893	T	Pv04	1165722	72	BRS Estilo	BRSMG Ouro Vermelho

Source: Costa et al. (2021).

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

Validation of the SNP marker ss715646893 associated with the Lv238 region

The SNP ss715646893 displayed a high level of association with disease resistance, and thus, it underwent an additional validation process. This specific SNP was converted into a TaqMan® probe (ThermoFisher) (SHEN et al., 2009; APPLIED BIOSYSTEMS, 2021). The probe design was based on aligning sequences that contained the targeted SNP to the common bean reference genome (SCHMUTZ et al., 2014), using the BLAST command. A search for repetitive elements was performed using RepeatMasker, with both analyses available on the Phytozome platform (Phytozome v12.1: Home: https://phytozome.jgi.doe.gov/). Probes were designed using the online Custom TaqMan Assay Design Tool (ThermoFisher) available from: [https://www.thermofsher.com/order/customgenomicproducts/tools/genotyping/].

Segregating population and contrasting lines

The SNP marker associated with the genomic region responsible for resistance to anthracnose was validated using an F_2 population of 210 individuals. This population was obtained by crossing BRS Valente ♀ (female parent, susceptible to isolate Lv238, of *C. lindemuthianum* race 65) with BRS Estilo ♂ (male parent, containing the genomic region responsible for resistance to isolate Lv238). The plants were assessed for the reaction to isolate Lv238, of race 65 of *C. lindemuthianum* in greenhouse conditions at the Biology Departament of UFLA.

Phenotyping of anthracnose severity in common bean genotypes

The Lv238 isolate was grown in Petri dishes containing BDA culture medium. Posteriorly, aiming to obtain high sporulation rates, small pieces of mycelium were transferred to sterile common-bean pods placed in test tubes for an incubation period of 10–15 days at 22˚C, in the dark. Subsequently, the conidia suspensions (1.2×10^6 conidia.ml^{-1}).

The panel of the 236 common bean genotypes was carried out in 162-cell polystyrene trays, with Topstrato® commercial substrate, using one seed per genotype. Seedlings with fully expanded primary leaves (V2) were sprayed with a suspension of conidia to the point of oozing out of the stem on both surfaces of the unifoliate leaves with the aid of a manual sprayer. After inoculation, the seedlings were incubated in a humidity chamber for 48 hours with a 12-hour photoperiod and a temperature of 20 ºC. Subsequently, the seedlings were transferred to a greenhouse with controlled humidity and temperature and assessed 12 days

after inoculation according to the severity rating scale from 1 to 9 described by Van Schnoonhoven and Pastor-Corrales (1987) (Table 2).

Each plant was assessed individually. Plants with scores between 1 and 3 were considered resistant and the others susceptible.

Table 2 - Scale descriptive in grades to assess the severity of the anthracnose in seedlings in common bean (SCHOONHOVEN; PASTOR-CORRALES, 1987).

	Note Description
1	Absence of symptoms
2	Up to 1% of the main veins show necrotic spots, visible only on the underside of the leaves
3	Greater frequency of foliar symptoms described in the previous grade, with up to 3% of the veins affected.
4	Up to 1% of the veins show necrotic spots, noticeable on both sides of the leaf.
5	Greater frequency of foliar symptoms described in the previous grade, with up to 3% of the veins affected.
6	Necrotic spots on the veins, noticeable on both sides of the leaves, and the presence of some lesions on stems, branches, and petioles.
7	Necrotic spots in most of the veins and in a large part of the adjacent mesophilic tissue that ruptures. Presence of abundant lesions on the stalk, branches, and petioles.
8	Necrotic spots on almost all veins, are very abundant on stems, branches, and petioles, causing ruptures, defoliation, and reduced plant growth.
9	Dead plants

Genotyping of common bean genotypes

Before phenotyping, one primary leaf from each of the 259 genotypes was collected as a seedling. Each leaf was collected individually, wrapped in aluminum foil under liquid nitrogen, and kept in an ultra-freezer at -80°C. The genomic DNA of the 259 genotypes was extracted using the CTAB method, according to the protocol proposed by Doyle and Doyle (1990) and modified by IAC (1998). DNA concentration was estimated using a fluorometer Qubit® (Thermo Scientific®, Waltham, USA), and integrity was visualized by electrophoresis in 1.0% agarose gel stained with ethidium bromide.

Taqman® SNP genotyping assays were amplified with the Taqman® GTXpress ™ reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's guidelines. Amplification was performed 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 for allele analysis using the Genotyping Analysis Module, V.3.7.

Statistical Analysis

Observed frequencies of plants for the phenotypic classes, resistant and susceptible in the F_2 generation, have been used to test the hypothesis of phenotypic segregation 3Resistent_:1susceptible. The Chi-Square test (χ^2) was used, adopting a significance level of 5% with the help of the R software, version 4.1.2 (R Core Team 2021).

Linkage analysis between the SNP marker and the region was performed using the OneMap package (Margarido et al., 2007) and the estimated recombination frequency was converted to genetic distance (cM).

All analyses will be performed using the 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 (%) = $(1 - 4rf^{2}) \times 100$, where " rf " is the recombination frequency.

RESULTS

In a recent study conducted by Costa et al. (2021), the SNP ss715646893 emerged as the most significant genetic marker responsible for conferring resistance to the Lv238 isolate of race 65 of *C. lindemuthianum*. This marker accounts for 72% of the phenotypic variation and is located in a specific region between 1.13 Mb and 1.17 Mb at the end of chromosome Pv04. The region spans 31.2 Kb and is situated in a subtelomeric region where it is also present in the Co_L gene, which plays a crucial role in conferring resistance to the Lv238 strain. The study by Costa et al. (2021) provides a detailed insight into the role of this marker in conferring resistance to the Lv238 isolate.

The marker, located at position 1,165,722 bp, displays a C:T polymorphism. According to Costa et al. (2021), susceptibility is associated with the C allele, whereas the T allele is linked to resistance. This marker's significance lies in its potential application in marker-assisted selection (MAS) due to its polymorphic nature and its ability to explain a

significant proportion of phenotypic variance. TaqMAN probes have been developed specifically for this marker to enhance its relevance.

The analysis performed in Phytozome did not reveal the presence of the marker in repetitive regions. Based on these findings, the marker demonstrated an appropriate profile for genotyping. To validate the effectiveness of this marker, we crossed the cultivars BRS Estilo (resistant) and BRS Valente (susceptible), generating the F_1 and F_2 populations resulting from this crossing. We confirmed the reactions of the parental cultivars when inoculated with the Lv238 strain, obtaining results consistent with those presented by Costa et al. (2017). After designing the probe, we verified its amplification pattern, subjecting it to tests on the parents and the F_1 generation. The marker exhibited an appropriate amplification pattern, in which the T allele was associated with resistance, while the C allele was associated with susceptibility. The BRS Estilo cultivar showed an amplification pattern consistent with the T allele, indicating its resistance. The BRS Valente cultivar, in turn, presented the expected pattern of susceptibility, with amplification of the C allele, as detailed in Table 3.

Table 3 - Amplification pattern for marker ss714656893 for the BRS Estilo and BRs Valente genotypes and their respective allele and phenotype for resistance to Lv238 of race 65 of *C. lindemunthianum* .

Identification	**Allele**	**ss714656893**	**Phenotype**
BRS Estilo	T allele	T:T	Resistant
BRS Valente	C allele	C:C	Susceptible
Generation F_1	CT allele	C:T	Resistant
Control (H_2O)	-	NOAMP *	

*NOAMP – Not amplified

In this study, we assessed the severity of anthracnose in the F_2 generation obtained by crossing BRS Estilo and BRS Valente. This population, consisted of 210 plants was genotyped. Out of these, 159 plants were found to be resistant (R), while 51 were susceptible (S). Importantly, the observed ratio of 3R:1S was consistent with the expected ratio (χ^2 = 0.05; p = 0.81), as shown in Table 2. In terms of genotyping, we found 59 dominant homozygotes, 96 heterozygotes, and 55 recessive homozygotes. These results were also consistent with the expected segregation ratio of 1TT:2CT:1CC (χ^2 = 1.69; p = 0.42), as presented in Table 4.

Table 4 - Phenotypic and genotypic segregation of individuals from the F_2 population derived from the cross between BRS Estilo x BRS Valente), assessed for resistance to isolate Lv238, from race 65 of *Colletotrichum lindemuthianum*.

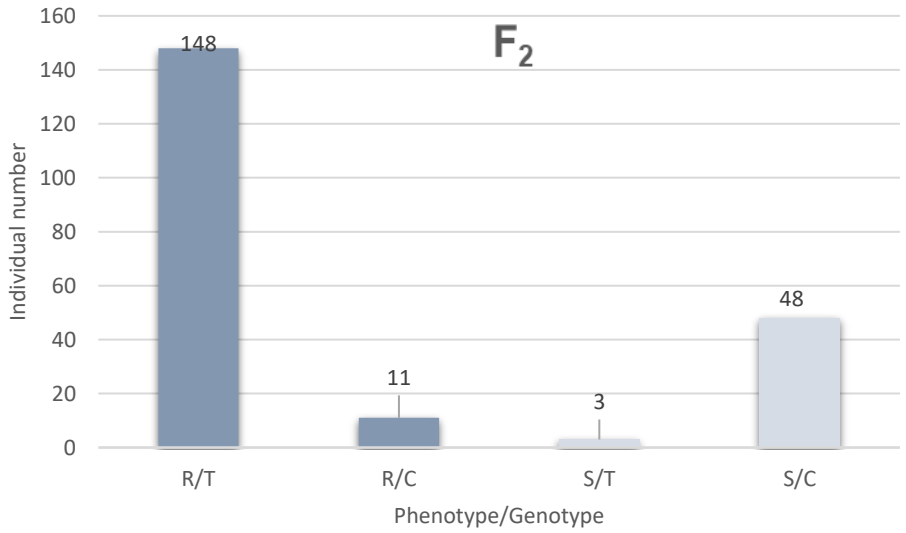
Genotype / Phenotype	OF [a]	EF [b]	Hypothesis	χ^2 [c]	P-value [d]	rf (%) [f]	SE(%) [g]
T:T	59	52.5	1TT:2CT:1TT	1.69	0.42	11.40cM	94.80
C:T	96	105					
C:C	55	52.5					
Class							
Resistant	159	157.5	3:1	0.05	0.81		
Susceptible	51	52.5					

[a] OF: Observed frequency [b] EF: Expected frequency [c] χ^2:Chi-square [d] P-value: associated with the null hypothesis (1TT:2CT:1CC for the molecular marker and 3R:1S for the phenotypic data); [f] rf: recombination frequency.

[g] SE: Selection efficiency.

Using the analysis of the F_2 population, frequency of recombination between the *Co$_L$* gene and the SNP marker ss714656893 was estimated (11.40%). This suggests that the distance between the *Co$_L$* gene and the marker is 11.40 cM, with a selection efficiency of 94.80%. Out of the 210 plants in the F_2 generation, 14 of them were identified as recombinants, making up 6.67% of the observed proportion. Among these 14 plants, 11 were found to be resistant and carried the C allele, while the remaining 3 were found to be susceptible and carried the T allele (see Figure 1). The majority of the plants, that is, 196, presented parental phenotypes, with 148 of them being resistant and having the T allele, while the remaining 48 were susceptible and contained the C allele (see Figure 1).

Fig. 1 - Proportion of individuals parental and recombinants distributed in the F_2 population derived from the cross between BRS Estilo x BRS Valente (Lv238).



Legend: R/C: parental plants, resistant and amplified for the C allele; R/T: recombinant, resistant plants amplified for the T allele; S/C: recombinant plants, susceptible and amplified for the C allele; S/T: parental plants, phenotypically susceptible and amplified for the T allele.

The SNP with ss715646893 was analyzed using simple linear regression. According to the regression model, this marker is statistically significant, explaining around 49.43% of the phenotypic variation (as shown in Table 5). The regression model's slope estimate was -3.79, which indicates that homozygous presence of the T reference allele leads to an average score that is 3.79 times lower than that of plants that lack this allele ($p < 0.05$) (Table 5).

Table 5 - Summary of the regression analysis between the SNP marker and the reaction for resistance to isolate Lv238 of *Colletotrichum lindemuthianum* race 65.

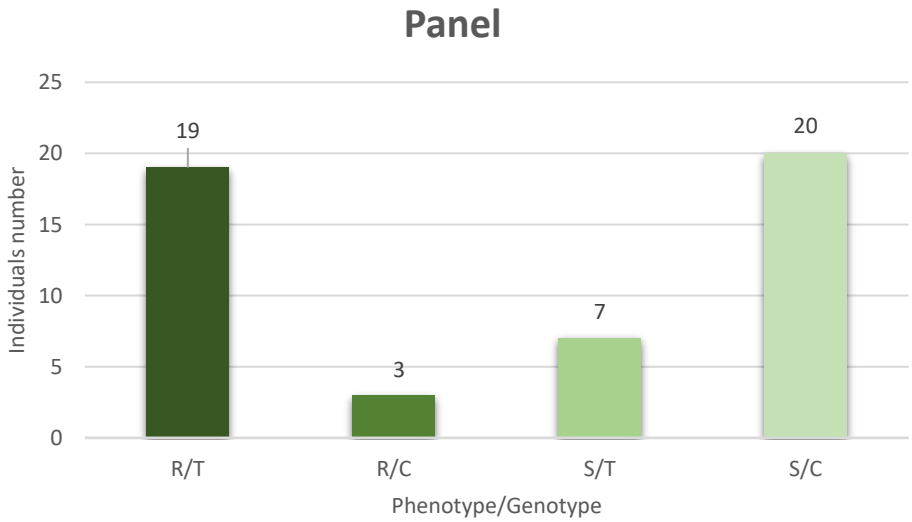
SV [1]	Df [2]	SS [3]	MS [4]	F-value	p-value	R^2 (%)	Slope [5]
C vs T [a]	1	192.8	192.8	76.9	3.85E-11	49.43	-3.79
Residue	209	592.1	2.83				

SV[1]: Source of variation; Df[2]: degree of freedom, SS[3]: Sum of square; MS [4]: mean square; Slope [5]: Angular coefficient of the regression equation; the negative sign on the slope indicates that the allele is associated with disease resistance [a]: contrast considered in the regression analysis between marker alleles and disease severity of *C. lindemuthianum* strain Lv238 race 65.

To further validate this marker, we employed an additional approach by using a panel consisting of a comprehensive set of 49 genotypes. These genotypes include cultivars and elite lines from various breeding programs such as UFLA, UFV, and EMBRAPA. Among

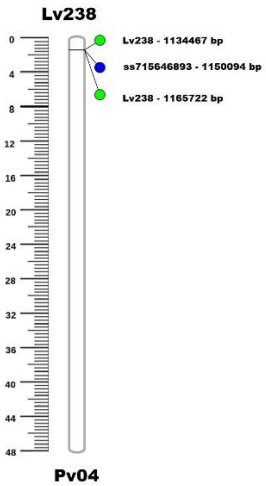
these genotypes, we identified 10 recombinant plants, which account for 20.41% of the panel. Out of these, 3 showed resistance without the presence of the T allele, while 7 showed susceptibility with the T allele. We obtained a total of 39 plants (representing 79.59% of the panel) that exhibited characteristics similar to their parents and whose genotypic profiles coincided. Among this group, 38.8% demonstrated resistance to the T allele, while 40.8% showed susceptibility in the absence of the T allele (Figure 2).

Fig. 2 - Proportion of individuals parental and recombinants distributed in the validation panel of common bean lines.



Legend: R/C: parental plants, resistant and amplified for the C allele; R/T: recombinant, resistant plants amplified for the T allele; S/C: recombinant plants, susceptible and amplified for the C allele; S/T: parental plants, phenotypically susceptible and amplified for the T allele.

Fig. 3 – Distribution of the ss715646893 marker within the region delimited by Costa et al. (2021) responsible for conferring resistance to isolate Lv238 of *Colletotrichum lindemuthianum* race 65.



DISCUSSION

In the work of Costa et al. (2017), duplicate genes were identified that confer resistance to the different isolates of race 65 of *C. lindemuthianum* assessed. In a later work, Costa et al. (2021) identified SNPs associated with a region that confers resistance to Lv238 isolates. The process of validation is necessary, since large linkage blocks are typical of biparental populations due to the low frequency of recombination (COSTA et al., 2021; PERSEGUINI et al., 2016). This happens in the F_2 population of Costa et al. (2021), SNPs were found associated with R^2 of 100%. According to the author, due to the small sample size, no recombinant individuals were identified.

A GWAS was performed as another strategy to identify SNPs associated with resistance to Lv238 (COSTA et al., 2021). However, unlike what was expected for a GWAS, the LD decay extended over several base pairs, around 400K. This happened due to the high degree of kinship between the accessions. Even though the UFLA Diversity Panel (UDP) is made up of accessions from the active germplasm bank of UFLA, EMBRAPA, and UFV, among others, presenting great diversity, 63% of the accessions came from different SR programs at these institutions. Even under these conditions, the SNP ss714656893 was identified, which was also found in the biparental population with a proportion of 72% explaining the phenotypic variance.

As previously mentioned, a validation process was carried out with the resistant parent BRS Estilo x BRS Valente and also through a panel of 49 lines of various origins. From the phenotyping and genotyping data of the F_2 population (BRS Estilo x BRS Valente) the estimate of the recombination frequency was 11.40%, which can be considered a low magnitude, which gave a high selection efficiency (94.6%). Estimates of selection efficiency between 97% and 99.8% have been reported in the literature in validation work on SNPs associated with resistance genes to common bean pathogens. Other validation works also found similar values between 97% and 99.8% of selection efficiency (ALVARES et al., 2019; GOMES-MESSIAS et al., 2022). This indicates that the value found allows the use of this probe in MAS aiming at resistance to the Lv238 isolate of race 65 of *C. lindemuthianum*.

The high LD guaranteed the necessary resolution for the identification of polymorphisms close to the causal gene Co_L , which confers resistance to this isolate. Using the allelism test carried out by Costa et al. (2017), the BRS Valente and BRSMG Ouro Vermelho strains have the same susceptibility reaction. However, these strains have different

genetic backgrounds. BRS Valente comes from mass selection from the crosses Engopa 201-Ouro, Ônix and the line NA 512586. BRSMG Ouro Vermelho comes from the recurrent selection program of the parents AN9022180 x Vermelhinho backcrossed with Vermelhinho.

For the F_2 population of Costa et al. (2021), the frequency of recombination between the SNP marker ss714656893 and the *Co$_L$* gene was 1% and in the present work, it was 11.40%. This difference can be explained by the different genetic background and the location of these genes at the ends of the chromosome, also known as subtelomeric regions (BISNETA et al., 2021; SILVA et al., 2023). In these regions, the process of proliferation of R genes occurs due to intra- and interchromosomal recombination events (CHEN et al., 2017; MEZIADI et al., 2016; RAHMANZADEH et al., 2022).

As has already been described for the B4 cluster on the Pv04 chromosome, where the *Co$_L$* gene is located, it is derived from an ectopic recombination process with the Pv11 chromosome and therefore comes from the Co-2 cluster (CHEN et al., 2017; MEZIADI et al., 2016; RICHARD et al., 2017). Furthermore, it appears that the Pv04 chromosome has at its end, terminal Knobs (heterochromatic blocks) and also a set of repetitive regions forming satellite DNA, called Khipu (CHEN et al., 2018; MEZIADI et al., 2016). In addition, to increase the variability and difference between the sequences of these regions, transposable elements, and miRNAs may be present, acting as factors for the gene expression regulation process (CHEN et al., 2018; MEZIADI et al., 2016; PARKER et al., 2022; YI and RICHARDS, 2007). All of these factors can interfere with obtaining closely associated markers, due to the large number of repetitive regions and because they are hotspots for recombination (CHEN et al., 2018; MEZIADI et al., 2016; MURUBE; CAMPA; FERREIRA, 2019).

These recombination events and the difference in genome size between cultivars can change the distance in centiMorgans (cM) between the marker and the *Co$_L$* gene, influencing marker efficiency. The regression analysis also shows the high efficiency of this marker, since the presence of the T allele causes an approximately 4 times reduction in the phenotypic mean. This value is of high magnitude and had an R^2 adjustment of 49%, a value above most of those reported in the literature (GOMES-MESSIAS et al., 2022).

The recombination frequency estimated with the Panel data was higher (FR=20.41%) and the selection efficiency was 83.83%. As already mentioned, due to the genomic plasticity of the subtelomeric region of Pv04, these differences may occur. When we change the genetic background, there is a reduction in selection efficiency due to the processes mentioned above.

Thus, when we change the genetic background, there is a reduction in the efficiency of the marker. However, from what we observed there was no drastic reduction. There were few individuals who presented the T allele that were susceptible, representing only 14.29% of the individuals in the Panel.

To guarantee the selection efficiency of this marker, it must be used in populations with a genetic background related to the BRS Estilo genotype, donor of the resistance allele. Another alternative is to develop markers closely associated with the *Co$_L$* gene through fine mapping. For this, recombinant individuals from the segregating population BRS Estilo x BRSMG Ouro Vermelho, BRS Estilo x BRS Valente or recombinant individuals from the panel can be used. It is necessary for other markers to be validated for this isolate and for other anthracnose races. Using more than one marker can increase MAS efficiency. Thus, fine mapping helps to identify the specific position of the *Co$_L$* gene, since only one candidate gene associated with this marker is known, *Phavu-004G011800g* , which encodes an enzyme from the glycosyl hydrolase family, which plays an important role in cell wall metabolism (COSTA et al., 2021).

Based on the Panel and allelism tests carried out by Costa et al. (2017) it is possible to state that the *Co $_F$* gene is a new gene on the Pv04 chromosome. For race 65, 9 genes that confer resistance have already been described, namely *Co-Pa* , *Co-1* , *Co-Ac* and *CoPv01 ^{CDRK}* in Pv01, *Co-13* in Pv-03, *Co-15* and *Co-6* in Pv-04, *Co-4* in Pv08 and *Co-2* in Pv11 (NUNES et al., 2022; PAULINO et al., 2022). Furthermore, there is a difference in reaction to resistance at the isolate level (COSTA et al., 2021). On the panel we included some differential cultivars to observe this distinction.

The cultivar Widusa of Andean origin has *Co-1^5* (GONÇALVES-VIDIGAL and KELLY, 2006), the cultivar To of Mesoamerican origin has *Co-4* (KELLY and VALLEJO, 2004) and the cultivar G2333 of Mesoamerican origin has the genes *Co-3 ^{5}*, *Co4 ^{2}* and *Co-5 ^{2}* (SOUZA et al., 2014). Based on the Panel results, these cultivars were resistant to Lv238, but did not amplify for the T allele. Therefore, they may not present the *Co$_L$* gene and may present another resistance gene to this isolate, which indicates that the gene *Co$_L$* is a different gene from the previously mentioned genes. These cultivars presented historical and evolutionary recombinations that may also have dissociated the T allele from the *Co$_L$* gene (MEZIADI et al., 2016; ZHU et al., 2008). The cultivar BRSMG Madrepérola was susceptible and amplified for the T allele. In this case, it probably has the SNP ss714656893, but due to historical and evolutionary recombination events, it did not present it associated with the *Co$_L$*

gene. In this sense, when the cultivar amplifies for the T allele, but is still susceptible, as for the lines VR-25, BRSMG Madrepérola, and CNFJ 15288. This indicates that the SNP ss715646893 is not linked to the *Co$_L$* resistance gene. Probably due to the processes already mentioned that increase genetic variability.

When we checked the individuals that did not show recombination, we observed that the BAT93 cultivar has the *Co-u*, *Co-3^3*, and *Co-3^{38B}* genes (GEFFROY et al., 2008; MURUBE; CAMPA; FERREIRA, 2019). It was susceptible to the Lv238 isolate and amplified to the C allele. This indicates that this gene is different from the *Co$_L$* gene that conditions resistance to the Lv238 isolate. The Perry Marrow cultivars of Andean origin have the *Co-1^3* gene (ZUIDERVEEN et al., 2016), which is located in Pv01 and the Cornell 49-242 cultivar of Mesoamerican origin has the *Co-2* gene which is located in Pv11 (KELLY and VALLEJO, 2004). These were resistant and amplified for the T allele. As the genes already described are on other chromosomes, it is an indication that the *Co$_L$* gene is another gene, as it is on Pv04 according to Costa et al. (2021). This fact also indicates that these cultivars have the same *Co$_L$* gene for resistance to Lv238 as the BRS Estilo associated with the T allele.

The Ouro Negro cultivar of Mesoamerican origin, has the *Co-3^4* gene located in Pv04 (VALENTINI et al., 2017), was resistant to Lv238 and amplified to the T allele. In this case, the *CoL* resistance gene is different of the *Co-3^4* resistance gene, since according to the allelism test carried out by Costa et al. (2017) the F_2 generation of BRS Estilo x Ouro Negro showed segregation of 15R:1S, which indicates that they are two different genes and that they are duplicated genes. The BRS Cometa cultivar showed resistance and amplified for the T allele. In the work carried out by Costa et al. (2017) the F_2 generation of BRS Cometa x BRS Estilo showed 1R:0S segregation, which indicates that it is just one gene and that they carry that same gene. This fact can be evidenced by the genealogy of these cultivars. The cultivar BRS Estilo was obtained by the bulk-segregant method from the crossing of EMP 250 /4/ A 769 /// A 429 / XAN 252 // V 8025 / PINTO VI 114, while the cultivar BRS Cometa was obtained by crossing A 769 / 4/ EMP 250 /// A 429 / XAN 252 // C 8025 / G 4449 /// WAF 2 / A 55 // GN 31 / XAN 170. We can state that the *Co$_L$* gene present in these two cultivars is identical by descent, since they have four parents in common. The BRS Esplendor cultivar also presented resistance and amplified for the T allele, showing that it may also have the *Co$_L$* gene, even though the cross that originated it has no parent in common with the previous ones, since it was obtained by the bulk-segregant method from the crossing of CB911863/AN9123293.

The use of molecular markers has some limitations. At different phenological stages, different genomic regions will be active (ALMEIDA et al., 2021; PÁDUA et al., 2022). The presence of the resistance gene is not enough, it needs to be active in the different tissues of the plant. At different phenological stages we will have different degrees of heterochromatization (PEREIRA et al., 2019). As most resistance genes are in subtelomeric regions, they present a high degree of heterochromatization forming Knobs and Khipu (MEZIADI et al., 2016; CHEN et al., 2018). Thus, a plant may even present the resistance gene, but it may be inactive in different tissues (VLASSOVA et al., 2016; SILVA et al., 2021). In addition to molecular markers, phenotyping is necessary, especially under natural conditions. Several epigenetic mechanisms influence the formation of heterochromatic blocks and the process of gene silencing (YI and RICHARDS, 2007). It is important to consider only one type of tissue when performing phenotyping since the expression pattern of resistance genes can change depending on the tissue.

Therefore, to select genotypes with durable resistance to anthracnose, breeding strategies are necessary that combine the crossing of more than one parent to guarantee the presence of different resistance genes in the progeny and transgressive segregation (NUNES et al., 2022; MUNGALU et al., 2020; PAULINO et al., 2022). Furthermore, MAS can be used to select individuals in the initial stages, aiming at gene pyramiding (GOMES-MESSIAS et al., 2022). As genetic control of anthracnose resistance is qualitative and quantitative, recurrent selection is a strategy for durable resistance (COSTA et al., 2021; NABI et al., 2022; NUNES et al., 2022; SHAFI et al., 2022), as it ensures recombination and accumulation of loci that confer resistance. However, this strategy must be associated with MAS and phenotypic assessment through races mixture. Selection via markers does not exclude phenotyping in a greenhouse with a mixture of races and under natural conditions.

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Supplementary Material

Table S1 - Phenotypic means to isolate Lv238 of *Colletotrichum lindemuthianum* race 65, amplification to allele ss715646893, and origin of common bean lines from the active germplasm bank of the Universidade Federal de Lavras-UFLA.

Number	Identification	Origin	Allele	Nota
1	BAT 93	**Differential**	CC	9
2	G2333	**Differential**	CC	3
3	A252		CC	9
4	Widusa	**Differential**	CC	3
5	TO	**Differential**	CC	3
6	México222	**Differential**	CC	8
7	BRSMG Realce	**Convênio**	CC	1
8	CNFRS 15558	**Embrapa**	CC	2
9	BRS União	**Embrapa**	CC	9
10	MAIII-17.159	**UFLA**	CC	6
11	CXIII 1.7A	**UFLA**	CC	9
12	CXII-13	**UFLA**	CC	7
13	RPXI-43	**UFLA**	CC	5
14	RPCVIII 13	**UFLA**	CC	6
15	VR-20	**UFV**	CC	4
16	Ouro vermelho	**UFV**	CC	9
17	UFV-6	**UFV**	CC	2
18	VC37	**UFV**	CC	9
19	VC 27	**UFV**	CC	8
20	VP 30	**UFV**	CC	7
21	CNFPMG -11-21	**Embrapa**	CC	1
22	ESAL503	**UFLA**	CC	9
23	MB 89	**UFLA**	CC	9
24	E09/10-28	**Embrapa**	CC	8
25	EMB9	**Embrapa**	CC	6
26	CV-6	**UFV**	CC	9
27	Roxo90		CC	9
28	OURO NEGRO		TT	3
29	BRS COMETA	**Embrapa**	TT	3
30	BRS ESPLENDOR	**Embrapa**	TT	3
31	Perry Marrow	**Differential**	TT	3
32	Cornell 49242	**Differential**	TT	3
33	VR-23	**UFV**	TT	2
34	VR-24	**UFV**	TT	2
35	VP-37	**UFV**	TT	1

Number	Identification	Origin	Allele	Nota
36	VR-25	**UFV**	TT	9
37	BRSFC 406	**Embrapa**	TT	3
38	BRSMG UAI	**Convênio**	TT	1
39	MADREPEROLA	**Convênio**	TT	4
40	MAXII-5	**UFLA**	TT	2
41	RPXI -6	**UFLA**	TT	3
42	VC20	**UFV**	TT	3
43	VP-33	**UFV**	TT	2
44	CNFP 15677	**Embrapa**	TT	2
45	CNFJ 15288	**Embrapa**	TT	9
46	CNFRX 15 275	**Embrapa**	TT	3
47	ESAL652	**UFLA**	TT	3
48	ESAL664	**UFLA**	TT	3
49	ESAL561	**UFLA**	TT	1