FRANCISCO HENRIQUE NUNES DA SILVA ALVES

COMMON BACTERIAL BLIGHT OF COMMON BEAN: INSIGHTS INTO HOST RESISTANCE AND BACTERIAL VIRULENCE MECHANISMS

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

Advisor: Jorge Luis Badel Pacheco

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Jorge Luis Badel Pacheco
Adviser

“O otimista é um tolo. O pessimista é um chato. Bom mesmo é ser um realista esperançoso.”
Ariano Suassuna

In memoriam of Bernarda Nunes, Rosemary Martins, and Alessandro,
To my parents Conceição and Romero,
To my sister Heloisa,
To my aunt Eliana,
I dedicate.

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RESUMO

ALVES, Francisco Henrique Nunes da Silva, D.Sc., Universidade Federal de Viçosa, outubro, 2023. **Crestamento bacteriano comum do feijão comum: Percepções sobre a resistência genética do hospedeiro e os mecanismos de virulência bacteriana**. Orientador: Jorge Luis Badel Pacheco.

O Crestamento Bacteriano Comum (CBC), causado pelas bactérias Gram-negativas *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*) e *Xanthomonas citri* pv. *fuscans* (*Xcf*), representa uma ameaça significativa para a produção de feijão comum no mundo. Para contribuir a desvendar os mecanismos moleculares da resistência do hospedeiro e da virulência bacteriana subjacentes à interação entre o feijão comum e *Xanthomonas*, neste estudo, primeiro (Capítulo 1) 80 cultivares de feijão comum dos grupos carioca e preto foram classificadas com base na sua resistência após inoculação com uma estirpe de *Xpp*. A doença foi avaliada com base na severidade dos sintomas e a Área Abaixo da Curva de Progresso da Doença (AACPD). Posteriormente, as cultivares foram agrupadas em quatro categorias de resistência/suscetibilidade segundo os valores de severidade da doença. Três cultivares do grupo carioca e três do grupo preto apresentaram os valores mais baixos de severidade da doença e AACPD, e foram classificados como altamente resistentes a *Xpp*. Após essa classificação, foi realizado um Estudo de Associação Genômica Ampla (*Genome-Wide Association Study*, GWAS) exploratório usando 384 marcadores de Polimorfismos de Nucleotídeo Único (*Single-Nucleotide Polymorphism*, SNP) e os dados de severidade da doença da mesma coleção de 80 cultivares. Cinco SNPs significativamente associados com a resistência foram encontrados em três cromossomos diferentes de feijoeiro comum. Esses SNPs se encontram em genes que codificam para funções bioquímicas potenciais, incluindo uma proteína Lir1 regulada pela luz, uma proteína da família de transportadores de oligopeptídeos dependentes de prótons, uma fosfatase dependente de metal, uma proteína WPP que interage com o domínio de ancoragem da cauda e uma proteína da superfamília das pectinas liases. Na segunda fase (Capítulo 2), visando explorar os mecanismos de virulência bacteriana, 35 sequências genômicas de *Xcf* e 36 sequências genômicas de *Xpp* foram utilizadas em análises de pangenômica e construção de dendrogramas usando presencia/ausência de genes. A análise de

pangenômica revelou uma significativa diversidade e plasticidade genômica em ambos os patovares. Os dendrogramas não indicaram um agrupamento evidente dos isolados segundo a sua origem geográfica. Além disso, genes ativados por HrpG e HrpX nos genomas de *Xpp* e *Xcf* foram preditos por meio de bioinformática, buscando sequências de caixas *hrp* em regiões promotoras. No total, 51 genes dependentes de HrpG e HrpX em *Xcf* e 49 genes em *Xpp* foram preditos. Além disso, para identificar efetores secretados pelo sistema de secreção do tipo III (*Type III secretion Effectors*, T3SEs), buscas Blastp de todas as proteínas anotadas nos genomas de *Xpp* e *Xcf* foram conduzidas contra os bancos de dados de efetores conhecidos de *Xanthomonas* e *Pseudomonas*. Essas buscas resultaram na identificação de um total de 39 T3SEs, sendo que o efetor XopAL2 foi encontrado exclusivamente em *Xcf*, seis efetores foram exclusivos de *Xpp* e sete efetores foram compartilhados com *Pseudomonas*. Este estudo oferece contribuições significativas à comunidade científica, fornecendo informações sobre genótipos de feijão comum com altos níveis de resistência a *Xpp*, genes candidatos potencialmente associados à resposta de resistência do feijão comum a *Xpp* e sobre os possíveis mecanismos que as espécies de *Xanthomonas* podem empregar para causar doença em plantas de feijão comum.

Palavras-chave: *Xanthomonas citri* pv. *fuscans*. *Xanthomonas phaseoli* pv. *phaseoli*. *Phaseolus vulgaris.*

ABSTRACT

ALVES, Francisco Henrique Nunes da Silva, D.Sc., Universidade Federal de Viçosa, October 2023. **Common bacterial blight of common bean: Insights into host resistance and bacterial virulence mechanisms**. Advisor: Jorge Luis Badel Pacheco.

Common Bacterial Blight (CBB), induced by the Gram-negative bacteria *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*) and *Xanthomonas citri* pv. *fuscans* (*Xcf*) is a substantial menace to common bean production worldwide. To contribute to unveiling the molecular mechanisms of host resistance and bacterial virulence subjacent to the interaction between common bean with *Xanthomonas*, in this study, first (Chapter 1) 80 common bean cultivars of the carioca and black groups were classified based on their resistance upon inoculation with a *Xpp* strain. The disease was assessed based on symptom severity and the Area Under Disease Progress Curve (AUDPC). The cultivars were subsequently grouped into four resistance/susceptibility categories according to their disease severity values. Three carioca and three black bean cultivars exhibited the lowest disease severity and AUDPC values and were classified as highly resistant to *Xpp*. A positive correlation between AUDPC and disease severity was observed. Following this classification, an exploratory Genome-Wide Association Study (GWAS) was undertaken using 384 Single Nucleotide Polymorphism (SNP) markers and data on disease severity from the same set of 80 cultivars. Five SNPs significantly associated with resistance were found on three different common bean chromosomes. These SNPs are within genes coding for a range of potential biochemical functions, including a light-regulated Lir1 protein, a proton-dependent oligopeptide transporter family protein, a metallo-dependent phosphatase-like protein, a WPP domain-interacting tail-anchored protein 2, and a pectin lyase-like superfamily protein. In the second phase (Chapter 2), aiming to explore the bacterial virulence mechanisms, 35 genome sequences of *Xcf* and 36 genome sequences of *Xpp* were utilized in pangenomic analyses and dendrogram constructions using gene presence/absence. The pangenomic analyses revealed significant genomic diversity and plasticity for both pathovars. The dendrograms did not indicate an evident clustering by geographic origin. Additionally, *Xcf* and *Xpp* genes activated by HrpG and HrpX were predicted bioinformatically searching for *hrp* box sequences within the

promoter regions in their genomes. A total of 51 HrpG- and HrpX-dependent genes were found in *Xcf* and 49 genes in *Xpp*. Furthermore, to identify Type III Secreted Effectors (T3SEs), Blastp searches of all annotated proteins from the *Xpp* and *Xcf* genomes were conducted against databases of known *Xanthomonas* and *Pseudomonas* T3SEs. These searches led to the identification of 39 T3SEs, with the effector XopAL2 found exclusively in *Xcf*, six effectors were unique to *Xpp*, and seven effectors were shared with phytopathogenic *Pseudomonas*. This study brings significant contributions to the scientific community. It offers insights into common bean genotypes displaying high levels of resistance to *Xpp*, identifies candidate genes potentially associated with common bean resistance to *Xpp*, and sheds light on the mechanisms that *Xanthomonas* species might employ to cause disease in common bean plants.

Keywords: *Xanthomonas citri* pv. *fuscans*. *Xanthomonas phaseoli* pv. *phaseoli*. *Phaseolus vulgaris*

SUMMARY

1. GENERAL INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important legumes for human consumption. This species has two distinct genetic origins: the Mesoamerican gene pool located in Mexico and the Andean gene pool covering the regions of southern Bolivia and northern Argentina (BITOCCHI *et al.,* 2013). Brazil is the fourth largest producer of beans in the world, with an estimated production of 2.89 million tons in the 2022/2023 crop season (FAO, 2023).

In Brazil, common bean consumption predominantly centers on two commercial groups: carioca beans, and black beans, both belonging to the Mesoamerican gene pool (ALMEIDA *et al.,* 2020; PEREIRA *et al.,* 2019). Carioca beans are characterized by their cream-colored grains with brown stripes and accounting for nearly 70% of total consumption of common bean. It is grown in various regions, with their market price closely tied to domestic crop performance (ALMEIDA *et al.,* 2020; PIRES *et al*., 2005). Black beans, recognized for their abundant anthocyanin content, are popular in numerous Latin American countries. In Brazil, the states of Minas Gerais, Paraná, Santa Catarina, and Rio Grande do Sul lead the production of this type of bean (PIRES *et al*., 2005).

A significant portion of common bean production is threatened by biotic stresses, with Common Bacterial Blight (CBB) standing out as the most important bacterial disease. CBB was first described in Brazil in 1954 in the state of Pará, and it is currently known to be caused by two distinct Gram-negative phytopathogenic bacteria, namely *Xanthomonas citri* pv. *fuscans* (*Xcf*) and *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*) (BELETA and BASKAS, 2017, BRIAND *et al.,* 2021, PAIVA *et al.,* 2020).

The bacteria penetrate through wounds and natural openings, mainly by stomata and hydathodes, multiply in the intercellular spaces (AKHAVAN *et al.,* 2013), and cause symptoms whose development is favored by temperatures between 28-32ºC. The first symptom of CBB on leaves is the formation of water-soaked spots, which are more evident on the underside and can progress to form necrotic spots surrounded by chlorotic haloes. Under favorable conditions and with disease progression, intense defoliation can occur (AKHAVAN *et al.,* 2013). Water-soaked symptoms can also be observed on the pods, where the tissue becomes dark green

with longitudinal fissures (KOBAYASTI *et al.,* 1999). The pathogen can infect the seeds remaining viable for long periods, representing the primary source of long-distance dissemination and primary source of inoculum (MARQUES *et al.,* 2005).

Management of CBB in regions characterized by a high inoculum potential is hindered due to the inefficiency of chemical compounds when either foliar spraying or seed treatment are utilized (MUTLU *et al.,* 2005; SILVA *et al.,* 2009). Given the absence of effective methods for CBB control in common bean crops, the recommended approaches to disease management involve the utilization of disease-free seeds, planting in areas free from the pathogens, and cultivation of varieties displaying high levels of resistance (MARINGONI AND LAURETTI, 1999). The low cost for the producer and minimal environmental impact, makes the use of resistant genotypes an optimal strategy for CBB control (COSTA AND RAVA, 2003; MONTEIRO *et al.,* 2021).

Qualitative resistance of common bean to CBB appears to be rare or nonexistent. Only one study has provided evidence that resistance to *Xpp* in common bean is of qualitative nature and governed by a single dominant gene. However, the precise identification of this gene remains to be described (ZAPATA *et al.,* 2011). These observations underscore the importance of understanding the mechanisms involved in the resistance of common bean to CBB, which is primarily considered quantitative. Due to the different levels of severity of symptoms expressed by different genotypes of the host species, distinguishing between resistance levels can be challenging at times. Therefore, it is important to monitor the disease progression over time, typically by estimating the area under the disease progress curve (AUDPC) (ANDRADE *et al*., 2017; MONTEIRO *et al.,* 2020, TRYPHONE *et al.,* 2012).

In research conducted in Brazil, high levels of resistance to CBB were observed in different common bean genotypes both under field and greenhouse conditions, including the cultivars BRS Radiante, IAPAR 16 and Diamante Negro. Conversely, the cultivar Carioca MG exhibited high susceptibility (MONTEIRO *et al*., 2020). However, it remains unclear whether the same genes responsible for resistance against *Xpp* are also effective against *Xcf*. Studies suggest the population of *X. phaseoli* pv. *phaseoli* is genetically more diverse and exhibits more significant variability in aggressiveness to *P. vulgaris* plants when compared to that of *X. citri* pv. *fuscans* (MUTLU *et al*., 2008; PAIVA *et al*., 2020).

Traditionally, identification of genes involved in the resistance response have been identified by positional cloning utilizing segregating populations derived from crosses between parents with contrasting phenotypes (DEMIRJIAN *et al.,* 2023). The development of cutting-edge next-generation sequencing (NGS) technologies and statistical methods in quantitative genetics during the last decade made it possible the application of new alternative strategies. Among these, Genome-wide association studies (GWAS) are aimed at detecting variants at genome loci that are associated with complex traits in the population. Using molecular markers, such as single-nucleotide polymorphisms (SNPs), and disease severity data, GWAS can identify causal mutations that affect the resistance phenotype (VISSCHER *et al.,* 2017, ISIDRO-SÁNCHEZ *et al.,* 2017). In Brazil, GWAS has already been used in *P. vulgaris* to identify 10 SNPs potentially associated with common bean resistance to *Xcf*, located close to nines genes previously linked to plant resistance to pathogens (MONTEIRO *et al.,* 2021), but it is not known if the same SNPs are associated with resistance to *Xpp*.

The *Xanthomonas* genus displays a remarkable level of genetic diversity (MKANDAWIRE *et al.,* 2004). Over the course of their evolutionary history, strains of the genus have either acquired or lost biochemical functions to better suit specific environmental conditions (AZARIAN *et al.,* 2020). Insights into the gain and loss of genes in bacterial genomes can be achieved by pangenomic analysis. The pangenome concept is instrumental in the study of genomic diversity, adaptability, and evolution within a specific taxon (AGARWAL *et al.,* 2023). By examining the presence of universally shared and dispensable genes within a specific taxon, it becomes feasible to delineate the profile of a particular pathogen and gain insights into the mechanisms underpinning disease development (AN *et al.,* 2019). Gene functions involved in pathogenicity are frequently gained and lost in bacterial populations through mutation or recombination.

Many *Xanthomonas* rely on a type III secretion system (T3SS) to cause disease in their hosts. Type III secretion effectors (T3SE) are translocated into host cells by the T3SS, where they support pathogen nutrition and virulence by suppressing the host immune response (ALAVI *et al.,* 2008 PAIVA *et al.,* 2021). Certain effectors, designated avirulence proteins, are specifically recognized by resistance proteins in the plant triggering a resistant phenotype. This recognition activates the plant defense

mechanisms, typically leading to the hypersensitive response (HR), a rapid and localized cell death that restricts the tissue colonization by the pathogen (HAN AND HWANG, 2017).

In the *Xanthomonas* genus, the T3SS is coded by a cluster of *hrp* genes whose activation *in planta* is mediated by two regulatory proteins coded by the genes *hrpG* and *hrpX* (TEPER *et al.,* 2021). HrpG activates the expression of *hrpX*, whose regulatory function results from its binding to the promoter regions of genes that possess a conserved sequence (so-called PIP box) in their promoter regions. Upon activation of the T3SS, effectors are injected directly into the host cell cytoplasm, and therefore, are commonly referred to as *Xanthomonas* outer proteins (Xop) (KOEBNIK *et al.,* 2006; KVITKO & COLLMER, 2023). The host specificity of particular species or pathovars of the genus is determined by the set of T3SE they carry. For example, *X. citri* pv. *citri*, is comprised of three distinct pathotypes exhibiting varying host ranges, being the effector AvrG1 identified as a host-limiting factor (ESCALON *et al.,* 2013; TIMILSINA *et al.,* 2020). Up to this point, investigations into the diversity of T3SE in *Xpp* and *Xcf* have primarily centered on the variability of ten specific effectors (PAIVA *et al.,* 2021; TUGUME *et al.,* 2019). This restricted focus has resulted in an incomplete understanding of the full arsenal of effectors that contribute to their pathogenicity towards common bean plants.

Thus, the objectives of this work were: (i) to investigate whether sources of resistance against *Xpp* exist in a select group of Mesoamerican *P. vulgaris* cultivars of the carioca and black bean groups by inoculating 80 common bean cultivars with an *Xpp* strain; (ii) to identify genes potentially associated with resistance to *Xpp* by conducting an exploratory GWAS analysis using 384 SNPs; (iii) to estimate the diversity in the T3SE repertoire of these two bacterial pathogens that cause CBB, by pangenomic analysis and comprehensive predictions of genes activated by HrpX and HrpG; and (iv) to gain insights into T3SEs that could target conserved function across divergent plant species by identifying those sharing significant similarity with known effectors from phytopathogenic *Pseudomonas*. The findings are presented in two chapters, each delving into distinct yet interconnected facets of the interaction between common bean and *Xanthomonas*.

2. REFERENCES

AGARWAL, V.; STUBITS, R.; NASSRULLAH, Z.; DILLON, M. M. Pangenome insights into the diversification and disease specificity of worldwide *Xanthomonas* outbreaks. Frontiers in Microbiology 14:1-18. 2023.

AKHAVAN, A.; BAHAR, M.; ASKARIAN, H.; LAK, M. R.; NAZEMI, A.; ZAMANI, Z. Bean common bacterial blight: Pathogen epiphytic life and effect of irrigation practices. SpringerPlus 2:1–9. 2013.

ALMEIDA, C. P.; PAULINO, J. F. C.; CARBONELL, S. A. M.; CHIORATO, A. F.; SONG, Q.; VITTORI, V. D.; RODRIGUEZ, M.; PAPA, R.; BENCHIMOL-REIS, L. L. Genetic diversity, population structure, and Andean introgression in Brazil common bean cultivars after half a century of genetic breeding. Genes 11:1-22. 2022.

AN, S-Q.; POTNIS, N.; DOW, M.; VORHÖLTER, F-J.; HE, Y-Q.; BECKER, A.; TEPER, D.; LI, Y.; WANG, N.; BLERIS, L.; TANG, J-L. Mechanistic insights into host adaptation, virulence and epidemiology of the phytopathogen *Xanthomonas*. FEMS Microbiology Reviews 44:1-32. 2019.

AZARIAN, T.; HUANG, I-T.; HANAGE, W. P. Structure and dynamics of bacterial populations: Pangenome ecology. In: TETTELIN, H., MEDINI, D. (eds) The Pangenome. Springer. 115-128. 2020.

BITOCCHI, E.; RAU D.; BELLUCCI, E.; RODRIGUEZ, M.; MURGIA, L. M.; GIOIA, T.; SANTO, D.; NANNI, L.; ATTENE, G.; PAPA, R. Beans (*Phaseolus* ssp.) as a model for understanding crop evolution. Frontiers in Plant Science, 8:722, 2017.

BELETA, T.; BASKAS, K. K. Common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) of beans with special focus on Ethiopian condition. Journal of Plant Pathology & Microbiology, 8:1000403, 2017.

BRIAND, M.; RUH, M., DARRASE, A.; JACQUES, M-A.; CHEN, N. W. G. Complete and circularized sequence of 17 *Xanthomonas* strains responsible for common bacterial blight of bean. Microbiology Resource Announcements, 10:e00371-21. 2021.

COSTA, J. G. C.; RAVA, C. A. Linhagens de feijoeiro comum com fenótipos agronômicos favoráveis ao crestamento bacteriano comum e antracnose. Ciência Agrotécnica 27:1176-1182. 2003.

DEMIRJIAN, C.; VAILLEAU, F.; BERTHOMÉ, R.; ROUX, F. Genome-wide association studies in plant pathosystems: Success or failure? Trends in Plant Science 28:471-485. 2023.

ESCALON, A.; JAVEGNY, S.; VERNIÈRE, C.; NOËL, L. D., VITAL, K.; POUSSIER, S.; HAJRI, A.; BOUBEAU, T.; PRUVOST, O.; ARLAT, M.; GAGNEVIN, L. Variations in type III effector repertoires, pathological phenotypes and host range of *Xanthomonas citri* pv. *citri* pathotypes. Molecular Plant Pathology 14:483-496. 2013.

FAO – Food and Agriculture Organization of the United Nations. Available at: <http://www.fao.org/faostat/en/#data/QC>. Accessed on: 13 de September, 2023.

HAN, S. W.; HWANG, B. K. Molecular functions of *Xanthomonas* type III effector AvrBsT and its plant interactions in cell death and defense signaling. Planta 245:237-253. 2017.

ISIDRO-SÁNCHEZ, J.; AKDEMIR, D.; MONTILLA-BASCÓN, G. Genome-wide association analysis using R. Methods in Molecular Biology 1536:189-207. 2017.

KOBAYASTI, L. M.; SOUZA, R. M.; SANTOS, J. B. Avaliação de cultivares e linhagens de feijoeiro quanto à reação foliar e de vagens a *Xanthomonas axonopodis* pv. *phaseoli* var. fuscans. Ciência e Agrotecnologia, Lavras, 23:40-47, 1999.

KOEBNIK, R.; KRÜGER, A.; THIEME, F.; URBAN, A.; BONAS, U. Specific binding of the *Xanthomonas campestris* pv. *vesicatoria* AraC-type transcriptional activator HrpX to plant-inducible promoter boxes. Journal of Bacteriology 188:7652-7660. 2006.

KVITKO, B. H.; COLLMER, A. Discovery of the Hrp type III secretion system in phytopathogenic bacteria: How investigation of hypersensitive cell death in plants led to a novel protein injector system and a world of inter-organismal molecular interactions within plant cells. Phytopathology 113: 626-636. 2023.

MARINGONI, A. C.; LAURETTI, R. L. B. Reação de genótipos de feijoeiro comum a *Fusarium oxysporum* f. sp. *phaseoli*, *Macrophomina phaseolina* e *Xanthomonas campestris* pv. *phaseoli*. Pesquisa Agropecuária Brasileira 34:532-535. 1999.

MARQUES, A. S. A.; GUIMARÃES, P. M.; SANTOS, J. P. S.; VIEIRA, T. M. Sobrevivência e viabilidade de *Xanthomonas axonopodis* pv. *phaseoli* em sementes de feijão armazenadas sob condições controladas. Fitopatologia Brasileira. v. 30, n. 5, p. 527-531, 2005.

MKANDAWIRE, A. B. C.; MABAGALA, R. B.; GUZMÁN, P.; GEPTS, P.; GILBERTSON, R. L. Genetic diversity and pathogenic variation of common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) suggests pathogen coevolution with the common bean. Phytopathology 94:593-603. 2004.

MONTEIRO, A. L. R.; CHAVES, F. S.; PANTALEÃO, A. S. L.; CARNEIRO, P. C. S.; CARNEIRO, J. E. S.; BADEL, J. L. Sources, spectrum, genetics, and inheritance of *Phaseolus vulgaris* resistance against *Xanthomonas citri* pv. *fuscans*. Phytopathology 110:1428-1436, 2020.

MONTEIRO, A. L. R; PANTALEÃO, A. S. L.; BADEL, J. L.; SOARES, P. H. M.; CARNEIRO, V. Q.; CARNEIRO, P. C. S.; CARNEIRO, J. E. S. Genome-wide association study (GWAS) of *Phaseolus vulgaris* resistance to *Xanthomonas citri* pv. *fuscans*. Plant Pathology, 70:1733-1744, 2021.

MUTLU, N.; MIKLAS, P. N.; REISER, J.; COYNE, D. P. Backcross breeding for improved resistance to common bacterial blight in pinto bean (*Phaseolus vulgaris* L.). Plant Breeding 124:282-287, 2005.

MUTLU, N.; VIDAVER, A. K.; COYNE, D. P.; STEADMAN, J. R.; LAMBRECHT, P. A.; REISER, J. Differential pathogenicity of *Xanthomonas campestris* pv. *phaseoli* and *X. fuscans* pv. *fuscans* strains for bean genotypes with common blight resistance. Plant Disease 92:546-554, 2008.

PAIVA, B. A. R.; WENDLAND, A.; TEIXEIRA, N. C.; FERREIRA, M. A. S. V. Rapid detection of *Xanthomonas citri* pv. *fuscans* and *Xanthomonas phaseoli* pv. *phaseoli* in common bean by loop-mediated isothermal amplification. Plant Disease 104:198-203, 2020.

PAIVA, B. A. R.; WENDLAND, A.; ROSSATO, M.; FERREIRA, M. A. S. V. Virulence and type III effector diversities of *Xanthomonas citri* pv. *fuscans* and *X. phaseoli* pv. *phaseoli* in Brazil. Journal of Phytopathology 170: 1-14. 2021.

PEREIRA, H. S.; MOTA, A. P. S.; RODRIGUES, L. A.; DE SOUZA, T. L. P. O.; MELO, L. C. Genetic diversity among common bean cultivars based on agronomic traits and molecular markers and application to recommendation of parent lines. Euphytica 215:1-16. 2019.

PIRES, C. V.; OLIVEIRA, M. G. A.; CRUZ, G. A. D. R.; MENDES, F. Q.; DE REZENDE, S. T.; MOREIRA, M. A. Composição físico-química de diferentes cultivares de feijão (*Phaseolus vulgaris* L.). Alimentos e Nutrição Araraquara 16:157-162. 2005.

SILVA, A.; SANTOS, L.; BALBINOT, A. L.; MATEI, G. OLIVEIRA, P. H. Reação de genótipos de feijão ao crestamento bacteriano comum, avaliado por dois métodos de inoculação. Ciência Agrotécnica 33:2019-2024. 2009.

SINGH, S. P.; MIKLAS, P. N. Breeding common bean for resistance to common bacterial blight: A review. Crop Science 55:971-984. 2015.

TIMILSINA, S.; POTNIS, N.; NEWBERRY, E. A.; LIYANAPATHIRANAGE, IRUEGAS-BOCARDO, F.; WHITE, F. F.; GOSS, E. M.; JONES, J. B. *Xanthomonas* diversity, virulence and plant-pathogen interactions. Nature Reviews Microbiology 18:415-427. 2020.

TRYPHONE, G. M.; CHILAGANE, L. A.; PROTAS, D.; MBOGO, P.; NCHIMBI-MSOLLA, S. Introgression of common bacterial blight *(Xanthomonas axonopodis* pv. *phaseoli*) resistance to common bean *(Phaseolus vulgaris* L.) adapted to Tanzania facilitated by marker assisted selection. International Journal of Agricultural Science 2:285-290. 2012.

TUGUME, J. K.; TUSIIME, G.; SEKAMATE, A. M.; BURUCHARA, R.; MUKANKUSI, C. M. Diversity and interaction of common bacterial blight disease-causing bacteria (*Xanthomonas* spp.) with *Phaseolus vulgaris* L. Crop Journal 7:1-7. 2019.

VISSCHER, P. M.; WRAY, N. R.; ZHANG, Q. 10 years of GWAS discovery: Biology, function and translation. American Journal of Human Genetics. 101:5-22. 2017.

ZAPATA, M.; BEAVER, J. S.; PORCH, T. G. Dominant gene for common bean resistance to common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli*. Euphytica 179:373-382. 2011.

3. Chapter 1 - Research Article

Genes putatively involved in *Phaseolus vulgaris* resistance against *Xanthomonas phaseoli* pv. *phaseoli* revealed by a Genome-Wide Association Study

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ABSTRACT:

Carioca and black groups play a significant role in Latin American common bean (*Phaseolus vulgaris* L.) consumption, serving as excellent sources of nutrients. One of the most significant threats for common bean production is the occurrence of common bacterial blight (CBB), a disease caused by the Gram-negative bacteria *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*) and *Xanthomonas citri* pv. *fuscans* (*Xcf*). An effective method for managing this disease is the use of plant genetic resistance. However, the pathogenic bacteria are genetically diverse, raising the need for a continuous search of new sources of resistance. The objectives of this study were to assess the resistance to *Xpp* of carioca and black common bean cultivars and to identify genes potentially associated with the resistance response. For that, eighty cultivars were inoculated with strain *Xpp* CFBM-UFV-0002, CBB severity assessed over time and the area under the disease progress curve (AUDPC) calculated. Also, an exploratory genome-wide association study (GWAS) using single nucleotide polymorphisms (SNPs) and disease

severity data of the same set of cultivars was conducted. The cultivars were categorized into four groups based on the severity values. Three carioca and three black bean cultivars exhibited high levels of resistance to *Xpp*, A positive correlation between AUDPC and disease severity was observed. In addition, five SNPs with the highest -$\log_{10}(p)$ values unveiled genes across three chromosomes which code for diverse putative biochemical functions, including a light regulated Lir1 protein, a proton-dependent oligopeptide transporter family protein, a metallo-dependent phosphatase-like protein, a WPP domain-interacting tail-anchored protein 2, and a pectin lyase-like superfamily protein. These findings contribute knowledge about sources of carioca and black common bean resistance to *Xpp* and provide valuable insights into genes potentially involved in the resistance response.

KEYWORDS: Common bacterial blight, Common bean, GWAS, Resistance.

3.1. INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) represents the most significant grain legume for direct human consumption worldwide since it is a source of highly valuable proteins and micronutrients (BITOCCHI *et al.,* 2012). The origin of common bean can be traced back to the Americas, specifically to the central region of the continent. It is primarily found in Mexico, which is the origin of most small-grain bean varieties, and in northern Argentina and southern Peru, where large-grain cultivars originated (BITOCCHI *et al.,* 2013; TSUTSUMI *et al.,* 2015).

Common bean is grown in a variety of production systems and across all Brazilian regions, satisfying the needs of both small and large-scale farmers (BIANCHINI *et al.,* 2005). In Brazil, the consumption of common bean primarily revolves around two commercial groups: carioca beans, which represent almost 70% of total consumption, and black beans, both belonging to the Mesoamerican gene pool (ALMEIDA *et al*., 2020; PEREIRA *et al*., 2019). Carioca beans are identifiable by their cream-colored grain with brown stripes and are notable for their high levels of fibers, proteins, and calcium content (PIRES *et al.,* 2005). On the other hand, black beans are notable for their high iron and anthocyanin content, which confers the dark color to the grain (ALMEIDA *et al.,* 2020; PIRES *et al.,* 2005). Common bean plays an important role in the diversification and sustainability of intensive agriculture, favoring

environmentally sustainable agricultural practices such as crop rotation and intercropping. This is mainly attributed to its capacity for biological nitrogen fixation, soil-enriching effects, and weed control properties (BITOCCHI *et al.,* 2017).

The Gram-negative bacteria *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*) and *Xanthomonas citri* pv. *fuscans* (*Xcf*), etiological agents of the Common Bacterial Blight (CBB) disease, contribute significantly to the substantial losses occurring in the global common bean production (MONTEIRO *et al.,* 2021). The losses caused by the disease depend on the level of resistance of the planted cultivar and the climatic conditions prevalent in the cropping area (DÍAZ *et al.,* 2001).

At the outset of disease symptom development, small water-soaked lesions appear on the leaves, gradually evolving into necrotic lesions surrounded by chlorotic haloes. Under favorable conditions, there is a risk of substantial necrotic tissue loss and extensive defoliation. In the case of pods, lesions also initiate as water-soaked spots that darken over time. The bacteria can infect the seed inducing pronounced discoloration and remaining viable for extended periods (BIANCHINI *et al.,* 2005; MARQUES *et al.,* 2005; MONTEIRO *et al.,* 2021).

Common Bacterial Blight is primarily managed through preventive measures such as the utilization of pathogen-free seeds, the implementation of two-year crop rotations with non-leguminous plants, and the elimination of plant residues (FOUCHER *et al.,* 2020). When the pathogen is already present in an area, disease control becomes challenging, as curative chemical treatments are inefficient (MUTLU *et al.,* 2005). Seed treatment with antibiotics is only effective for eliminating externally associated bacteria, while there is currently no satisfactory seed treatment method to fully eliminate internally borne *Xcf* or *Xpp* cells without compromising seed viability (FOUCHER *et al.,* 2020; KAVINA *et al.,* 2011). In this context, the development and utilization of resistant cultivars represent the most cost-effective and ecologically sound strategy to control CBB (MONTEIRO *et al*., 2021).

Multiple sources of resistance to CBB have been identified in *P. vulgaris,* as well as in other closely related species like *P. coccineus, P. lunatus,* and *P. acutifolius* (SINGH & MIKLAS, 2015). In Ethiopia, an assessment of the resistance in 23 red bean cultivars revealed minimal severity in eight genotypes, indicating substantial potential as sources of CBB resistance (GIRMA *et al.,* 2022). In Brazil, a high level of resistance

was documented against *Xcf* and *Xpp* strains in controlled greenhouse environments, particularly in the genotypes BRS Radiante, IAPAR 16, and Diamante Negro. Conversely, susceptibility was noted in the Carioca MG cultivar (SILVA *et al.,* 2000; MONTEIRO *et al.,* 2020). However, the resistance expressed by a specific common bean genotype depends on the bacterial strain or population used for resistance assessment (CHEN *et al.,* 2021).

It is also widely known that the number of genes conditioning common bean resistance to CBB is dependent on the common bean genotype. For instance, the inheritance of resistance to the strain 3353 of *Xanthomonas axonopodis* pv. *phaseoli* (synonymous with *Xpp*) was determined through segregation analysis in the F4 and F5 generations resulting from the cross between the resistant and susceptible parents PR0313-58 and Rosada Nativa, respectively. The results supported the hypothesis that resistance to *Xpp* is conferred by a single dominant gene (ZAPATA *et al.,* 2010). Nonetheless, most inheritance studies on the resistance of common beans to CBB concluded that the resistance is mainly quantitative and polygenic (SILVA *et al.,* 2009; AZEVEDO *et al.,* 2015; CHEN *et al.,* 2021; MONTEIRO *et al*., 2020). In addition, distinct resistance profiles have been observed in the Mesoamerican and Andean gene pools, which may stem from significant variations in the content of resistance-associated genes within each genetic pool. Within the Mesoamerican gene pool, there is evidence of moderate resistance to CBB, whereas, as of now, only one resistant cultivar has been documented in the Andean gene pool (SINGH & MIKLAS, 2015; MONTEIRO *et al*., 2020; CHEN *et al.,* 2021).

Quantitative trait loci (QTL) for *P. vulgaris* resistance to CBB have been identified in several studies (CHEN *et al*., 2021; SINGH & MIKLAS, 2015; GIRMA *et al.,* 2022). In addition, eight and thirteen individual gene candidates associated with resistance against *Xcf* (MONTEIRO *et al*., 2021) and *Xpp* (BARBOSA *et al.,* 2022), respectively, have been proposed. However, in the study by Barbosa *et al.* (2022) no cultivars resistant to *Xpp* were identified, and a single SNP was used to predict the thirteen genes potentially associated with resistance, indicating the need to conduct additional screening for identification of resistant cultivars and prediction of resistance-associated SNPs. Hence, there is a significant need for the application of high throughput approaches to identify additional *P. vulgaris* genes potentially associated with resistance to *Xpp*. One such approaches is genome-wide association studies

(GWAS) (YANG *et al.,* 2022), which help identify markers potentially associated with resistance by examining natural genetic variations in pathogen populations, such as Single Nucleotide Polymorphisms (SNPs) (TAM *et al.,* 2019).

One of the main advantages of GWAS is that it minimizes the need for the long process of crossing between parents with differing phenotypes by leveraging the natural variation found within populations (MONTEIRO *et al.,* 2021). Once candidate SNPs are identified, genes linked to them can be retrieved from reference genome sequences, offering insights into their potential roles in the observed phenotypic traits (GONZÁLEZ *et al.,* 2017; DEMIRJIAN *et al.,* 2023). Nonetheless, one critical challenge in GWAS lies in managing false positives, which often arise due to the influence of population structure and kinship relationships. These two factors are known to introduce non-casual associations, leading to misleading outcomes (LIU *et al*., 2016; PRICE *et al.,* 2010). To address the challenge of false positives in GWAS, researchers have employed a Mixed Linear Model (MLM) that integrates both fixed and random effects (YANG *et al.,* 2014). This MLM effectively incorporates population structure and kinship among individuals to adjust association tests for genetic markers.

The Fixed and Random Model Circulating Probability Unification (FarmCPU) approach effectively minimizes kinship-related issues by implementing a fixed-effect model without utilizing kinship values derived from all markers or associated markers as in traditional methods (LIU *et al*., 2016). FarmCPU employs kinship using the maximum likelihood method (MLM). This innovative process resolves issues associated with stepwise regression models. Similarly, the BLINK method arranges markers with the most significant associations at the top as reference markers. Subsequently, it values the remaining markers for linkage disequilibrium with the top-associated marker (HUANG *et al.,* 2019). This iterative process continues until no more markers can be removed. BLINK optimizes computational efficiency by approximating MLM using Bayesian information content within a fixed-effect model (HUANG *et al.,* 2019; ALAVILLI *et al.,* 2022). By employing different models, the rate of false positive identification can be significantly reduced.

This study set out to investigate whether sources of resistance against *Xpp* exist in a select group of Mesoamerican *P. vulgaris* cultivars of the carioca and black bean groups. For this, 80 cultivars were inoculated with an *Xpp* strain, the severity of disease

symptoms was evaluated and the AUDPC was calculated. Additionally, an exploratory GWAS using 384 SNPs on the same set of common bean cultivars was conducted to identify genes potentially associated with resistance to *Xpp.* A comparison with previous studies was carried out to ascertain whether genes identified as associated with resistance to *Xcf* were also associated with resistance to *Xpp*.

3.2. MATERIALS AND METHODS

Bacterial strain

The *Xpp* strain CFBM-UFV-0002 utilized in this research is part of the culture collection of the Laboratory of Molecular Phytobacteriology, Universidade Federal de Viçosa (CFBM-UFV). It was routinely cultivated on solid 523 medium (KADO & HESKETT, 1970) at 28ºC for 48 h. A previous study demonstrated that this strain causes severe symptoms in susceptible *P. vulgaris* cultivars (MONTEIRO *et al*., 2020).

Plant material and cultivation

A total of 80 common bean cultivars (Table S1) were utilized. Seeds of these cultivars were sourced from the Common Bean Germplasm Bank at Universidade Federal de Viçosa (UFV), Viçosa, Brazil. These valuable accessions were kindly provided by Prof. José Eustáquio de Souza Carneiro, from the Department of Agronomy at UFV. The seeds were pre-germinated in a seed tray for 7 days and then transplanted into 1 L pots filled with a 1:1 mixture of substrate Tropstrato HT Hortaliças® (Vida Verde, Mogi Mirim, SP, Brazil) and soil. Throughout the cultivation period, the plants were fertilized with Niphokam® (Fênix-Agro-Pecus Industrial Ltda, Tietê, SP, Brazil) every 15 d and watering was adjusted to meet their daily hydration requirements. All these cultivation activities took place within a controlled greenhouse environment at the Department of Plant Pathology at UFV.

Plant phenotyping

Eighty common bean cultivars were planted between February and April 2023. For inoculation, the *Xpp* CFBM-UFV-0002 strain was grown on solid 523 medium (KADO & HESKETT, 1970) at 28ºC for 48 h. Bacterial cells were resuspended in 10 mM $MgCl_2$ solution, and the optical density at 600 nm (OD_{600}) was adjusted to 0.1. At the V3 phenological stage, the plants were inoculated with the *Xpp* CFBM-UFV-0002 strain by spraying the bacterial suspension onto both leaf surfaces with an atomizer

Jet Master (Schulz, Joinville, SC, Brazil) until the run-off point. At least five plants of each cultivar were treated with a 10 mM $MgCl_2$ solution to serve as the control group. The plants were placed in a mist chamber 24 h before and 24 h after inoculation and subsequently maintained in the greenhouse until the end of the experiments. Disease severity was assessed daily for up to 19 days using a rating scale ranging from 1 (resistance) to 9 (susceptibility), as adopted from Schoonhoven & Pastor-Corrales (1987).

The inoculated plants were arranged in a randomized design, with each experimental unit consisting of at least one plant per pot and five repetitions per genotype. At 19 days after inoculation (DAI), the most affected trifoliate leaf of each plant was selected for evaluation. The severity data were used to calculate the Area Under the Disease Progress Curve (AUDPC) according to the method described by Campbell and Madden (1990). To calculate the AUDPC, disease ratings at 9, 11, 13, 15, 17, and 19 DAI were considered. The entire experiment was replicated once.

Plant genotyping

The genotyping data for the 80 common bean accessions have been comprehensively documented in prior studies (NASCIMENTO *et al.,* 2018; MONTEIRO *et al.,* 2021) This specific SNP set has previously been meticulously curated to capture polymorphic sites across a selection of 88 Andean and Mesoamerican common bean genotypes (MÜLLER *et al.,* 2015). The extraction and analysis of genomic DNA from the 80 cultivars included in this study were conducted at the Biotechnology Laboratory of Embrapa, located in Santo Antônio, GO, Brazil. The genotyping data with the panel of 384 SNPs used in this study was directly obtained from NASCIMENTO *et al*. (2018). Genotype calls were executed utilizing Genome Studio software version 1.8.4 (Illumina, San Diego, CA, USA). The selection of the most pertinent SNPs was predicated on a Call Rate (CR) criterion, ranging from 0.9, and a Minimum Allele Frequency (MAF) threshold of 5%, following criteria adopted by Anderson *et al*. (2010).

Genome-wide association study

The association of SNPs with CBB resistance employed the linkage disequilibrium method and a random effect model (YU *et al.* 2000; HUANG *et al.,* 2019). This analysis was carried out with the GWAS run by GAPIT version 3 using the

BLINK and FarmCPU models, as described by Lil *et al*. (2016) and Huang *et al.* (2019) and implemented in the R programming environment version 4.3.1 (R DEVELOPMENT CORE TEAM, 2018).

Identification of candidate genes and their functions

The threshold of $-\log_{10}(p)$ for identifying SNPs significant at 5% probability was determined after applying the Bonferroni correction for multiples testing. SNPs with $-\log_{10}(p)$ values equal to or greater than 3.9 were chosen for subsequent gene identification. To identify genes potentially associated with resistance, the positions of significant SNPs were mapped in the reference genome sequence of the common bean cultivar G19833 0 (PhaVulg1_0;), which is available at the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/assembly/GCF_000499845.1). With the SNP positions on the chromosomes as reference loci, the DNA, mRNA and protein sequences of physically close genes were obtained. Next, the annotations of the gene models were retrieved from the *P. vulgaris* v2.1 available at Phytozome v13 (accessible at: https://phytozome-next.jgi.doe.gov/). The gene annotations were validated by performing Blastp searches at NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using the gene model sequences.

Statistical analysis

For phenotypical data, statistical differences among cultivars were determined using analysis of variance (ANOVA) and mean comparisons were conducted using the Scott-Knott test, with a significance level set at $P \leq 0.05$. The packages agricolae (MENDIBURU, 2017) and ExpDes (FERREIRA *et al.,* 2018) were used for ANOVA and ScottKnott (JELIHOVSCHI *et al.,* 2014) for mean comparisons. The ggplot2 package (WICKHAM, 2016) was utilized for the graphical visualization of the data. Severity values were log transformed before ANOVA and mean comparisons.

3.3. RESULTS

Resistance of common bean cultivars to *Xanthomonas phaseoli* pv. *phaseoli*

The 80 common bean cultivars were subjected to two rounds of inoculation with *Xpp* CFBM-UFV-0002 during February and April of year 2023, yielding similar outcomes. Early symptoms of CBB manifested as water-soaked lesions at 9 DAI.

Noticeable variations in disease severity were detected among the common bean genotypes at 15 DAI (data not shown). The mean disease severity of the 80 cultivars were divided into four categories according to the Scott-Knott test grouping (Table 1). Out of the 80 evaluated cultivars, 37 were designated as highly susceptible, 31 as moderately susceptible, 6 as moderately resistant, and 6 as highly resistant. The mean severity scores for the highly resistant cultivars ranged from 2.2 to 3.0, whereas those for the highly susceptible cultivars ranged between 5.2 and 7.2. The cultivars classified as highly resistant to *Xpp* CFBM-UFV-0002 were BRS Campeiro, IPR Gralha, IAC Imperador, IAC Formoso, BR2 Grande Rio and BRSMG Talismã (Figure 1).

Significant differences were also detected in the AUDPC values among the tested common bean cultivars. The mean AUDPC values spanned from 19.0 to 77.6 and were separated into four groups by the Scott-Knott test (Table 2). The group with the highest mean AUDPC values consisted of 37 cultivars, while the lowest mean AUDPC values were attributed to six cultivars. The cultivars classified as highly resistant by severity scores also exhibited the lowest AUDPC values. A positive and significant correlation between disease severity ratings and AUDPC values, with an R^2 = 0.87, was observed (Figure 2).

Association of common bean SNPs with resistance against *X. phaseoli* pv. *phaseoli*

Five SNPs significantly associated with resistance to *Xpp* were found in three different *P. vulgaris* chromosomes using two different models, FarmCPU and BLINK (Figure 3). Application of the two models resulted in different SNP data distributions, with BLINK seemingly showing a greater deviation of significant SNPs from the expected -log(*p*) values (Figure 4). Out of the five SNPs potentially associated with *Xpp* resistance, three (74, 308 and 311) reside at distant positions from each other on chromosome 1. These SNPs are located within genes predicted to code for functions annotated as light-regulated Lir1 protein, proton-dependent oligopeptide transporter family protein, and metallo-dependent phosphatase-like protein. One SNP (300) is on chromosome 2 within a gene that putatively codes for a WPP domain-interacting tail-anchored protein 2. Lastly, the remaining significant SNP (16) is located on chromosome 9 within a gene coding for a putative pectin lyase-like superfamily protein (Table 3).

3.4. DISCUSSION

In this study, out of 80 Mesoamerican common bean cultivars investigated, six were classified as highly resistant to *Xpp* CFMB-UFV-0002 based on disease severity. Furthermore, a GWAS analysis, considering 384 SNPs and disease severity data for the same common bean cultivars, revealed five SNP markers exhibiting the highest -$\log_{10}(p)$ values for association with CBB resistance, which reside within genes encoding proteins with various biochemical functions.

Among the six cultivars with the lowest mean disease severity scores, three were black beans, BRS Campeiro, IPR Gralha, and BR2 Grande Rio, and three were carioca beans, IAC Imperador, IAC Formoso, and BRSMG Talismã. Among the six cultivars that exhibited a highly resistance response in this study, four were classified as moderately resistant to *Xcf* in a previous study, namely BRS Campeiro, IAC Formoso, IPR Gralha e BRSMG Talismã (MONTEIRO *et al.,* 2021). In contrast, cultivars IAC Imperador and BR2 Grande Rio, were previously considered susceptible to *Xcf* (MONTEIRO *et al.,* 2021). These observations suggest some *P. vulgaris* resistance mechanisms may be effective against distinct *Xanthomonas* species that cause CBB.

The AUDPC, an invaluable metric for tracking disease severity over time, is extensively used to access information on diverse components of quantitative resistance (JEGER AND VILJANEN-ROLLISON, 2010). In this study, the common bean cultivars exhibited a wide range of AUDPC values caused by the same *Xpp* strain, which is characteristic of quantitative resistance, and aligns with findings from prior studies (MONTEIRO *et al.,* 2021; GIRMA *et al.,* 2022). Furthermore, similar ranges in mean AUDPC values exhibited by common bean genotypes have previously been reported (DURHAM, 2011; MONTEIRO *et al.,* 2021). Interestingly, the six cultivars displaying the lowest disease severity also showed the lowest AUDPC values. These findings imply that some components of the *P. vulgaris* quantitative resistance may effectively restrain the progression of disease symptoms and also restrict the extent of tissue damage caused by *Xpp*. The highly resistant cultivars here identified could serve as a valuable source for common bean resistance breeding programs.

A total of eight genes associated with resistance to a *Xcf* strain were described when the same cultivars were analyzed with the set of SNP markers employed in the present study (MONTEIRO *et al.,* 2021). Here, none of those eight genes were found associated with the resistance against *Xpp*. The five SNPs with the largest -$\log_{10}(p)$ values identified in the present study are within genes coding for functions not reported by Monteiro *et al*. (2021). Barbosa *et al*. (2022) identified a single SNP on chromosome 7 (Pv07) that potentially correlates with resistance to *Xpp* in a GWAS with only 18 common bean cultivars. This SNP was found close to various genes previously associated with plant disease resistance. Notably, none of these genes are predicted to code for functions similar to those reported in our study. Previous studies have reported the presence of genes potentially linked to resistance against CBB across multiple chromosomes, including Pv01, Pv02, Pv03, Pv04, Pv06, Pv07, Pv08, Pv09, Pv10, and PV11, each with diverse biochemical functions (AMBACHEW *et al.,* 2021; MONTEIRO *et al.,* 2021; WU *et al.,* 2017; ZHU *et al.,* 2016).The diversity of genes potentially associated with *P. vulgaris* resistance to *Xpp* identified in different studies reinforce the notion that such a resistance is complex and bacterial strain-dependent.

Out the SNPs identified in this study, SNPs 74, 308 and 311 are located on chromosome 1 residing respectively within the genes *Phvul.001G085200*, predicted to code for a light-regulated Lir1 protein; *Phvul001G163400*, which encodes a putative proton-dependent oligopeptide transporter family protein; and *Phvul.001G267900*, annotated as a metallo-dependent phosphatase-like protein-coding gene. *Light-regulated (LIR1)* genes have been described in numerous angiosperm and gymnosperm species, displaying a remarkable level of conservation across various plant species (YANG *et al.,* 2016). *LIR1* exhibits circadian expression patterns, with its transcript levels increasing during daylight hours, peaking toward the end of the day, and declining in the absence of light. Plant LIR1 deficiency results in retarded growth, reduced photosynthetic electron transfer, and decrease accumulation of Leaf-type Ferrodoxin-NADP+ Oxidoreductases (LFNR) (CIANNAMEA *et al.,* 2007; YANG *et al.,* 2016). Nevertheless, *LIR1* has not previously been associated with plant resistance against pathogens.

The oligopeptide transporter family (OPT) is one of the most extensive transporter families within the plant realm, playing a pivotal role in various aspects of specialized plant metabolism (KANSTRUP AND NOUR-ELDIN, 2022). Among the

members of the OPT family, OPT3 is responsible for transporting iron (Fe) from the xylem to the phloem in *Arabidopsis*, with specific involvement in essential biological processes like the regulation of reactive oxygen species (ROS), plant stress responses, and basal pathogen resistance (KURT, 2021). It is well known that ROS production is one of the early events that occur during the plant defense response (O'BRIEN *et al.,* 2012). However, to the best of our knowledge, the involvement of OPT proteins in ROS regulation and defense response to pathogens in plant species other than *Arabidopsis*, such as *P. vulgaris,* remains to be elucidated.

The phosphoprotein metallophosphatase family includes the Mg^{2+} and Mn^{2+} dependent type 2C protein phosphatases (PP2C) (FUCHS *et al*., 2013). These PP2Cs serve as co-receptors for the phytohormone abscisic acid, contributing to stress signaling. For instance, in rice, PP2C XB15 regulates plant innate immunity. Deficiency of XB15 or reduced expression of *XB15* results in spontaneous cell death symptoms and leads to the constitutive expression of pathogen recognition receptors (PRR) genes, like *XA21*, whose protein product recognizes the *Xanthomonas oryzae* pv. *oryzae* avirulence protein AvrXa21 (RaxX). Conversely, plants overexpressing XB15 exhibit enhanced susceptibility to *X. oryzae* pv. *oryzae* strains carrying the AvrXa21 activity (PARK *et al*., 2008).

Single-nucleotide polymorphism 300 was identified within gene *Phvul.002G083200* and predicted to code for a WPP domain-interacting tail-anchored protein 2. In *Arabidopsis*, WPP domain-interacting tail-anchored protein 2 (WIT2) is characterized by the presence of a coiled-coil domain and a predicted transmembrane domain at its C-terminal. These structures play a critical role in facilitating the association between RanGAP1 and the nuclear envelope in root tips (BRKLJACIC *et al*., 2009). The Ran GTPase activating protein (RanGAP) is a significant component of the Ras superfamily and plays a key role in processes such as nucleocytoplasmic transport, spindle organization, and assembly of nuclei (BORUC *et al*., 2015). It is known that protein nucleocytoplasmic trafficking is an important process during the activation of plant defense responses (WU *et al.,* 2022). Interestingly, knockdown of RanGAP1 results in reduced susceptibility to cyst nematode and PVX infection in *N. benthamiana* plants, showing that RanGAP1 could act as common host target of evolutionary distinct effectors originating from two plant pathogens with differing lifestyles (SUKARTA *et al*., 2021). However, as of now, no reports on the association

of either WIT2 or RanGAP1 with common bean resistance to pathogens have been reported.

Phvul009G151200, the gene within which SNP 16 was mapped, encodes a putative pectin lyase-like superfamily protein. Pectin lyases are a group of enzymes that degrade pectin, which constitutes a significant portion of the primary cell walls in higher plants (WILLATS *et al*., 2001). A comprehensive differential expression analysis in *Arabidopsis* using quantitative real-time RT-PCR revealed that certain pectin lyase genes were up-regulated during wound stress, suggesting their potential role in triggering plant defense mechanisms (CAO *et al*., 2012). Nonetheless, so far, no relationship between *P. vulgaris* pectin lyase genes with resistance to pathogen attack has been reported.

In summary, this research successfully identified six common bean cultivars expressing high levels of quantitative resistance to *Xpp*, as indicated by their low severity and AUDPC values. It is demonstrated that sources of resistance against *Xpp* are present in carioca and black cultivars of the Mesoamerican common bean gene pool. Also, five SNPs significantly associated with resistance to *Xpp* were identified within genes that code for diverse functions, some of which had not previously been associated with plant resistance to bacterial pathogens. These findings not only enhance our understanding of common bean resistance to *Xpp*, but also provide valuable insights into the genes potentially contributing to the resistance response. In future studies, the association of the identified SNPs with the resistance response as well as the involvement of the identified genes will be investigated.

3.5. REFERENCES

AMBACHEW, D.; JOSHUA, J.; MMBAGA, M. T. BLAIR, M. W. Sources of resistance to common bacterial blight and charcoal rot disease for the production of Mesoamerican common beans in the southern United States. Plants 10:998. 2021.

ALAVILLI, H.; LEE, JL.; YOU CR.; POLI, Y.; KIM, HJ, JAIN, A.; SONG, K. GWAS reveals a novel candidate gene *CmoAP2/ERF* in pumpkin (*Cucurbita moschata*) involved in resistance to powdery mildew. International Journal of Molecular Sciences 23:2-18. 2022.

ALMEIDA, C. P.; PAULINO, J. F. C.; CARBONELL, S. A. M.; CHIORATO, A. F.; SONG, Q.; VITTORI, V. D.; RODRIGUEZ, M.; PAPA, R.; BENCHIMOL-REIS, L. L. Genetic diversity, population structure, and Andean introgression in Brazil common bean cultivars after half a century of genetic breeding. Genes 11:1-22. 2020.

ANDERSON, C. A.; PETTERSSON, F. H.; CLARKE, G. M.; CARDON, L. R.; MORRIS, A. P.; ZONDERVAN, K. T. Data quality control in genetic case-control association studies. Nature Protocols 5:1564-1573. 2010.

AZEVEDO, C. V.G.; RIBEIRO, T. D. A.; CARBONELL, S. A. M.; CHIORATO, A. F. Adaptability, stability and pathogen resistance in common bean genotypes. Pesquisa Agropecuária Brasileira 50:912-922. 2015.

BARBOSA, C. C. F.; PAULINO, J. F. C.; ALMEIDA, C. P.; CARNONELL, S. A. M.; CHIORATO, A. F.; BENCHIMOL-REIS, L. L. Association mapping for common bacterial blight in carioca beans. Crop Breeding and Applied Biotechnology 22:1-7. 2022.

BIANCHINI, A.; MARINGONI, A. C.; CARNEIRO, S. M. T. P. G. Doenças do feijoeiro. in: KIMATI, H.; AMORIM, L.; REZENDE, J. A. M.; BERGAMIN FILHO, A.; CAMARGO, L. E. A. (eds). Manual de Fitopatologia. v. 2. Doenças das Plantas Cultivadas. 4. ed. São Paulo: Agronômica Ceres, p. 333-349. 2005.

BITOCCHI, E.; BELLUCCI, E.; GIARDINI, A.; RAU, D.; RODRIGUEZ, M.; BIAGETTI, E.; SANTILOCCHI, R.; ZEULI, P. S.; GIOIA, T.; LOGOZZO, G.; ATTENE, G.; NANNI, L.; PAPA, R. Molecular analysis of the parallel domestication of the common bean (*Phaseolus vulgaris*) in Mesoamerica and the Andes. New Phytologist 197:300-313. 2013.

BITOCCHI, E.; NANNI, L.; BELLUCCI, E.; ROSSI, M.; GIARDINI, A.; ZEULI, P. S.; LOGOZZO, G.; STOUGAARD, J.; McCLEAN, P.; ATTENE, G.; PAPA, R. Mesoamerican origin of the common bean *(Phaseolus vulgaris* L.) is revealed by sequence data. Proceedings of the National Academy of Sciences U. S. A. 109:788-796. 2012.

BITOCCHI, E.; RAU D.; BELLUCCI, E.; RODRIGUEZ, M.; MURGIA, L. M.; GIOIA, T.; SANTO, D.; NANNI, L.; ATTENE, G.; PAPA, R. Beans (*Phaseolus* ssp.) as a model for understanding crop evolution. Frontiers in Plant Science, 8:722, 2017.

BORUC, J.; GRIFFIS, A. H. N.; RODRIGO-PEIRIS, T.; ZHOU, X.; TILFORD, B.; DAMME, D. V.; MEIER, I. GAP Activity, but not subcellular targeting, is required for

Arabidopsis RanGAP cellular and developmental functions. The Plant Cell 27:1985-1998. 2015.

BRKLJACIC, J.; ZHAO, Q.; MEIER, I. WPP-domain proteins mimic the activity of the HSC70-1 chaperone in preventing mistargeting of RanGAP1-anchoring protein WIT1. Plant Physiology 151:142-154. 2009.

CAO, J. The pectin lyases in *Arabidopsis thaliana*: Evolution, selection and expression profiles. PLOS One 7:e46944. 2012.

CIANNAMEA, S.; JENSEN, C. S.; AGERSKOV, H.; PETERSEN, K.; LENK, I.; DIDION, T.; IMMINK, R. G. H.; ANGENENT, G. C.; AND NIELSEN, K. K. A new member of the LIR gene family from perennial ryegrass is cold-responsive, and promotes vegetative growth in *Arabidopsis*. Plant Science 172:221-227. 2007.

CHEN, N. W. G.; RUH, M.; DARRASE, A.; FOUCHER, J.; BRIAND, M.; COSTA, J.; STUDHOLME, D. J.; JACQUES, M. Common bacterial blight of bean: A model of seed transmission and pathological convergence. Molecular Plant Pathology. 22:1464-1480. 2021.

DEMIRJIAN, C.; VAILLEAU, F.; BERTHOMÉ, R.; ROUX, F. Genome-wide association studies in plant pathosystems: Success or failure? Trends in Plant Science 28:471-485. 2023.

DÍAZ, C. G.; RENATO, B.; BASSANEZI, C. V. G.; LOPES, D. B.; FILHO, A. B. Quantificação do efeito do crestamento bacteriano comum na eficiência fotossintética e na produção do feijoeiro. Fitopatologia Brasileira 26:71-76. 2001.

DURHAM, K. M. Evaluation of common bacterial blight resistance inter-cross population of common bean. MS Thesis. The Faculty of Graduate Studies of The University of Guelph. 2011.

FERREIRA, E. B.; CAVALCANTI, P. P.; NOGUEIRA, D. A. ExpDes: Experimental Designs. Applied Mathematics 5:2952. 2018.

FOUCHER, J.; RUH, M.; PRÉVEAUX, A.; CARRÈRE, S.; PELLETIER, S.; BRIAND, M.; SERRE, R.; JACQUES, M.; CHEN, N. W. G. Common bean resistance to *Xanthomonas* is associated with upregulation of the salicylic acid pathway and downregulation of photosynthesis. BMC genomics 21:1-19. 2020.

FUCHS, S.; GRILL, E.; MESKIENE, I.; SCHWEIGHOFER, A. Type 2C protein phosphatases in plants. FEBS Journal 280:681-693. 2013.

GIRMA, F.; FININSA, C.; TEREFE, H.; AMSALU, B. Evaluation of common bean (*Phaseolus vulgaris*) genotypes for resistance to common bacterial blight and angular leaf spot diseases, and agronomic performances. Heliyon 8:e10425. 2022.

GONZÁLEZ, A. M.; GODOY, L.; SANTALLA, M. Dissection of resistance genes to *Pseudomonas syringae* pv. *phaseolicola* in UI3 common bean cultivar. International Journal of Molecular Sciences 18:2503. 2017.

HUANG, M.; LIU, X.; ZHOU, Y.; SUMMER, R. M.; ZHANG, Z. BLINK: A package for the next level of genome-wide association studies with both individuals and markers in the millions. GigaScience 8:giy154. 2019.

JEGER, M. J., VILJANEN-ROLLISON, S. L. H. The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. Theoretical and Applied Genetics 102:32-40. 2010.

JELIHOVSCHI, E. G.; FARIA, J. C.; ALLAMAN, I. B.; ScottKnott: A Package for performing the Scot-Knott clustering algorithm in R. Trends in Applied and Computational Mathematics 15:3-17. 2014.

KADO, C. I.; HESKETT, M. G. Selective media for isolation of *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas* and *Xanthomonas*. Phytopathology 60:969-976. 1970.

KANSTRUP, C.; NOUR-ELDIN, H. H. The emerging role of the nitrate and peptide transporter family: NPF in plant specialized metabolism. Current Opinion in Plant Biology 68:102243. 2022.

KAVINA, C.; MANDUMBU, R.; PARWADA, C.; TIBUGARI, H.; A review of the occurrence, biology and management of common bacterial blight. Journal of Agricultural Technology 7:459-1474. 2011.

KERK, D.; TEMPLETON, G.; MOORHEAD, G. B. G. Evolutionary radiation pattern of novel protein phosphatases revealed by analysis of protein data from the completely sequence genomes of humans, green algae, and higher plants. Plant Physiology 146:351-367. 2008.

KURT, F. An Insight into Oligopeptide Transporter 3 (OPT3) family proteins. Protein & Peptide Letters 28:43-54. 2021.

LIU, X.; HUANG, M.; FAN, B.; BUCKLER, E. S.; ZHANG, Z. Iterative usage of fixed and random effect models for power and efficient genome-wide association studies. PloS Genetics 12:e1005767. 2016.

MARQUES, A. S. A.; GUIMARÃES, P. M.; SANTOS, J. P. S.; VIEIRA, T. M. Sobrevivência e viabilidade de *Xanthomonas axonopodis* pv. *phaseoli* em sementes de feijão armazenados sob condições controladas. Fitopatologia Brasileira. 30:527-531. 2005.

MENDIBURU, F. Agricolae: Statistical procedures for agriculture research. R Package Version 1:2-3. 2017.

MONTEIRO, A. L. R.; CHAVES, F. S.; PANTALEÃO, A. S. L.; CARNEIRO, P. C. S.; CARNEIRO, J. E. S.; BADEL, J. L. Sources, spectrum, genetics, and inheritance of *Phaseolus vulgaris* resistance against *Xanthomonas citri* pv. *fuscans*. Phytopathology 110:1428-1436, 2020.

MONTEIRO, A. L. R; PANTALEÃO, A. S. L.; BADEL, J. L.; SOARES, P. H. M.; CARNEIRO, V. Q.; CARNEIRO, P. C. S.; CARNEIRO, J. E. S. Genome-wide association study (GWAS) of *Phaseolus vulgaris* resistance to *Xanthomonas citri* pv. *fuscans*. Plant Pathology 70:1733-1744, 2021.

MOURAD, A. M. I.; SALLAM, A.; BELAMKAR, V.; WEGULO, S.; BOWDEN, R.; JIN, Y.; MAHDY, E.; BAKHEIT, B.; EL-WAFAA, A. A.; POLAND, J.; BAEZIGER, P. S. Genome-wide association study for identification and validation of novel SNP markers for *Sr6* stem rust resistance gene in bread wheat. Frontiers in Plant Science 9:380.2018.

MÜLLER, B. S.F.; PAPPAS, G. J.; VALDISSER, P. A. M. R. An operational SNP panel integrated to SSR marker for the assessment of genetic diversity and population structure of the common bean. Plant Molecular Biology Reporter 33:1697-1711. 2015.

MUTLU, N.; MIKLAS, P.; REISER, J.; COYNE, D. Backcross breeding for improved resistance to common bacterial blight in pinto bean (*Phaseolus vulgaris* L.). Plant Breeding 124:282-287. 2005.

NASCIMENTO, M.; NASCIMENTO, A. C. C.; SILVA, F. F. Quantitative regression for genome-wide association study of flowering time-related traits in common bean. PloS One 3:e0190303. 2018.

O'BRIEN, J. A.; DAUDI, A.; BUTT, V. S.; BOLWELL, G. P. Reactive oxygen species and their role in plant defense and cell wall metabolism. Planta 236:765-779. 2012.

PARK, C. J.; PENG, Y.; CHEN, X.; DARDICK, C.; RUAN, D.; BART, R.; CANLAS, P. E.; RONALD, P. C. Rice XB15, a protein phosphatase 2C, negatively regulates cell death and XA21-mediated innate immunity. PloS Biology 6:e231. 2008.

PEREIRA, H. S.; MOTA, A. P. S.; RODRIGUES, L. A.; DE SOUZA, T. L. P. O.; MELO, L. C. Genetic diversity among common bean cultivars based on agronomic traits and molecular markers and application to recommendation of parent lines. Euphytica 215:1-16. 2019.

PIRES, C. V.; OLIVEIRA, M. G. A.; CRUZ, G. A. D. R.; MENDES, F. Q.; DE REZENDE, S. T.; MOREIRA, M. A. Composição físico-química de diferentes cultivares de feijão (*Phaseolus vulgaris* L.). Alimentos e Nutrição Araraquara 16:157-162. 2005.

PRICE, A. L. ZAITLEN, N.; REICH, D.; PATTERSON, N. New approaches to population stratification in genome-wide association studies. Nature Reviews Genetics 11:459-463. 2010.

R DEVELOPMENT CORE TEAM. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. 2018.

SINGH, S. P.; MIKLAS, P. N. Breeding common bean for resistance to common bacterial blight: A review. Crop Science 55:971-984. 2015.

SILVA, S. A. G.; MORAIS, O. P.; RAVA, C. A.; COSTA, J. G. C. Generalized method for analysis of unbalanced diallels. Pesquisa Agropecuaria Brasileira 35:80-89. 2000.

SILVA, A.; SANTOS, I.; BALBINOT, A. L.; MATEI, G.; OLIVEIRA, P. H. Reaction of bean genotypes to the common bacterial blight, evaluated by two inoculation methods. Ciência e Agrotecnologia 33:2019-2024. 2009.

SCHOONHOVEN, V.; PASTOR-CORRALES, M. A. Sistema estándar para la evaluación de germoplasma de frijol. Colombia: Centro Internacional de Agricultura Tropical, 86p. 1987.

SUKARTA, O. C. A.; DIAZ-GRANADOS, A.; SLOOTWEG, E. J.; OVERMARS, H.; SCHAIK, C. V.; POKHARE, S.; ROOSIEN, J.; POMP, R.; ELASHRY, A.; SMANT, G.; GOVERSE, A. Two evolutionary distinct effectors from a nematode and virus target RanGAP1 and 2 via the WPP domain to promote disease. bioRxiv 06.24.449730. 2021.

TAM, V.; PATEL, N.; TURCOTTE, M.; BOSSÉ, Y.; PARÉ, G.; MEYRE, D. Benefits and limitations of genome-wide association studies. Nature Reviews Genetics 20:467-484. 2019.

TSUTSUMI, C. Y.; BULEGON, L. G.; PIANO, J. T.; Melhoramento genético do feijoeiro: Avanços, perspectivas e novos estudos, no âmbito nacional. Nativa 3:217-223. 2015.

WICKHAM, H. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York. 2016.

WILLATS, W. G.; McCARTNEY, I.; MACKIE, W.; KNOX, J. P. Pectin: Cell biology and prospects for functional analysis. Plant Molecular Biology 47:9-27. 2001.

WU, J. ZHU, J. WANG, L.; WANG, S. Genome-wide association study identifies NBS-LRR-encoding genes related with anthracnose and common bacterial blight in the common bean. Frontiers in Plant Science 8:1398. 2017.

WU, X.; HAN, J.; GUO, C. Function of nuclear pore complexes in regulation of plant defense signaling. International Journal of Molecular Sciences 23:1-17. 2022.

YANG, L.; QU, Q.; HAO, Z.; SHA, K.; LI, Z.; LI, S. Powerful Identification of large quantitative trait loci using genome-wide R/glmnet-based regression. Journal of Heredity 113:472-478. 2022.

YANG, C.; HU, H.; REN, H.; KONG, Y.; LIN, H.; GUO, J.; WANG, L.; HE, Y.; DING, X.; GRABSZTUNOWICZ, M.; MULO, P.; CHEN, T.; LIU, Y.; WU, Z.; WU, Y.; MAO, C.; WU, P.; MO, X. LIGHT-INDUCED RICE1 Regulated light-dependent attachment of LEAF-TYPE FERRODOXIN-NADP+ OXIDOREDUCTASE to the thylakoid membrane in rice and *Arabidopsis*. The Plant Cell 28:712-728. 2016.

YANG, J.; ZAITLEN, N.; GODDARD, M. E.; VISSCHER, P. M.; PRICE, A. L. Advantages and pitfalls in the application of mixed-model association methods. Nature Genetics 36:512-517. 2014.

YU, K.; PARK, S. J.; POYSA, V. Marker-assisted selection of common beans for resistance to common bacterial blight: Efficacy and economics. Plant Breeding 119:411-415. 2000.

ZAPATA, M.; BEAVER, J. S.; PORCH, T. G. Dominant gene for common bean resistance to common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli*. Euphytica 179:373-382. 2011.

ZHU, J.; WANG, L.; BLAIR, M. W.; ZHU, Z.; WANG, S. QTL and candidate genes associated with common bacterial blight resistance in the common bean cultivar Longyundou 5 from China. The Crop Journal 4:344-352. 2016.

3.6. TABLES

Table 1. Severity of Common Bacterial Blight symptoms in common bean cultivars inoculated with *Xanthomonas phaseoli* pv. *phaseoli* CFBM-UFV-0002.

Cultivar	Severity*	Classification	Cultivar	Severity*	Classification
Carioca MG	7.2 a	HS	IAPAR 16	5.0 b	MS
BRS Estilo	6.6 a	HS	BR-IPA 10	5.0 b	MS
Pérola	6.0 a	HS	IPR Eldorado	5.0 b	MS
FT 120	6.0 a	HS	VP33	5.0 b	MS
BRS Majestoso	6.0 a	HS	Rudá R	5.0 b	MS
IAC Tunã	6.0 a	HS	IAC-Apuã	5.0 b	MS
Iraí	6.0 a	HS	BRS Requinte	5.0 b	MS
IAC-Carioca Pyatã	5.8 a	HS	BRS Pontal	5.0 b	MS
Carioca 130	5.8 a	HS	BR IPA 11 Brígida	4.8 b	MS
IAC – Alvorada	5.8 a	HS	Macumino	4.8 b	MS
IAC-Una	5.7 a	HS	IAC Votuporanga	4.7 b	MS
BRS Notável	5.7 a	HS	Varre-Sai	4.7 b	MS
IAC-Ybaté	5.6 a	HS	IPR Tuiuiú	4.6 b	MS
IPR Uirapurú	5.6 a	HS	IPR Campos Gerais	4.6 b	MS
IPR 139	5.6 a	HS	BRS Grafite	4.6 b	MS
Rico 1735	5.6 a	HS	IAPAR8 Rio Negro	4.6 b	MS
Moruna	5.6 a	HS	Rio Tibagi	4.6 b	MS
IAC Carioca	5.6 a	HS	VP 22	4.6 b	MS
BRS Cometa	5.5 a	HS	Aporé	4.5 b	MS
Milionário 1732	5.4 a	HS	Onix	4.4 b	MS
IAPAR 57	5.4 a	HS	IPR Saraçura	4.4 b	MS
IAC-Carioca Akytá	5.4 a	HS	Capixaba Precoce	4.4 b	MS
IPR Tiziu	5.4 a	HS	IAPAR 65	4.4 b	MS
Rio Doce	5.3 a	HS	Rico 23	4.2 b	MS
Rudá	5.2 a	HS	Diamante Negro	4.2 b	MS
VC 15	5.2 a	HS	BRS Expedito	4.2 b	MS

IAPAR 81	5.2	a	HS	FT Bonito	4.2	b	MS
IPR Andorinha	5.2	a	HS	IPR Graúna	4.2	b	MS
BRSMG	5.2	a	HS	BRS Esplendor	4.0	c	MR
Madrepérola	5.2	a	HS	BR- 3 Ipanema	4.0	c	MR
BR1 Xodó	5.2	a	HS	Preto Uberabinha	4.0	c	MR
IPR Colibri	5.2	a	HS	BRS Supremo	4.0	c	MR
IAPAR 44	5.2	a	HS	BRS Esplendor	4.0	c	MR
Carioca 80	5.2	a	HS	BRSMG Pioneiro	3.8	c	MR
Pampa	5.2	a	HS	BRS Valente	3.6	c	MR
IPR Tangará	5.2	a	HS	BRS Campeiro	3.0	d	HR
Carioca 1070	5.2	a	HS	IPR Gralha	3.0	d	HR
Ouro Negro	5.2	a	HS	IAC Imperador	2.8	d	HR
IAPAR 20	5.0	b	MS	IAC Formoso	2.6	d	HR
SCS Guará	5.0	b	MS	BR2 Grande Rio	2.4	d	HR
IAPAR 31	5.0	b	MS	BRSMG Talismã	2.2	d	HR

* Within this column, means followed by the same letter are not significantly different according to the Scott-Knott test ($p \leq 0.05$) on the log-transformed data. ** HS, highly susceptible; MS, moderately susceptible; MR, moderately resistant; HR, highly resistant.

Table 2. Area under the disease progress curve (AUDPC) in common bean cultivars in response to *Xanthomonas phaseoli* pv. *phaseoli* CFBM-UFV-0002.

Cultivars	AUDPC*	Cultivars	AUDPC*
BRS Estilo	77.6 a	IAPAR 20	49.2 b
FT120	70.8 a	IAPAR 44	49.2 b
IAC Una	70.5 a	IAC-Apuã	49.2 b
BRS Notável	70.5 a	SCS Guará	48.8 b
BRS Cometa	69.0 a	Rio Doce	48.8 b
BRS Majestoso	66.8 a	BR IPA 11 Brígida	48.4 b
IAC Alvorada	66.8 a	Moruna	48.4 b
Carioca MG	66.4 a	VP 33	48.0 b
IAC Tunã	66.4 a	Rudá R	47.2 b
IAC Carioca Pyatã	65.6 a	BR-IPA 10	46.8 b
Pérola	65.2 a	IAC-Carioca Akytá	46.4 b
Carioca 1030	60.8 a	FT Bonito	45.6 b
IAPAR 57	60,4 a	IPR Tuiuiú	45.2 b
IAC Carioca	60.4 a	BRS-AGRO Pampa	45.2 b
IPR 139	60.0 a	Diamante Negro	45.0 b
IRAÍ	60.0 a	BR-3 Ipanema	44.8 b
IAC Ybaté	59.6 a	IAPAR 8-Rio Negro	44.8 b
Carioca 80	57.6 a	Aporé	43.0 b
IRP Uirapurú	57.2 a	IPR Saracura	41.6 b
BRS Pontal	56.0 a	BRSMG Pioneiro	40.8 b
IPR Colibri	55.6 a	BRS Esplendor	40.4 b
BRS Grafite	55.6 a	IPR Tiziu	40.4 b
IAC Votuporanga	55.5 a	Milionário 1732	39.6 b
VC 15	55.2 a	VP22	39.2 b
Madrepérola	55.2 a	Onix	38.8 b
BRS Requinte	54.8 a	IAPAR 65	38.8 b
Carioca 1070	54.8 a	IPR Campos Gerais	38.0 b
IPR Eldorado	54.4 a	IPR Graúna	37.2 c
Capixaba Precoce	53.2 a	Ouro Negro	37.2 c
BR1 Xodó	53.2 a	BRS Supremo	36.8 c
Rudá	52.8 a	Rico 23	36.5 c
IAPAR 31	51.6 a	Rio Tibagi	36.4 c

IPR Andorinha	51.6	a	Preto Uberabinha	33.6	c
BRS Expedito	51.0	a	BRS Valente	31.2	c
Varre-Sai	51.0	a	BRS Campeiro	29.0	d
IPR Tangará	50.8	a	IAC Formoso	23.6	d
Rico 1735	50.4	a	IAC Imperador	20.8	d
IAPAR 16	50.0	b	BR2 Grande Rio	20.0	d
Macanudo	50.0	b	BRSMG Talismã	19.2	d
IAPAR 81	49.2	b	IPR Gralha	19.0	d

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

Table3. Common bean genes linked to Single Nucleotide Polymorphism (SNP) markers with the highest -$\log_{10}(p)$ values and putatively associated with *Xanthomonas phaseoli* pv. *phaseoli* resistance.

SNP marker	Chr.*	Genome position (pb)	Candidate gene	Putative Function
74	1	15214491	*Phvul.001G085200*	Light regulated Lir1 protein
308	1	42448516	*Phvul.001G163400*	Proton-dependent oligopeptide transporter family protein
311	1	52001027	*Phvul.001G267900*	Metallo-dependent phosphatase-like protein
300	2	12806073	*Phvul.002G083200*	WPP domain-interacting tail-anchored protein 2
16	9	21976209	*Phvul.009G151200*	Pectin lyase-like superfamily protein

*Chr, Chromosome.

3.7. FIGURES

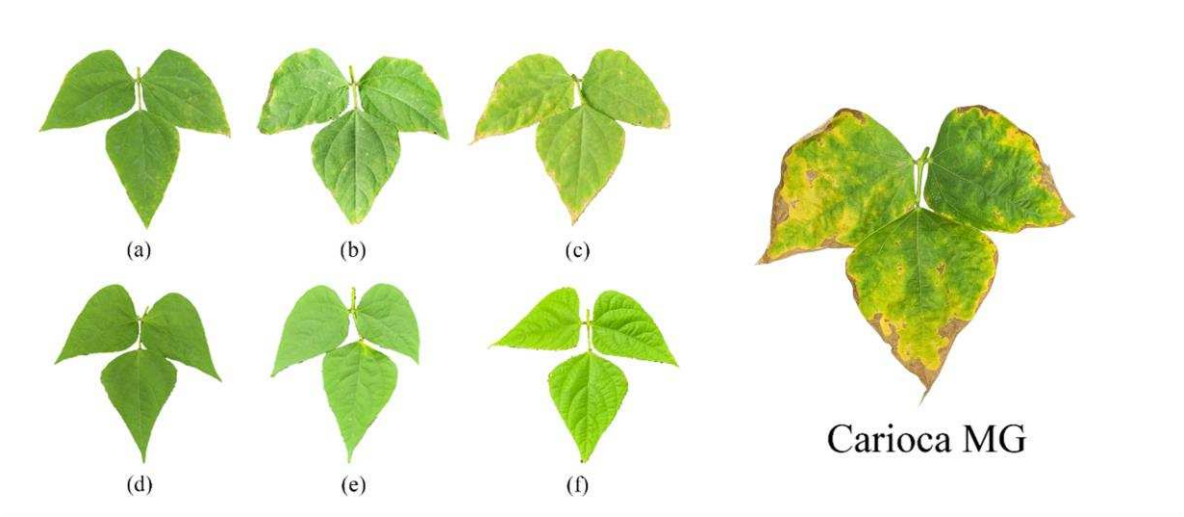

Figure 1. Comparison of Common Bacterial Blight symptoms on highly resistant common ben cultivars with the susceptible control Carioca MG at 19 days after inoculation with *Xanthomonas phaseoli* pv. *phaseoli* CFBM-UFV-0002. (a) BRS Campeiro; (b) IPR Gralha; (c) BR2 Grande Rio; (d) IAC Formoso; (e) BRSMG Talismã; (f) IAC Imperador.

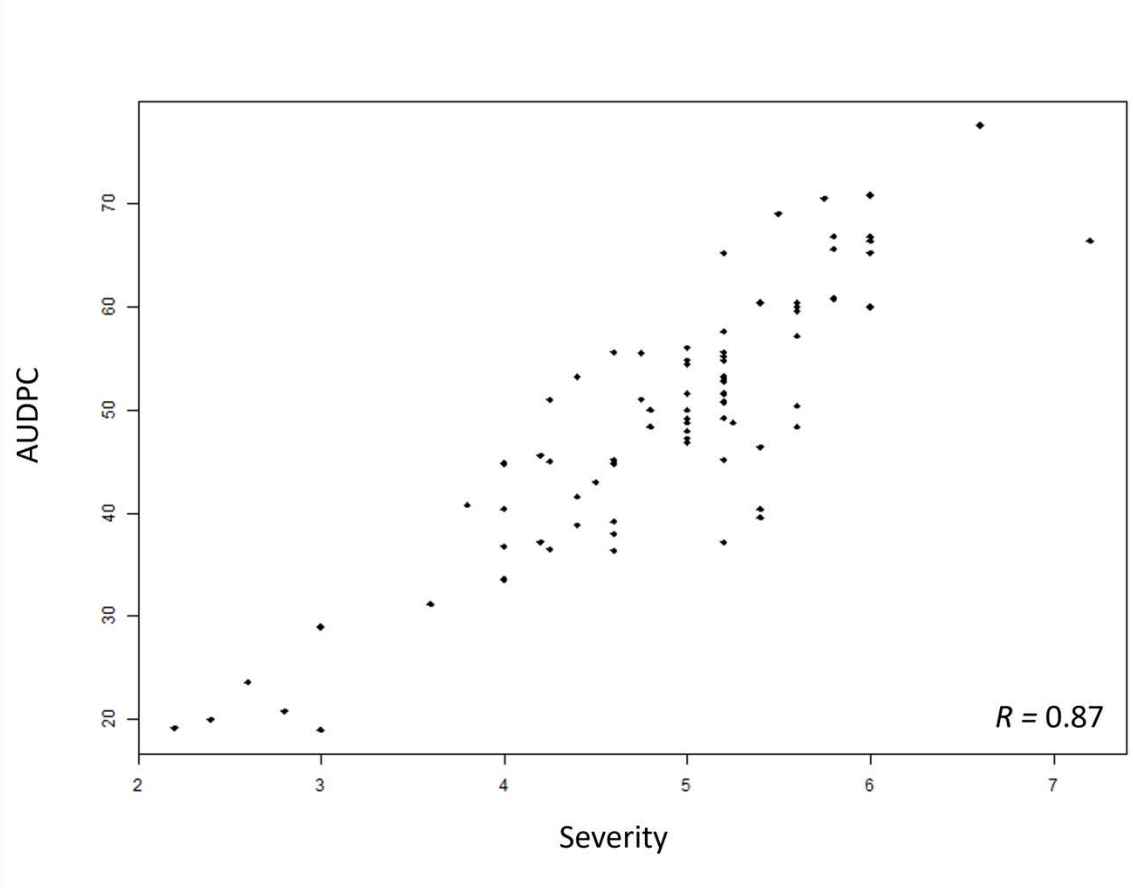

Figure 2. Relationship between disease severity and area under the disease progress curve for 80 *Phaseolus vulgaris* cultivars inoculated with *Xanthomonas phaseoli* pv. *phaseoli* CFBM-UFV-0002.

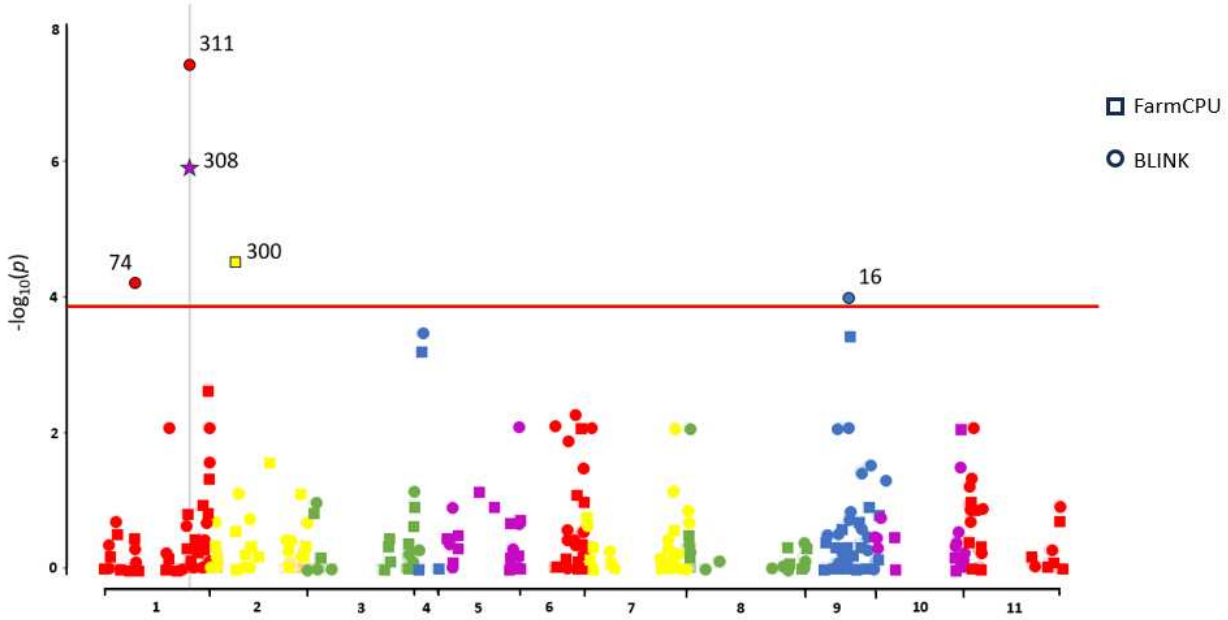

Figure 3. Manhattan plot of the genomic association of 384 common bean single-nucleotide polymorphisms (SNPs) with resistance to *Xanthomonas phaseoli* pv. *phaseoli* CFBM-UFV-0002. Squares, circles and stars indicate SNPs identified using the FarmCPU model, the BLINK model, and both models, respectively. The horizontal red line indicates the $-\log_{10}(p)$ threshold of 3.9 used to select SNPs for gene identification. The numbers indicate the SNPs identification.

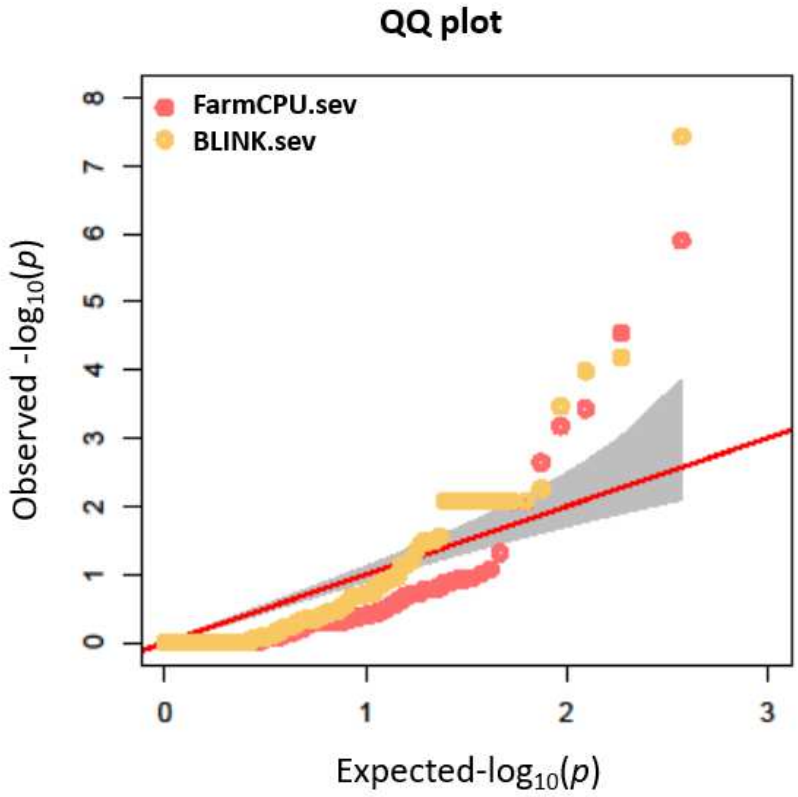

Figure 4. Quantile-Quantile plot for the association of single-nucleotide polymorphisms (SNPs) with resistance of 80 common bean cultivars to *Xanthomonas phaseoli* pv. *phaseoli* CFBM-UFV-0002 using the two models FarmCPU and BLINK.

3.8. SUPPEMENTARY MATERIAL

Table S1. Common bean cultivars used in this study.

Cultivar	Source	Group	Cultivar	Source	Group
Aporé	EMGOPA	Carioca	IAC–Una	IAC	Preto
BR IPA 11 Brígida	IPA	Carioca	IAC–Ybaté	IAC	Carioca
BR-IPA 10	IPA	Preto	IAPAR 16	IAPAR	Carioca
BR–3 Ipanema	PESAGRO	Preto	IAPAR 20	IAPAR	Preto
BR–IPAGRO 1–Macanudo	IPAGRO	Preto	IAPAR 31	IAPAR	Carioca
BR1 Xodó	PESAGRO	Preto	IAPAR 44	IAPAR	Preto
BR2 Grande Rio	PESAGRO	Preto	IAPAR 57	IAPAR	Carioca
BRS Campeiro	EMBRAPA	Preto	IAPAR 65	IAPAR	Preto
BRS Cometa	EMBRAPA	Carioca	IAPAR 8–Rio Negro	IAPAR	Carioca
BRS Esplendor	EMBRAPA	Preto	IAPAR 81	IAPAR	Carioca
BRS Estilo	EMBRAPA	Carioca	IPR 139	IAPAR	Carioca
BRS Expedito	EMBRAPA	Preto	IPR Andorinha	IAPAR	Carioca
BRS Grafite	EMBRAPA	Preto	IPR Campos Gerais	IAPAR	Carioca
BRS Majestoso	EMBRAPA	Carioca	IPR Colibri	IAPAR	Carioca
BRS Notável	EMBRAPA	Carioca	IPR Eldorado	IAPAR	Carioca
BRS Pontal	EMBRAPA	Carioca	IPR Gralha	IAPAR	Preto
BRS Requinte	EMBRAPA	Carioca	IPR Graúna	IAPAR	Preto
BRS Supremo	EMBRAPA	Preto	IPR Saracura	IAPAR	Carioca
BRS Valente	EMBRAPA	Preto	IPR Tangará	IAPAR	Carioca
BRS–IPAGRO 2–Pampa	EMCAPA	Preto	IPR Tiziu	IAPAR	Preto
BRSMG Madrepérola	UFV	Carioca	IPR Tuiuiú	IAPAR	Preto
BRSMG Pioneiro	EMBRAPA	Carioca	IPR Uirapurú	IAPAR	Preto
BRSMG Talismã	UFV/UFLA	Carioca	IRAÍ	IPAGRO	Preto
Capixaba Precoce	EMCAPA	Preto	Milionário 1732	EPAMIG	Preto
Carioca 1030	IAC	Carioca	Moruna	IAC	Preto
Carioca 1070	IAC	Carioca	Onix	EMGOPA	Preto
Carioca 80	IAC	Carioca	Ouro Negro	EMBRAPA	Preto

Carioca MG	EMBRAPA	Carioca	Pérola	EMBRAPA	Carioca
Diamante Negro	EMBRAPA	Preto	Preto Uberabinha	IPEGRO	Preto
FT 120	FT - sementes	Preto	Rico 1735	EPAMIG	Preto
FT bonito	FT - sementes	Carioca	Rico 23	UFV	Preto
IAC – Carioca Akytá	IAC	Carioca	Rio doce	EMCAPA	Carioca
IAC – Carioca Pyatã	IAC	Carioca	Rio Tibagi	UFV	Preto
IAC Alvorada	IAC	Carioca	Rudá	EMCAPA	Carioca
IAC Carioca	IAC	Carioca	Rudá R	EMCAPA	Carioca
IAC Formoso	IAC	Carioca	SCS Guará	EPGRI	Carioca
IAC Imperador	IAC	Carioca	Varre–Sai	PESAGRO	Preto
IAC Tunã	IAC	Preto	VC 15	UFV	Carioca
IAC Votuporanga	IAC	Carioca	VP 22	UFV	Preto
IAC–Apuã	IAC	Carioca	VP 33	UFV	Preto

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

4. Chapter 2 - Research Article

Differences and commonalities in type III secretion effector repertoires between *Xanthomonas* that cause common bacterial blight in common bean.

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ABSTRACT:

Common Bacterial Blight (CBB) is a serious disease affecting common bean (*Phaseolus vulgaris* L.) production. The disease is attributed to the Gram-negative bacteria *Xanthomonas citri* pv. *fuscans* (*Xcf*) and *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*). Type III secretion effectors (T3SE) play a pivotal role in the pathogenicity of *Xanthomonas* species during interactions with their host plants. These *Xanthomonas* T3SE are activated *in planta* by HrpG and HrpX, two global regulatory proteins. Gaining insights into the genomic diversity, plasticity and T3SE richness of *Xcf* and *Xpp* can significantly enhance our understanding on how CBB is caused by its etiological agents. The objective of this study was to examine the genome diversity and plasticity of *Xpp* and *Xcf* and to identify genes that may be associated with their phytopathogenicity. For that, 35 genome sequences of *Xcf* and 36 genome sequences of *Xpp* were employed for pangenomic analysis and dendrogram construction using presence/absence gene repertoires. Furthermore, predictions of genes activated by HrpG and HrpX were conducted by bioinformatically searching for *hrp* box sequences in gene promoter regions. To identify T3SE, Blastp searches were conducted against databases of known *Xanthomonas* and *Pseudomonas* effectors. The pangenome

analysis showed that both species exhibit significant genetic diversity and plasticity. The dendrogram using pangenomes did not reveal a clear clustering by geographic origin for either pathogen. Sequence analysis of the *hrp* box indicated the presence of 51 and 49 HrpG- and HrpX-dependent genes in *Xcf* and *Xpp*, respectively. The Blastp searches identified a total of 39 T3SEs, with effector XopAL2 being unique to *Xcf*, six effectors exclusive to *Xpp*, and seven effectors shared with phytopathogenic *Pseudomonas*. Collectively, our findings provide important insights into the mechanisms that *Xanthomonas* species may utilize to cause disease in common bean plants.

KEYWORDS: *Phaseolus vulgaris*, *Xanthomonas phaseoli* pv. *phaseoli*, *Xanthomonas citri* pv. *fuscans*, type III effectors.

4.1. INTRODUCTION

Xanthomonas is a Gram-negative bacterial genus of the Gammaproteobacteria class (RYAN *et al.,* 2011) that comprises 33 validly published species according to the List of Prokaryotic Names with Standing Nomenclature (https://www.bacterio.net/), which may be further subdivided into distinct pathovars. Although *Xanthomonas* species can Infect more than 400 different plant species, including economically important crops, such as rice, wheat, citrus, tomato, pepper, and banana, each pathovar exhibits considerable host specificity (JACQUES *et al.,* 2016; TIMILSINA *et al.,* 2020). Many *Xanthomonas* are distinguished by the production of the yellow pigment xanthomonadin, although strains of some pathovars, such as *X. phaseoli* pv. *manihotis* (sin. *X. axonopodis* pv. *manihotis*) and *X. campestris* pv. *mangiferaindicae*, lack the production of such a pigment (TIMILSINA *et al.,* 2020).

Common bean (*Phaseolus vulgaris* L.), which ranks among the world's most widely consumed agricultural products, is also affected by *Xanthomonas* species. Common Bacterial Blight (CBB) of common bean is a disease of widespread occurrence worldwide, significant negative impact on production, and limited control due to the low effectiveness of the chemical products currently available (YU *et al.,* 2000; DE PAIVA *et al.,* 2018). The causal agents of CBB are the Gram-negative bacteria *X. phaseoli* pv. *phaseoli* (*Xpp*) (YOUNG *et al.,* 1996) and *X. citri* pv. *fuscans*

(*Xcf*) (GILBERTSON and MAXWELL, 1992). Although both pathogens can cause the same disease symptoms and similar damage to common bean plants, they can be differentiated based on some distinctive phenotypic traits, more notoriously the *in vitro* melanin production by *Xcf* (DE PAIVA *et al.,* 2018).

Under elevated humidity and temperature conditions, CBB has the potential to trigger severe disease outbreaks, resulting in substantial reductions in crop yield, with susceptible genotypes experiencing a decrease of up to 80% in production (YANG *et al*., 2022). *Xpp* and *Xcf* primarily gain entry to the plant tissue through stomata and wounds, colonizing mesophyll cells and spreading systemically throughout the plant. The bacteria reach various types of plant tissue, including stem where cankers form, pods that develop water-soaked lesions, and seeds that may exhibit extensive discoloration or deformation (MONTEIRO *et al*., 2020; YANG *et al*., 2022).

Bacteria, like other prokaryotes, exhibit a remarkable degree of genetic diversity, which can vary significantly even within individual lineages (AZARIAN *et al.,* 2020). Along their evolutionary history, these microorganisms can gain or lose biochemical functions to adapt themselves to specific environmental conditions or energy demands to sustain an efficient metabolism. It has become increasingly clear that variations in genes are intricately linked to the ecological niche of the organism, driven by continuous processes of niche exploration, diversification, and adaptation (LASSALLE & DIDELOT, 2020). In this regard, the pangenome encompasses two fundamental genome elements: core genes, a set of genes universally present across all strains of the same taxon, and accessory genes, which are shared among two or more strains of the same taxon, besides unique genes present exclusively in a particular strain of the taxon (RASKO *et al.,* 2008). Together, these elements collectively help define the taxon genetic diversity, plasticity and evolutionary history, and can be applied at different taxonomic levels, such as genus, species or pathovar. Accessory genes can encompass various genetic elements, such as those originating from transposons, plasmids, and genes that have undergone substantial mutations and recombination (AZARIAN *et al.,* 2020).

In order to determine these sets of genes, individual strains from populations are sequentially interrogated for the presence of conserved, shared and unique genes. Several studies suggest the core genome of *Xanthomonas* is unlikely to undergo

significant changes with further sampling, while the *Xanthomonas* accessory genes represent a substantial resource that confers unparalleled plasticity to enhance adaptability and, potentially, influencing aspects such as pathogenicity, virulence, and host specificity (AGARWAL *et al.,* 2023; JACKSON *et al.,* 2010). This high genomic plasticity highlights the abundance of strategies *Xanthomonas* has evolved to suppress host immunity.

Protein secretion systems are pivotal to the intricate interplay between pathogens and their hosts (KAY & BONAS, 2009). Within the Gram-negative bacteria, *Xanthomonas* spp. are notorious for harboring genes for all recognized protein secretion systems (KOEBNIK *et al.,* 2006). Among these, the type III secretion system (T3SS) and its secreted effectors (T3SE) have emerged as key pathogenicity determinants, significantly shaping host specificity, restriction and expansion (AN *et al.,* 2019). In *Xanthomonas* spp., the T3SS is coded by a cluster of genes known for their essential role in the hypersensitive response in nonhost plants and in pathogenicity in host plants (hence *hrp*, for hypersensitive response and pathogenicity).

The *Xanthomonas hrp* cluster consists of a minimum of six *hrp* operons, named *hrpA* to *hrpF* (BONAS *et al.,* 1991; KOEBNIK *et al.,* 2006). The expression of these *hrp* operons occurs within the host plant upon activation by proteins coded by the two regulatory genes *hrpG* and *hrpX* (TEPER *et al.,* 2021). HrpG belongs to the OmpR family of two-component signal transduction response regulators and activates the expression of *hrpX*. Subsequently, HrpX, classified as an AraC-type transcriptional regulator, directs the expression of genes of the downstream *hrp* operons that code for the components of the T3SS, T3SE, and proteins that aid in the secretion process (BONAS *et al.,* 1991; KOEBNIK *et al.,* 2006). The regulatory function of HrpX results from its binding to a *cis*-regulatory element, known as the plant-inducible promoter (PIP) box, which is located within the promoter region of genes controlled by HrpX (TEPER *et al.,* 2021). A specific sequence motif, resembling the PIP box, has also been identified in various *Xanthomonas* species and is referred to as the hrp_{II} box (KOEBNIK *et al.,* 2006; KVITKO & COLLMER, 2023).

Although most *Xanthomonas* T3SEs are commonly referred to as Xop (*Xanthomonas* outer proteins), there are some exceptions, notably AvrBs1, AvrBs2,

and AvrBs3 (ESCALON *et al.,* 2013). These particular effectors have long been demonstrated to confer an avirulence phenotype to the strains carrying them, and hence, the Avr designation. The avirulence of the strains is due to the effector recognition by cognate resistance proteins within host plants, leading to the initiation of effector-triggered immunity (ETI) (WHITE *et al.,* 2009). Currently, a total of 74 Xop families are recognized, each bearing an alphabetical nomenclature ranging from XopA to XopBA (ESCALON *et al.,* 2013; TIMILSINA *et al.,* 2020).

With respect to the *Xanthomonas* pathogenic to common bean, Paiva *et al*. (2021) analyzed the T3SE profile of 44 strains belonging to *Xcf* and *Xpp*. Using PCR amplification targeting 10 T3SE, namely, XopR, XopV, XopE1, XopN, XopQ, XopAK, XopA, XopL, AvrBs2, and XopX, commonly found in *Xanthomonas* strains pathogenic to common bean, the researchers were able to differentiate between fuscans and nonfuscans strains. Their findings also highlighted a notable diversity in the T3SE profile among *Xpp* strains when compared to *Xcf*. Other effectors have also been reported to be present in strains of *Xpp* and *Xcf*, such as XopC and XopJ, which may play a significant role in pathogenicity (ALAVI *et al.,* 2008). So far, research on T3SE diversity in *Xpp* and *Xcf* primarily revolves around variability of the aforementioned 10 effectors and little attention has been given to unique effectors like XopC and XopF (ALAVI *et al.,* 2008; PAIVA *et al.,* 2021; TUGUME *et al.,* 2019). This limited focus has failed to capture the true extent of effector diversity found in these two common bean pathogens.

A better knowledge of the range of the effector arsenals *Xpp* and *Xcf* carry and a more comprehensive understanding of the differences between these two bacterial pathovars can shed light on how they manipulate the host, which in turn may guide genetic breeding programs for common bean resistance. Given the similarity of the symptoms caused by *Xcf* and *Xpp* on common bean, it was hypothesized that these two pathogens shared a set of T3SE genes that may be fundamental to cause disease on this plant species. Hence, to comprehend the diversity in the T3SE repertoire of these two bacterial pathogens, and to determine their commonalities, pangenomic analyses and comprehensive predictions of genes activated by HrpX and HrpG were conducted. Also, searches for *Xcf* and *Xpp* sequences with significant similarity with previously described *Xanthomonas* and *Pseudomonas* T3SE effectors were carried

out to gain some insights into virulence functions that could have conserved targets across divergent plant species.

4.2. MATERIALS AND METHODS

Bacterial genome sequences

Genome sequences of 36 *Xpp* and 35 *Xcf* isolates obtained from common bean were retrieved from the National Center for Biotechnology Information (NCBI) database (GenBank; https://www.ncbi.nlm.nih.gov/). Curated RefSeq sequences were utilized in the analyses, whose relevant information is provided in supplementary Tables S1 and S2.

Pangenomic analysis

The web-based Galaxy - Australia platform (https://usegalaxy.org.au/) was employed to conduct the pangenomic analysis. First, all *Xpp* and *Xcf* genome sequences were reannotated to obtain gff3 files using the prokaryotic genome annotation program Prokka (Galaxy Version 1.14.6+galaxy1), with default parameters. To generate the alignment of the core genomes, Roary (Galaxy Version 3.13.0+galaxy2) was used with settings such that a gene must be present in 100% of isolates analyzed to be considered part of the core genome. Subsequently, the genome alignment file was subjected to Gubbins (Galaxy Version 3.2.1+galaxy0) to enable tree visualization. The Gubbins final_tree.treeoutput file and the Roary gene_presence_absence.csv output files were uploaded to the Phandango website version 1.3.0 (https://jameshadfield.github.io/phandango/#/) for tree and gene presence/absence visualization. In addition, the script *create_pan_genome_plots* (https://github.com/sanger-pathogens/Roary/blob/master/bin/roary-create_pan_genome_plots.R) with some modifications and the ggplot2 library were used to build the pangenome plots in R version 4.3.1 (R DEVELOPMENT CORE TEAM, 2018).

Prediction and comparison of HrpX- and Hrp-G dependent gene repertoires

To identify genes dependent on HrpX and HrpG activation, a customized Python script was developed to search for conserved *hrp* boxes sequences within gene promoter regions and previously described as being activated by these regulatory

proteins (Table S3). Besides unveiling the *hrp* box sequences, the script also retrieves the sequences of the two downstream genes.

Prediction of T3SE repertoires

For the identification of T3SE, a comprehensive Blastp (Altschul *et al*. 1990) search was conducted using all the annotated proteins of each *Xpp* and *Xcf* genome included in this study against known *Xanthomonas* effector sequences deposited in the EuroXanth DokuWiki database (https://euroxanth.ipn.pt/doku.php?id=start) (Costa *et al.*, 2021, 2022). In addition, to identify *Xpp* and *Xcf* effectors shared with phytopathogenic bacteria from the *Pseudomonas* genus, Blastp was performed using known *P. syringae* effector sequences deposited in the *Pseudomonas syringae* Genome Resource database (http://www.pseudomonas-syringae.org/) against all proteins of the *Xanthomonas* pathogenic to common bean from the NCBI. A Venn diagram was constructed using the Jvenn tool (http://jvenn.toulouse.inra.fr/app/example.html) (Bardou *et al*., 2014) to represent the comparison between the T3SE gene repertoires of *Xpp* and *Xcf*.

4.3. RESULTS

The pangenomes of *Xpp* and *Xcf* exhibit high plasticity for acquisition of accessory genes.

Analysis of the *Xcf* genomes revealed that the pathovar pangenome was comprised of 3,252 core genes and 5,482 accessory genes, totaling 8,734 genes (Figure 1). In this analysis, a deep reduction in the number of core genes was observed until the addition of five genomes with little reduction in the number of genes afterwards. Conversely, the gain in accessory genes remained at a high rate even until addition of the last genome. On the other hand, when the *Xpp* genomes were analyzed, the pangenome consisted of 2,531 core genes and 7,309 accessory genes, summing 9,840 genes in total (Figure 2). In this case, a deep reduction in the number of core genes was observed until the addition of nine genomes while the number of accessory genes continued to grow at a stably rate until all genomes were added.

The pangenomes of *Xpp* and *Xcf* do not exhibit geographic differentiation.

The analysis of the pangenomes revealed clustering patterns among *Xcf* and *Xpp* isolates. In the *Xcf* dendrogram four clusters were formed, with isolates from the

same country spread among different clusters, for instance, those collected in Brazil, Canada, and Réunion Island (Figure 3). In contrast, the pangenomes of the *Xpp* isolates were separated into three clusters, the largest of which comprised isolates from several different countries, another cluster comprised isolates from Sudan and Zimbabwe, and the last one contained isolates from the United Kingdom and Réunion Island. Only the isolates from Sudan and the United Kingdom clustered together by geographic origin in the dendrogram (Figure 4).

Large sets of *Xpp* and *Xcf* genes are putatively activated by HrpG and HrpX.

The search for *hrp* box sequences within the promoter regions of 35 *Xcf* genome sequences, indicative of genes potentially activated by HrpG and HrpX, identified 51 genes. Among these, 10 code for structural components of the T3SS apparatus, 5 code for T3SEs, 2 code for T3SS-associated components, and 34 are genes that have not yet been characterized (Table 1). When analyzing 36 *Xpp* genome sequences, 49 genes were found, including 9 coding for structural components of the T3SS apparatus, 7 coding for T3SEs, 4 coding for T3SS-associated components, and 29 genes that have not previously been characterized (Table 2).

Xcf* and *Xpp* share T3SE candidates between themselves and with *Pseudomonas.

Comparison of the predicted effector repertoires revealed the presence of a total of 33 and 38 T3SE candidates in *Xcf* and *Xpp*, respectively. When the datasets for the two pathovars were combined, 39 distinct effectors were found in the set of isolates analyzed (Figure 5). Thirty-two effector candidates were found in both *Xcf* and *Xpp*. Only one effector candidate (XopAL2) was exclusively found in *Xcf* whereas six effector candidates were found only in *Xpp* (XopAG2, XopAI2, XopAO, XopAW, XopS, and XopBA).

When the genome sequences of the *Xanthomonas* pathogenic to common bean were interrogated for the presence of T3SE families previously described in phytopathogenic *Pseudomonas*, seven effector families were found (Table 4). Of these common effectors, five (HopQ, HopR1, HopAF1, HopAO2, and HopH1) were found in both *Xcf* and *Xpp* while HopX2 was found in *Xcf* only and HopG1 was found in *Xpp* only. Some of the effectors identified in *Xcf* and/or *Xpp* show significant sequence similarity with effectors previously described in *Xanthomonas* that cause disease in

other commercially important plant species, such as pepper, eggplant, radish and tomato (Table 3).

4.4. DISCUSSION

In this study, pangenomic analyses, using 35 genome sequences of *Xcf* and 36 of *Xpp*, revealed significant genome plasticity within the two pathovars. In the dendrogram of each pathovar generated based on gene presence/absence, distinct clusters were formed but a clear separation of isolates according to their geographic origins was not evident; isolates from the same country were spread among different clusters in the dendrograms. A higher variability, as indicated by the separation of isolates in a larger number of clusters, was evident for *Xcf*. In addition, most *Xpp* isolates were grouped in the same cluster. These observations are not consistent with reports indicating higher *Xpp* genetic diversity when compared with that of *Xcf* (MUTLU *et al*., 2008; MONTEIRO *et al.,* 2020).

In recent years, a significant amount of whole-genome sequencing data has become available, shedding light on the genetic diversity among various strains of *Xanthomonas* species and established a strong foundation for delving deeper in the diversity analysis within the genus (TIMILSINA *et al.,* 2020). In this investigation, the genetic richness of *Xcf* and *Xpp* was delved by conducting a pangenomic analysis of 71 *Xanthomonas* isolates responsible for infecting common bean. During the analysis, it was found that the genome of *Xcf* and *Xpp* contain 3,252 and 2,531 core genes, respectively. The results suggest that the core genomes of *Xcf* and *Xpp* are almost closed, as indicated by the little loss in the number of core genes when adding additional genome sequences. However, further sampling is still required in order to achieve the complete closure of their core genomes. The pangenomic analysis also indicated significant *Xcf* and *Xpp* genome plasticity, suggesting a high frequency of gene gain and loss during evolution. Consistently, recurrent recombination events have been noted in several *X. citri*, and *X. phaseoli* species (AGARWAL *et al.,* 2023; HUANG *et al.,* 2015; TIMILSINA *et al.,* 2015).

The genomic plasticity observed in this study is also in line with the genetic diversity of the pathovars of the *Xanthomonas* species that affect common bean. For instance, examining the genetic diversity within a collection of isolates from various

locations, Alavi *et al*. (2008) observed substantial genetic diversity in *X. axonopodis* pv. *phaseoli* (synonym *Xpp*), based on fluorescent amplified fragment length polymorphism (F-AFLP), leading to the categorization of isolates into three distinct genetic lineages, with those of *X. fuscans* pv. *fuscans* (synonym *Xcf*) forming a separate cluster from *Xpp*. Characteristics such as brown pigmentation and virulence may be encoded in accessory genes that could be transmitted by horizontal gene transfer, which has already been shown to occur among *Xpp* and *Xcf* strains (CHEN *et al*., 2018). Acquisition of virulence-associated genes could facilitate rapid host range shifts and promote the convergence of genetically distant strains toward a common host (ESCALON *et al.,* 2013).

It is known that some *Xanthomonas* genes coding for virulence factors, such as those coding for the T3SS apparatus, T3SEs and proteins that aid in the secretion process, depend on HrpG and HrpX for *in planta* activation (TEPER *et al.,* 2021). When searching for *hrp* boxes in the genome sequences of 35 *Xcf* and 36 *Xpp* isolates, genes putatively activated by HrpG and HrpX coding for all three aforementioned biological functions were identified. The nature and number of Hrp induced genes identified here are consistent with similar bioinformatics predictions conducted for other phytopathogenic bacteria of the genera *Pseudomonas* (ALVES *et al*., 2021), *Erwinia* (ALVES *et al*., 2022), and *Xanthomonas* (KOEBNIK *et al*., 2006; ZOU *et al*., 2006; TEPER *et al*., 2016).

Among the T3SS structural components, HrcP was found exclusively in *Xpp*, although its biological function has not yet been demonstrated. As for T3SEs, only two of them, XopAK and XopQ, are exclusive to *Xpp* and have been associated with host specificity and virulence in *Xanthomonas* species (TEPER *et al*., 2016; JIANG *et al*., 2009). Among the proteins that facilitate effector secretion, HpaA forms a pilus for secretion and translocation of effector proteins (LORENZ *et al.,* 2008), and XopA is a harpin that has been reported to limit the growth of *X. euvesicatoria* pv*. perforans* isolates (RYBAK *et al*., 2009). These results confirm the validity of the prediction approach utilized in this study. On the other hand, sets of uncharacterized genes of each pathovar were also retrieved by the bioinformatics pipeline employed, suggesting virulence factors unrelated to the T3SS are also induced by HrpX and HrpG or that novel T3SEs were identified.

The effectors injected by the T3SS into the plant cells can either activate (in the case of Avrs) or inhibit (in the case of non Avrs) signal transduction pathways related to plant defense (MEDINA *et al.,* 2018). This can result in the induction of disease resistance or the promotion of disease symptoms upon altering the plant physiology to favor pathogen development (RODRÍGUEZ-PUERTO *et al.,* 2022). During the evolution of their pathogenicity, a specific bacterial species or pathovar may develop its unique set of T3SEs (LIAO *et al.,* 2020). Here, when examining the T3SE repertoire of the set of *Xcf* and *Xpp* isolates investigated, a total of 39 effectors were identified, 32 of them being shared between the two common bean pathogens. This effector conservation suggests at least one or a few of these common effectors may be essential for pathogenicity towards common bean. The large number of effectors shared between the two bacterial pathogens may reflect functional redundancy, which has already been demonstrated for phytopathogenic *Pseudomonas* (BADEL *et al*. 2006, KVITKO *et al*., 2009). On the other hand, effector XopAL2, exclusively identified in *Xcf*, has been reported to be associated with disease development in *X. campestris* pv. *campestris* (GUY *et al.,* 2013). Also, the six effectors, XopAG2, XopAI2, XopAO, XopAW, XopS, and XopBA uniquely found in *Xpp*, have been shown to play roles in target protein recognition, Pathogen-Associated Molecular Pattern (PAMP)-Triggered Immunity (PTI) suppression, and stomatal closure suppression (LIU *et al.,* 2019; MEDINA *et al.,* 2018; POPOV *et al.,* 2018; RAFFEINER *et al.,* 2022). The lack of these effectors in either *Xcf* or *Xpp* suggests they are dispensable for pathogenicity.

Studies have shown that conservation of T3SE families occurs among distantly related bacterial species (WASHINGTON *et al*., 2016). When comparing the repertoire of *Xcf* and *Xpp* predicted T3SEs with those previously described for *Pseudomonas*, effectors from both common bean pathogens shared significant similarity with the effectors HopQ, HopR1, HopAF1, HopAO2, and HopH1. Among these, HopQ is conserved across multiple bacterial plant pathogens and plays a role in promoting virulence in *Pseudomonas syringae* pv. *tomato* (LI *et al*., 2013). HopAF1 has previously been identified in *Xanthomonas campestris* pv. *campestris* and several *Pseudomonas* species, where it suppresses plant immunity by blocking the induction of ethylene production (WASHINGTON *et al*., 2016; POTNIS *et al*., 2011). HopAO2 has been reported in *P. syringae* pv. *tomato* and *Pseudomonas savastanoi* pv. *savastanoi* and shown to inhibit the production of reactive oxygen species (ROS) and callose

deposition (CASTAÑEDA-OJEDA *et al*., 2017). Interestingly, only a sequence from *Xcf* was found significantly similar to the *Pseudomonas* effector HopX2. Conversely, only a sequence from *Xpp* showed significant similarity to the *Pseudomonas* effector HopG1. HopX2 has been shown to share a 69% sequence similarity with XopE1, which plays a pivotal role in chlorosis development elicited by *Xanthomonas euvesicatoria* on tomato plants (DUBROW *et al*., 2018; THIEME *et al*., 2007). In *P. syringae* pv. *tomato*, HopG1 suppresses plant defense responses and promotes disease symptom development in *Arabidopsis thaliana* (RODRÍGUEZ-PUERTO *et al*., 2022). The conservation of T3SE families in other phytopathogenic *Xanthomonas* and *Pseudomonas* suggests the *Xanthomonas* pathogenic to common bean may target functions conserved in other plant species.

Few studies have comprehensively explored the effector repertoire of *Xanthomonas* causing CBB. In general, most studies have focused on the identification of only 10 T3SE (ALAVI *et al*., 2008; PAIVA *et al*., 2021; TUGUME *et al*., 2019). These studies have primarily utilized PCR for amplification of effector sequences, which not only limits the discovery of novel effectors but also fails to fully explain the true effector diversity within collections of pathogenic strains. This limitation hinders our understanding of how *Xcf* and *Xpp* effectively manipulate the immune system of common bean to promote disease. The in-depth exploration of *Xcf* and *Xpp* T3SE repertoires in our study, and the description of their commonalities and differences provides clearer insights into the identification of key effectors that enable a bacterium to cause disease in common bean.

4.5. REFERENCES

AGARWAL, V.; STUBITS, R.; NASSRULLAH, Z.; DILLON, M. M. Pangenome insights into the diversification and disease specificity of worldwide *Xanthomonas* outbreaks. Frontiers in Microbiology 14:1-18. 2023.

ALAVI, S. M.; SANJARI, S.; DURAND, F.; BRIN, C.; MANCEAU, C.; POUSSIER, S. Assessment of the genetic diversity of *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas fuscans* subsp. *fuscans* as a basis to identify putative pathogenicity genes and a type III secretion system of the SPI-1 family by multiple suppression substrative hybridizations. Applied and Environmental Microbiology 74:3295-3301. 2008.

ALTSCHUL, S. F.; GISH, W.; MILLER, W.; MYERS, E. W.; LIPMAN, D. J. Basic local alignment search tool. Journal of Molecular Biology 215:403-410. 1990.

ALVES, F. H. N. S.; MONTEIRO, A. L. R.; PEREIRA, I. C.; SILVA, J. C. F.; ALMEIDA, R. N. D.; McTAVISH, K. J.; GUTTMAN, D. S.; RAIMUNDI, M. K.; SOUZA, R. M.; RESENDE, M. L. V.; BADEL, J. L. Genomic variability and plasticity of *Pseudomonas* causing coffee leaf spots in Minas Gerais state, Brazil. Plant Pathology 71:934-948. 2021.

ALVES, F. H. N. S.; PEREIRA, I. C.; SANTOS, S. A.; MONTEIRO, A. L. R.; VIDIGAL, P. M. P.; ALFENAS, A. C.; BADEL, J. L. Comprehensive prediction of plant cytoplasmic and apoplastic effectors underlying *Erwinia psidii* pathogenicity. Plant Pathology 72:130-143. 2022.

AN, S-Q.; POTNIS, N.; DOW, M.; VORHÖLTER, F-J.; HE, Y-Q.; BECKER, A.; TEPER, D.; LI, Y.; WANG, N.; BLERIS, L.; TANG, J-L. Mechanistic insights into host adaptation, virulence and epidemiology of the phytopathogen *Xanthomonas*. FEMS Microbiology Reviews 44:1-32. 2019.

AZARIAN, T.; HUANG, I-T.; HANAGE, W. P. Structure and dynamics of bacterial populations: Pangenome ecology. In: TETTELIN, H., MEDINI, D. (eds) The Pangenome. Springer. 115-128. 2020.

BADEL, J. L, SHIMIZU, R.; OH, H-S.; COLLMER, A. A *Pseudomonas syringae* pv. *tomato avrE1/hopM1* mutant is severely reduced in growth and lesion formation in tomato. Molecular Plant-Microbe Interactions 19:99-111. 2006.

BARDOU, P.; MARIETTE, J.; ESCUDIE, F.; DJEMIEL, C.; KLOPP, C. Jvenn: An interactive Venn diagram viewer. BMC Bioinformatics 15:293. 2014.

BONAS, U.; SCHULTE, R.; FENSELAU, S.; MINSAVAGE, G. V.; STASKAWICZ, B. J.; STALL, R. E. Isolation of a gene cluster from *Xanthomonas campestris* pv. *vesicatoria* that determines pathogenicity and the hypersensitive response on pepper and tomato. Molecular Plant-Microbe Interactions 4:81-88. 1991.

CASTAÑEDA-OJEDA, M. P.; MORENO-PÉREZ, A.; RAMOS, C.; LÓPEZ-SOLANILLA, E. L. Suppression of plant immune response by the *Pseudomonas savastanoi* pv. *savastanoi* NCPPB 3335 type III effector tyrosine phosphatases HopAO1 and HopAO2. Frontiers in Plant Science 8:1-15. 2017.

CHEN, N. W. G.; SERRES-GIARDI, L.; RUH, M.; BRIAND, M. BONNEAU, S.; DARRASSE, A.; BARBE, V.; GAGNEVIN, L.; KOEBNIK, R.; JACQUES, M-A. Horizontal gene transfer plays a major role in the pathological convergence of *Xanthomonas* lineages on common bean. BMC Genomics 19:1-20. 2018.

COSTA, J, POTHIER, J. F.; BOCH, J.; STEFANI, E.; JACQUES, M. A.; CATARA, V.; KOEBNIK, R. Integrating science on Xanthomonadaceae for sustainable plant disease management in Europe. Molecular Plant Pathology 22:1461-1463. 2021.

COSTA, J, POTHIER, J. F.; BOCH, J.; STEFANI, E.; KOEBNIK, R. Integrating science on *Xanthomonas* and *Xylella* for integrated plant disease management. Microorganisms 11:6 2022.

DE PAIVA, B. A. R.; WENDLAND, A.; TEIXEIRA, N. C.; FERREIRA, M. A. S. V. Rapid detection of *Xanthomonas citri* pv. *fuscans* and *Xanthomonas phaseoli* pv. *phaseoli* in common bean by loop-mediated isothermal amplification. Plant Disease 104:198-203. 2020.

DUBROW, Z.; SUNITHA, S.; KIM, J-G.; AAKRE, C. D.; GIRIJA, A. M.; SOBOL, G.; TEPER, D.; CHEN, Y. C.; OZBAKI-YAGAN, N.; VANCE, H.; SESSA, G.; MUDGETT, M. B. Tomato 14-3-3 proteins are required for Xv3 disease resistance and interact with a subset of *Xanthomonas euvesicatoria* effectors. Molecular Plant-Microbe Interactions 12:1301-1311. 2018.

ESCALON, A.; JAVEGNY, S.; VERNIÈRE, C.; NOËL, L. D., VITAL, K.; POUSSIER, S.; HAJRI, A.; BOUBEAU, T.; PRUVOST, O.; ARLAT, M.; GAGNEVIN, L. Variations in type III effector repertoires, pathological phenotypes and host range of *Xanthomonas citri* pv. *citri* pathotypes. Molecular Plant Pathology 14:483-496. 2013.

FOURIE, D.; HERSELMAN, L. Pathogenic and genetic variation in *Xanthomonas axonopodis* pv. *phaseoli* and its fuscans variant in Southern Africa. African Crop Science Journal 19:393-407. 2011.

GILBERTSON, R. L.; MAXWELL, D. P. Common blight of bean. In: CHAUBE, H. S.; SINGH, U. S., MUKHOPADHYAY, A. N. Plant diseases of international importance v2. Inglewood Cliffs. Prentice Hall. 18-39. 1992.

GUY, E.; GENISSEL, A.; HAJRI, A.; CHABANNES, M.; DAVID, P.; CARRERE, S.; LAUTIER, M.; ROUX, B.; BOUREAU, T.; ARLAT, M.; POSSIER, S.; NÖEL, L. D. Natural genetic variation of *Xanthomonas campestris* pv. *campestris* pathogenicity on *Arabidopsis* revealed by association and reverse genetics. mBio 4:1-13. 2013.

HUANG, C-L.; PU, P-H.; HUANG, H-J., SUNG, H-M.; LIAW, H-J, CHEN, Y-M, CHEN, C-M.; HUANG, M-B.; OSADA, N.; GOJOBORI, T.; PAI, T-W.; CHEN, Y-T.; HWANG, C-C.; CHIANG, T-Y. Ecological genomics in *Xanthomonas*: the nature of genetic adaptation with homologous recombination and host shifts. BMC Genomics 16:188. 2015.

JACQUES, M-A.; ARLAT, M.; BOULANGER, A.; BOUREAU, T.; CARRÈRE, S.; CESBRON, S.; CHEN, N. W. G.; COCIANCICH, S.; DARRASSE, A.; DENANCÉ, N.; SAUX, M. F-L.; GAGNEVIN, L.; KOEBNIK, R.; LAUBER, E.; NOËL, L. D.; PIERETTI, I.; PORTIER, P.; PRUVOST, O.; RIEUX, A.; ROBÈNE, I.; ROYER, M.; SZUREK, B.; VERDIER, V.; VERNIÈRE, C. Using ecology, physiology, and genomics to understand host specificity in *Xanthomonas*. Annual Review of Phytopathology. 54:163-187. 2016.

JACKSON, R. W.; JOHNSON, L. J.; CLARKE, S. R.; ARNOLD, D. L. Bacterial pathogen evolution: Breaking news. Trends in Genetics 27:32-40. 2010.

JIANG, W.; JIANG, B-L.; XU, R-Q.; HUANG, J-D.; WEI, H-Y., JIANG, G-F.; CEN, W-J.; LIU, J.; GE, Y-Y.; LI, G-H.; SU, L-L.; HANG, X-H.; TANG, D-J.; LU, G-T.; FENG, J-X.; HE, Y-Q.; TANG, J-L. Identification of six type III effector genes with the PIP box in *Xanthomonas campestris* pv. *campestris* and five of them contribute individually to full pathogenicity. Molecular Plant-Microbe Interactions 22:1401-1411. 2009.

KAY, S.; BONAS, U. How *Xanthomonas* type III effectors manipulate the host plant. Current Opinion in Microbiology 12:37-43. 2009.

KOEBNIK, R.; KRÜGER, A.; THIEME, F.; URBAN, A.; BONAS, U. Specific binding of the *Xanthomonas campestris* pv. *vesicatoria* AraC-type transcriptional activator HrpX to plant-inducible promoter boxes. Journal of Bacteriology 188:7652-7660. 2006.

KVITKO, B. H.; COLLMER, A. Discovery of the Hrp Type III secretion system in phytopathogenic bacteria: How investigation of hypersensitive cell death in plants led to a novel protein injector system and a world of inter-organismal molecular interactions within plant cells. Phytopathology 113:626-636. 2023.

KVITKO, B. H.; PARK, D. H.; VELÀSQUEZ, A. C.; WEI, C-F.; RUSSELL, A. B.; MARTIN, G. B.; SCHNEIDER, D. J.; COLLMER, A. Deletions in the repertoire of *Pseudomonas syringae* pv. *tomato* DC3000 type III secretion effector genes reveal functional overlap among effectors. PLOS Pathogens 5:1-16. 2009.

LASSALLE, F.; DIDELOT, X. Bacterial microevolution and the pangenome. In: TETTELIN, H., MEDINI, D. (eds) The Pangenome. Springer. 129-149. 2020.

LI, W.; YADETA, K. A.; ELMORE, J. M.; COAKER, G. The *Pseudomonas syringae* effector HopQ1 promotes bacterial virulence and interacts with tomato 14-3-3 proteins in a phosphorylation-dependent manner. Plant Physiology 161:2062-2074. 2013.

LIAO, Z-Y.; LI, J-Y., MO, X-Y.; NI, Z.; JIANG, W.; HE, Y-Q.; HUANG, S. Type III effectors *xopN* and *avrBs2* contribute to the virulence of *Xanthomonas oryzae* pv. *oryzicola* strain GX01. Research in Microbiology 171:102-106. 2020.

LIU, J-H., YANG, J-Y.; HSU, D-W., LAI, Y-H., LI, Y-P.; TSAI, Y-R., HOU, M-H. Crystal structure-based exploration of arginine-containing peptide binding in the ADP-ribosyltranferase domain of the type III effector XopAI protein. International Journal of Molecular Sciences 20:1-21. 2019.

LORENZ, C.; KIRCHNER, O.; EGLER, M.; STUTTMANN, J.; BONAS, U.; BÜTTNER, D. HpaA from *Xanthomonas* is a regulator of type III secretion. Molecular Microbiology 69:344-360. 2008.

MEDINA, C. A.; REYES, P. A.; TRUJILLO, C. A.; GONZALEZ, J. L.; BEJARANO, D. A.; MONTENEGRO, N. A.; JACOBS, J. M.; JOE, A.; RESTREPO, S.; ALFANO, J. R.; BERNAL, A. The role of type III effectors from *Xanthomonas axonopodis* pv. *manihotis* in virulence and suppression of plant immunity. Molecular Plant Pathology 19:593-606. 2018.

MONTEIRO, A. L. R.; CHAVES, F. S.; PANTALEÃO, A. S. L.; CARNEIRO, P. C. S.; CARNEIRO, J. E. S.; BADEL, J. L. Sources, spectrum, genetics, and inheritance of

Phaseolus vulgaris resistance against *Xanthomonas citri* pv. *fuscans*. Phytopathology 110:1428-1436, 2020.

MONTEIRO, A. L. R; PANTALEÃO, A. S. L.; BADEL, J. L.; SOARES, P. H. M.; CARNEIRO, V. Q.; CARNEIRO, P. C. S.; CARNEIRO, J. E. S. Genome-wide association study (GWAS) of *Phaseolus vulgaris* resistance to *Xanthomonas citri* pv. *fuscans*. Plant Pathology 70:1733-1744, 2021.

PAIVA, B. A. R.; WENDLAND, A.; ROSSATO, M.; FERREIRA, M. A. S. V. Virulence and type III effector diversities of *Xanthomonas citri* pv. *fuscans* and *X. phaseoli* pv. *phaseoli* in Brazil. Journal of Phytopathology 170:1-14. 2021.

POPOV, G.; MAJHI, B. B.; SESSA, G. Effector gene *xopAE* of *Xanthomonas euvesicatoria* 85-10 is part of an operon and encodes an E3 ubiquitin ligase. Journal of Bacteriology 200:1-19. 2018.

POTNIS, N.; KRASILEVA, K.; CHOW, V.; ALMEIDA, N. F.; PATIL, P. B.; RYAN, R. P.; SHARLACH, M.; BEHLAU, F.; DOW, J. M.; MOMOL, MT.; WHITE, F. F.; PRESTON, J. F.; VINATZER, B. A.; KOEBNIK, R.; SETUBAL, J. C.; NORMAN, D. J.; STASKAWICZ, B. J.; JONES, J. B. Comparative genomics reveals diversity among xanthomonads infecting tomato and pepper. BMC Genomics 12:146. 2011.

RAFFEINER, M.; ÜSTÜN, S.; GUERRA, T.; SPINTI, D.; FITZNER, M.; SONNEWALD, S.; BALDERMANN, S.; BÖRNKE, F. The *Xanthomonas* type-III effector XopS stabilizes CaWRKY40a to regulate defense responses and stomatal immunity in pepper (*Capsicum annuum*). Plant Cell 34:1684-1708. 2022.

RASKO, D. A.; ROSOVITZ, M. J.; MYERS, G. S. A.; MONGODIN, E. F.; FRICKE, W. F.; GAJER, P.; CRABTREE, J.; SEBAIHIA, M.; THOMSON, N. R.; CHAUDHURI, R.; HENDERSON, I. R.; SPERANDIO, V.; RAVEL, J. The pangenome structure of *Escherichia coli*: Comparative genomic analysis of *E. coli* commensal and pathogenic isolates. Journal of Bacteriology 190:6881-6893. 2008.

R DEVELOPMENT CORE TEAM. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. 2018.

RODRÍGUEZ-PUERTO, C.; CHAKRABORTY, R.; SINGH, R.; ROCHA-LOYOLA, P.; ROJAS, C. M. The *Pseudomonas syringae* type III effector HopG1 triggers necrotic cell death that is attenuated by AtNHR2B. Scientific Reports 12:1-14. 2022.

RYAN, R. P.; VORHÖLTER, F-J, POTNIS, N.; JONES, J. B.; SLUYS, M-A, V.; BOGDANOVE, A. J.; DOW, J. M. Pathogenomics of *Xanthomonas*: Understanding bacterium-plant interactions. Nature Reviews Microbiology 9:344-355. 2011.

RYBAK. M.; MINSAVAGE, G. V.; STALL, R. E.; JONES, J. B. Identification of *Xanthomonas citri* sp. *citri* host specificity genes in a heterologous expression host. Molecular Plant Pathology 10:249-262. 2009.

TEPER, D.; BURSTEIN, D.; SALOMON, D.; GERSHOVITZ, M.; PUPKO, TAL.; SESSA, G.; Identification of novel *Xanthomonas euvesicatoria* type III effector proteins by a machine-learning approach. Molecular Plant Pathology 17:398-411. 2016.

TEPER, D.; PANDEY, S. S.; WANG, N. The HrpG/HrpX regulon of *Xanthomonads* - an insight to the complexity of regulation of virulence traits in phytopathogenic bacteria. Microorganisms 9:1-16. 2021.

THIEME, F.; SZCZESNY, R.; URBAN, A.; KIRCHNER, O.; HAUSE, G.; BONAS, U. New Type III Effectors from *Xanthomonas campestris* pv. *vesicatoria* trigger plant reactions dependent on a conserved N-myristoylation motif. Molecular Plant-Microbe Interactions 20:1250-1261. 2007.

TIMILSINA, S.; JIBRIN, M. O.; POTNIS, N.; MINSAVAGE, G. V.; KEBEDE, M.; SCHWARTZ, A.; BART, R.; STASKAWICZ, B.; BOYER, C.; VALLAD, G. E.; PRUVOST, O.; JONES, J. B.; GOSS, E. M. Multilocus sequence analysis of xanthomonads causing bacterial spot of tomato and pepper plants reveals strains generated by recombination among species and recent global spread of *Xanthomonas gardneri*. Applied and Environmental Microbiology 81:1520-1529. 2015.

TIMILSINA, S.; POTNIS, N.; NEWBERRY, E. A.; LIYANAPATHIRANAGE, IRUEGAS-BOCARDO, F.; WHITE, F. F.; GOSS, E. M.; JONES, J. B. *Xanthomonas* diversity, virulence and plant-pathogen interactions. Nature Reviews Microbiology 18:415-427. 2020.

TUGUME, J. K.; TUSIIME, G.; SEKAMATE, A. M.; BURUCHARA, R.; MUKANKUSI, C. M. Diversity and interaction of common bacterial blight disease-causing bacteria (*Xanthomonas* spp.) with *Phaseolus vulgaris* L. Crop Journal 7:1-7. 2019.

WASHINGTON, E. J.; MUKHTAR, M. S.; FINKEL, O. M.; WAN, LI.; BANFIELD, M. J.; KLEBER, J. J.; DANGL, J. L. *Pseudomonas syringae* type III effector HopAF1 suppress plant immunity by targeting methionine recycling to block ethylene induction. Proceedings of the National Academy of Sciences U. S. A. 113:3577-3586. 2016.

WHITE, F. F.; POTNIS, N.; JONES, J. B.; KOEBNIK, R. The type III effectors of *Xanthomonas*. Molecular Plant Pathology 10:749-766. 2009.

YANG, P.; CHANG, Y.; WANG, L.; WANG, S.; WU, J. Regulatory mechanisms of the resistance to common bacterial blight revealed by transcriptomic analysis in common bean (*Phaseolus vulgaris* L.). Frontiers in Plant Science 12:1-12. 2022.

YOUNG, J. M.; SADDLER, G. S.; TAKIAWA, Y.; DE BOER, S. H.; VOUTERIN, L.; GARDAN, L.; GVOZDYAK, R. I.; STEAD, D.E. Names of plant pathogenic bacteria. Review of Plant Pathology 75:721-763. 1996.

YU, K.; PARK, S. J.; POYSA, V. Marker-assisted selection of common beans for resistance to common bacterial blight: Efficacy and economics. Plant Breeding 119:411-415. 2000.

ZOU, L-F.; WANG, X-P.; XIANG, Y.; ZHANG, B.; LI, Y-R.; XIAO, Y-L.; WANG, J-S.; WALMSLEY, A. R.; CHEN, G. Elucidation of the *hrp* clusters of *Xanthomonas oryzae* pv. *oryzicola* that control the hypersensitive response in nonhost tobacco and pathogenicity in susceptible host rice. Applied and Environmental Microbiology 72: 6212-6224. 2006.

4.6. TABLES

Table 1. Genes predicted to be activated by HrpG and HrpX identified in *Xanthomonas citri* pv. *fuscans* genome sequences using a customized Python script.

Gene	Isolate	Genome sequence annotation	Blast Annotation
T3SS structural components			
WP_007965380.1	ISO118C5	Type III secretion inner membrane ring lipoprotein SctJ	HrcJ
WP_099800979.1	BRM48906	Type III secretion system protein SctP	HrcP
WP_033481717.1	ISO118C5	Type III secretion system cytoplasmic ring protein SctQ	HrcQ
WP_022558048.1	ISO118C5	Type III secretion system export apparatus subunit SctR	HrcR
WP_008575435.1	ISO118C5	Type III secretion system export apparatus SctS	HrcS
WP_099800980.1	BRM48906	Type III secretion system export apparatus subunit SctU	HrcU
WP_007965389.1	BRM48906	FHIPEP family type III secretion protein	HrcV
WP_022558052.1	ISO118C5	HrpB1 family type III secretion system apparatus protein	HrpB1
WP_007965382.1	ISO118C5	Type III secretion protein HrpB2	HrpB2
WP_022558053.1	ISO118C5	Type III secretion protein HrpB4	HrpB4
Type III effector genes			

WP_080949418.1	ISO118C5	XopAF/AvrXv3 family type III secretion system effector	XopAF
WP_022559897.1	ISO118C5	Hypothetical protein	XopAM
WP_089142678.1	NCPPB 1654	XopE/AvrPphe family type III secretion system	XopE
WP_089142623.1	NCPPB 1654	Type III secretion system YopJ family effector AvrXv4	XopJ
WP_022557945.1	ISO118C5	Type III secretion system effector protein XopR	XopR
T3SS-associated components			
WP_099801088.1	BRM48906	Hypothetical protein	XopB
WP_033481231.1	ISO118C5	Hypothetical protein	XopA
Uncharacterized genes			
WP_022558828.1	NCPPB 1056	Amino acid permease	Amino acid permease
WP_022558093.1	ISO118C5	Anthranilate synthase component I	Anthranilate synthase
WP_022560380.1	CFBP4884	tRNA 2-thiocytidine(32) synthetase TtcA	ATPase
WP_022557995.1	NCPPB 2665	Barstar family protein	Barstar family protein
WP_033481230.1	ISO118C5	Beta-eliminating lyase-related protein	Beta-eliminating lyase-related protein
WP_022560374.1	CFBP6996	BPSS1780 family membrane protein	BPSS1780 family membrane protein
WP_007971730.1	NCPPB 1056	Dipeptidase	Dipeptidase
WP_033481904.1	NCPPB 1056	DNA-binding protein	DNA-binding protein
WP_022559584.1	CFBP6960	DUF488 domain-containing protein	DUF488 domain-containing protein

WP_022559419.1	ISO118C5	Family 43 glycosylhydrolase	Family 43 glycosylhydrolase
WP_017159753.1	CFBP6996	Glutamine amidotransferase	Glutamine amidotransferase
WP_022558224.1	ISO118C5	Glycosyl hydrolase family 28 protein	Glycosyl hydrolase
WP_089141025.1	NCPPB 1654	HAMP domain histidine kinase	HAMP domain histidine kinase
WP_234710372.1	ISO118C5	Hypothetical protein	Hypothetical protein
WP_017158792.1	ISO118C5	Hypothetical protein	Hypothetical protein
WP_234832140.1	ISO118C5	Hypothetical protein	Hypothetical protein
WP_022559301.1	ISO118C5	Hypothetical protein	Hypothetical protein
WP_029996228.1	ISO118C5	Hypothetical protein	Hypothetical protein
WP_099801076.1	CFBP 6988	Hypothetical protein	Hypothetical protein
WP_162836986.1	CFBP6960	Hypothetical protein	Hypothetical protein
WP_046121237.1	NCPPB 1056	Hypothetical protein	Hypothetical protein
WP_157768205.1	ISO118C5	Hypothetical protein	Hypothetical protein
WP_022559420.1	ISO118C5	Ig-like domain-containing protein	Ig-like domain-containing protein
WP_007975311.1	ISO118C5	ISXfu2 family transposase	ISXfu2 family transposase
WP_022558225.1	ISO118C5	Lytic murein transglycosylase B	Lytic murein transglycosylase B
WP_099801090.1	BRM48906	MnmC family methyltransferase	MnmC family methyltransferase
WP_022559302.1	ISO118C5	Pectate lyase	Pectate lyase

WP_022559304.1	ISO118C5	Glycosyl hydrolase family 28 protein	Polygalacturonase
WP_007963324.1	CFBP4884	Recombination-associated protein RdgC	`Recombination-associated protein
WP_234705899.1	NCPPB 3660	Restriction endonuclease	Restriction endonuclease
WP_033481660.1	ISO118C5	S9 family peptidase	S9 family peptidase
WP_033480905.1	ISO118C5	Transglycosylase RlpA family protein	Transglycosylase RlpA family protein
WP_022559447.1	XCP631	Hypothetical protein	Site-specific integrase
WP_162808331.1	BRM48906	TldD/PmbA family protein	TldD/PmbA family protein

Table 2. Genes predicted to be activated by HrpG and HrpX identified in *Xanthomonas phaseoli* pv. *phaseoli* genome sequences using a customized Python script.

Gene	Isolate	Genome sequence annotation	Blast Annotation
T3SS structural components			
WP_099800981.1	CFBP6991	Type III secretion inner membrane ring lipoprotein SctJ	HrcJ
WP_039567144.1	CFBP6164	Type III secretion system cytoplasmic ring protein SctQ	HrcQ
WP_003485742.1	CFBP6164	Type III secretion system export apparatus SctR	HrcR
WP_008575435.1	CFBP6164	Type III secretion system export apparatus SctS	HrcS
WP_039567146.1	CFBP6164	Type III secretion system export apparatus subunit SctU	HrcU
WP_046736071.1	CFBP6164	FHIPEP family type III secretion protein	HrcV
WP_007965384.1	CFBP6991	HrpB1 family type III secretion system apparatus protein	HrpB1
WP_007965382.1	CFBP6991	Type III secretion protein HrpB2	HrpB2
WP_099800982.1	CFBP6991	Type III secretion protein HrpB4	HrpB4
T3SS-associated components			
WP_039567163.1	CFBP6164	XopA/Hpa1	XopA
WP_039567143.1	NCPPB1811	Hypothetical protein	HpaA
WP_039567145.1	CFBP6164	Type III secretion system protein SctP	HpaP

WP_039567546.1	BB075a	Hypothetical protein	XopB
Type III effector genes			
WP_080949026.1	CFBP6164	XopAF/AvrXv3	XopAF/AvrXv3
WP_223842551.1	CFBP6164	XopAK family type III secretion system effector	XopAK
WP_039573022.1	CFBP6164	Hypothetical protein	XopAM
WP_236493349.1	CFBP6164	XopE/AvrPphe family type III secretion system effector	XopE
WP_080949173.1	CFBP6164	Type III secretion system YopJ family effector AvrXv4	XopJ
WP_039574546.1	CFBP6164	Type III secretion system effector XopQ	XopQ
WP_269467286.1	CFBP6164	Type III secretion system effector protein XopR	XopR
Uncharacterized genes			
WP_007974891.1	CFBP6991	Anthranilate synthase component I	Anthranilate synthase component I
WP_157860673.1	CFBP6546	Clp protease ClpP	Clp protease ClpP
WP_039576626.1	CFBP6546	Dihydroorotase	Dihydroorotase
WP_017155088.1	CFBP6546	DUF47 family protein	DUF47 family protein
WP_039581597.1	NCPPB2064	DUF4868 domain-containing protein	DUF4868 domain-containing protein
WP_099801929.1	CFBP6991	Family 43 glycosylhydrolase	Family 43 glycosylhydrolase
WP_039571164.1	CFBP6164	Glycosyl hydrolase family 28 protein	Glycosyl hydrolase family 28 protein
WP_099801078.1	CFBP6991	Hypothetical protein	Hypothetical protein

WP_157768394.1	CFBP6991	Hypothetical protein	Hypothetical protein
WP_131701848.1	NCPPB1158	Hypothetical protein	Hypothetical protein
WP_029996228.1	NCPPB556	Hypothetical protein	Hypothetical protein
WP_180301685.1	CFBP6164	Hypothetical protein	Hypothetical protein
WP_099802811.1	CFBP6991	Ig-like domain-containing protein	Ig-like domain-containing protein
WP_204368345.1	NCPPB1713	Integrase -type DNA-binding domain-	Integrase arm-type DNA-binding domain-
WP_099770664.1	CFBP6164	Lytic murein transglycosylase B	Lytic murein transglycosylase B
WP_039576625.1	CFBP6546	M23 family metallopeptidase	M23 family metallopeptidase
WP_046834028.1	CFBP6546	Membrane protein insertion efficiency factor YidD	Membrane protein insertion efficiency factor YidD
WP_080948768.1	CFBP6164	Hypothetical protein	Pectate lyase
WP_174232337.1	CFBP6991	S9 family peptidase	Peptidase
WP_099863750.1	CFBP6991	Phosphatidylserine/phosphatidylglycerophosphate	Phosphatidylserine/phosphatidylglycerophosphate
WP_040150053.1	NCPPB557	Recombinase family protein	Resolvase/recombinase
WP_039582378.1	CFBP6546	S8 family serine peptidase	S8 family serine peptidase
WP_039567602.1	PR1	SAM-dependent methyltransferase	SAM-dependent methyltransferase
WP_052764163.1	NCPPB557	SDR family NAD(P)-dependent oxidoreductase	SDR family NAD(P)-dependent oxidoreductase
WP_039573206.1	CFBP6164	Septal ring lytic transglycosylase RlpA family protein	Septal ring lytic transglycosylase RlpA family protein
WP_011050343.1	NCPPB2064	Beta-eliminating lyase-related protein	Threonine aldolase

WP_046834584.1	NCPPB1138v2	IS256-like element ISXax1 family transposase	Transposase
WP_022557821.1	CFBP6164	IS5-like element ISXfu1 family transposase	Transposase
WP_039581322.1	NCPPB1713	Tryptorubin family RiPP precursor	Tryptorubin family RiPP precursor

Table 3. Repertoire of type III effector candidates of *Xanthomonas* causing Common Bacterial Blight (CBB).

Effector	Function	Pathogen	Host	Reference
AvrBs2	PTI suppression	*Xanthomonas oryzae* pv. *oryzae*	Rice	Liao *et al.,* 2020
AvrBs3	Avirulence function	*Xanthomonas vesicatoria*	Pepper	Bonas *et al*., 1989
XopAD	Disease development	*Ralstonia solanacearum*	Eggplant	Chen *et al.,* 2021
XopAE	Disease symptom enhancement	*Xanthomonas euvesicatoria* pv*. euvesicatoria*	Arabidopsis	Popov *et al.,* 2018
XopAF2	Modulation of gene expression	*X. oryzae* pv. *oryzicola*	Tomato	Büther and Bonas, 2010
XopAG2	Unknown function			
XopAI	Target protein recognition	*Xanthomonas citri* pv *citri*		Liu *et al*., 2019
XopAK	Tissue specificity	*X. euvesicatoria* pv. *euvesicatoria*	Pepper	Teper *et al*., 2016
XopAL2	Disease development	*Xanthomonas campestris* pv. *campestris*	Arabidopsis	Guy *et al* 2013
XopAM	Enhanced virulence	*X. campestris* pv. *campestris*	Arabidopsis	Xie *et al* 2023
XopAO	PTI suppression	*Xanthomonas phaseoli* pv. *manihotis*	Pepper	Medina *et al* 2018
XopAP	Disease development	*X. euvesicatoria*	Pepper	Teper *et al*. 2015
XopAU	Disease symptoms	*X. euvesicatoria*	Pepper	Teper *et al*. 2018
XopAV1	Enhanced virulence	*X. campestris* pv. *campestris*	Radish	Yang *et al* 2015
XopAW	No significant effect	*X. euvesicatoria*	Pepper	Popov *et al*., 2018

XopAZ	Unknown function			
XopBA	Unknown function			
XopC1	Different mechanisms	*X. oryzae* pv. *oryzicola*	Pepper	Herzfeld, 2013
XopC2	Stomatal opening	*X. oryzae* pv. *oryzicola*	Pepper	Wang *et al* 2021
XopE1	Chlorosis	*X. euvesicatoria*	Tomato	Dubrow *et al*., 2018
XopE3	Unknown function			
XopF1	PTI suppression	*X. euvesicatoria*	Arabidopsis	Popov *et al*., 2016
XopG1	Immune response suppression	*X. oryzae* pv. *oryzicola*	Rice	Deb *et al*., 2020
XopH1	Disease symptom development	*X. euvesicatoria*	Arabidopsis	Popov *et al*., 2016
XopI1	Virulence	*X. vesicatoria*	Pepper	Büttner 2006
XopJ2	Callose deposition suppression	*X. vesicatoria*	Tomato	Kim *et al*., 2010
XopJ5	PTI suppression	*X. euvesicatoria*	Arabidopsis	Liu *et al*., 2017
XopK	PTI suppression	*X. oryzae* pv. *oryzae*	Rice	Qin *et al*., 2018
XopL	Cell death elicitation	*X. campestris* pv. *campestris*	*Nicotiana benthamiana*	Ortmann *et al*., 2023
XopM	Unknown function			
XopN	Immune response suppression	*X. oryzae* pv. *oryzicola*	Rice	Liao *et al.,* 2020
XopP	Immune response suppression	*X. oryzae* pv. *oryzicola*	Rice	Deb *et al*., 2020
XopQ	Virulence	*X. campestris* pv. *campestris*	Radish	Jiang *et al*., 2009

XopR	PTI suppression	*X. phaseoli* pv. *manihotis*	Pepper	Medina *et al* 2018
XopS	Stomatal closure inhibition	*X. vesicatoria*	Pepper	Raffeiner *et al*., 2022
XopT	Unknown function			
XopV	Immune response suppression	*X. oryzae* pv. *oryzicola*	Rice	Deb *et al*., 2020
XopX	Bacterial growth promotion	*X. citri* pv *citri*	*N. benthamiana*	Metz *et al*., 2005
XopZ1	PTI interfering	*X. oryzae* pv. *oryzae*	*N. benthamiana*	Song *et al*., 2010

Table 4. Shared effectors between *Xanthomonas* that cause Common Bacterial Blight (CBB) and phytopathogenic *Pseudomonas.*

Pathovar	*Pseudomonas* effector	Coverage (%)	E-value	Identity (%)	Protein ID
Xcf	HopQ	88	6,00E-179	61.4	WP_022560457.1
Xpp	HopQ	85	2,00E-175	62.3	WP_039574546.1
Xcf	HopR1	90	0	51.2	WP_022559897.1
Xpp	HopR1	90	0	51.1	WP_039573022.1
Xcf	HopAF1	99	5,00E-123	61.8	WP_080949418.1
Xpp	HopAF1	99	3,00E-123	61.5	WP_080949026.1
Xcf	HopAO2	77	1,00E-138	67.0	WP_099801749.1
Xpp	HopAO2	77	1,00E-138	67.0	WP_099801749.1
Xcf	HopH1	99	2,00E-66	49.6	WP_022558460.1
Xpp	HopH1	99	6,00E-67	50.0	WP_005997167.1
Xcf	HopX2	100	5,00E-139	63.1	WP_022557953.1
Xpp	HopG1	86	3,00E-146	48.6	WP_197687824.1

Xcf, *Xanthomonas citri* pv. *fuscans*; *Xpp*, *Xanthomonas phaseoli* pv. *phaseoli*

4.7. FIGURES

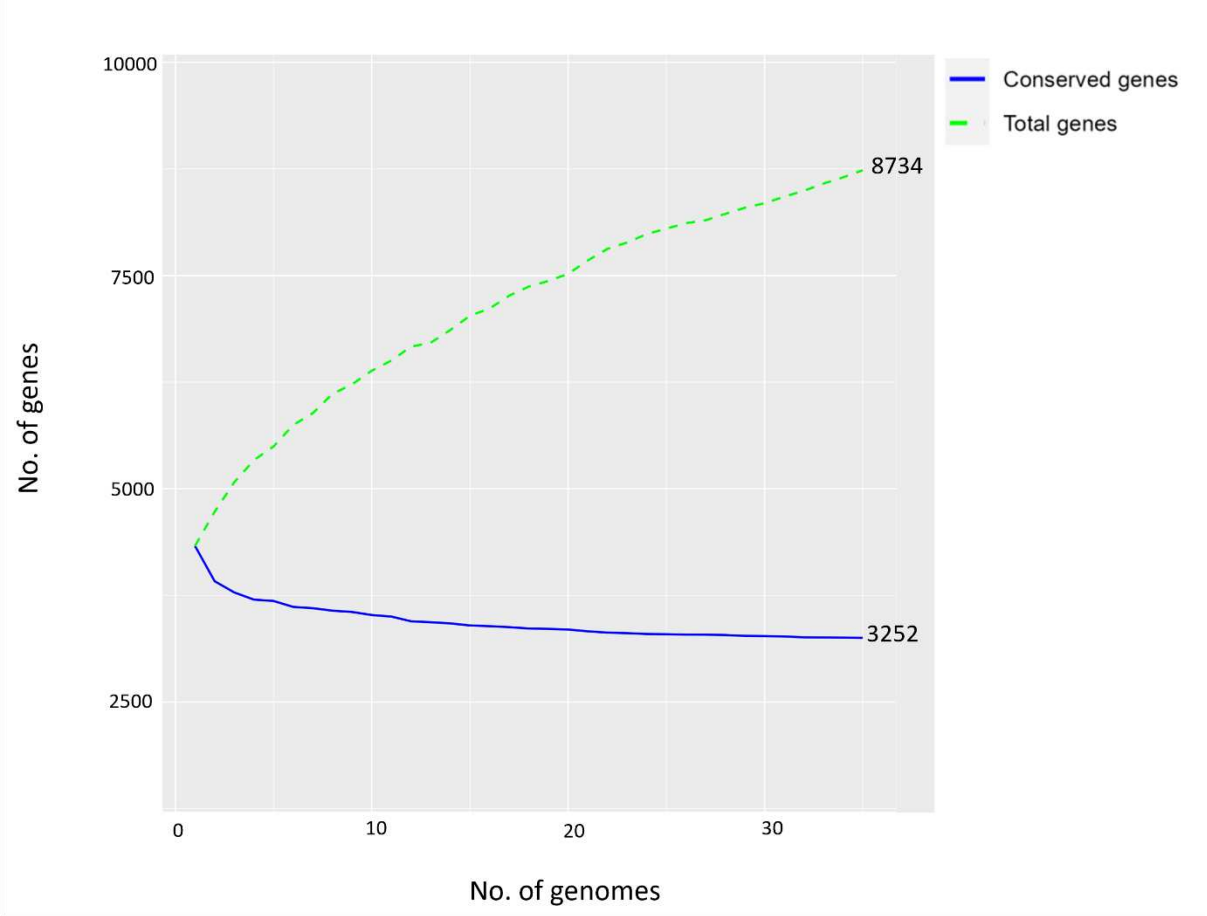

Figure 1. *Xanthomonas citri* pv. *fuscans* pangenome using 35 genome sequences. The green line shows the increase in the total number of genes and the blue line depicts the decrease in the total number of genes shared among all strains investigated (core genes), as the number of genomes added to the analysis increases.

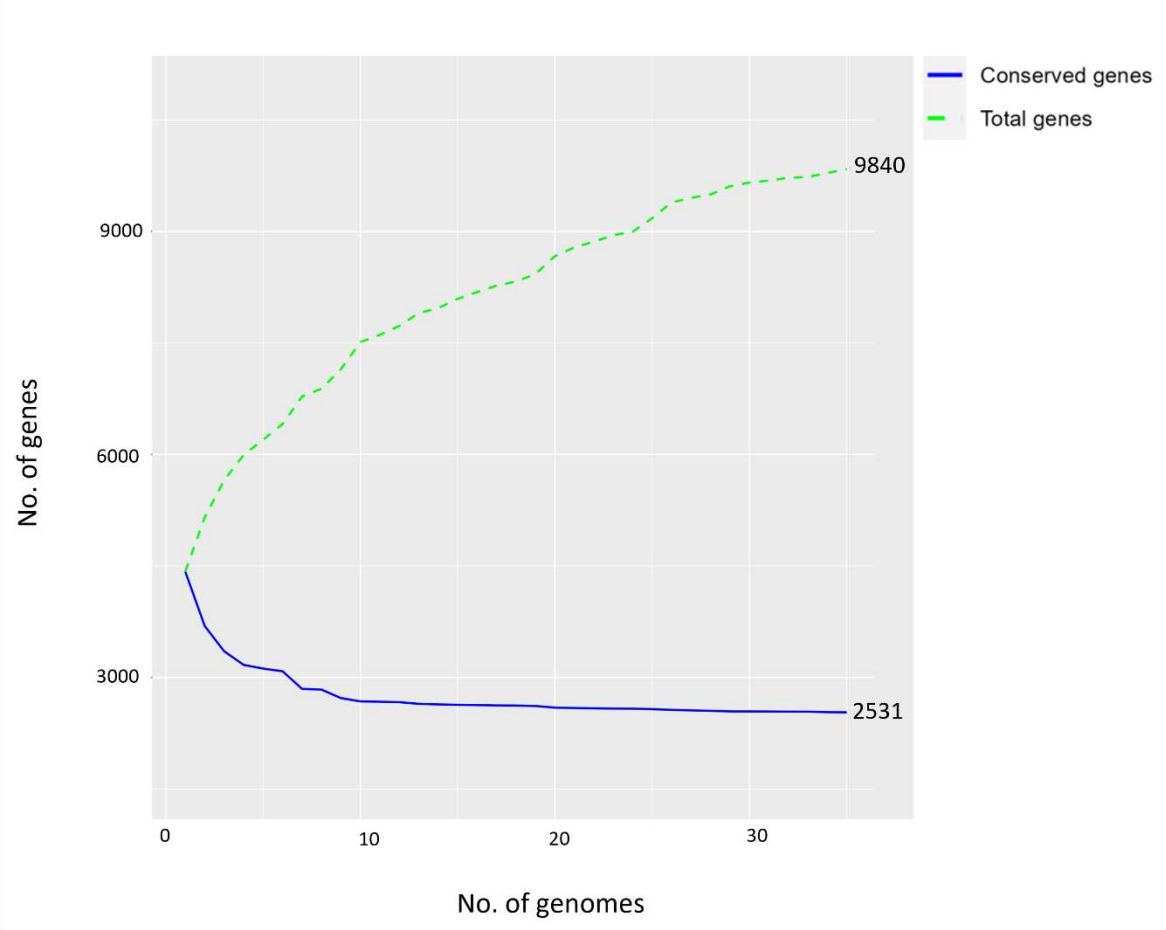

Figure 2. *Xanthomonas phaseoli* pv. *phaseoli* pangenome using 36 genome sequences. The green lines show the increase in the total number of genes and the blue line depicts the decrease in the total number of genes shared among all strains investigated (core genes), as the number of genomes added to the analysis increases.

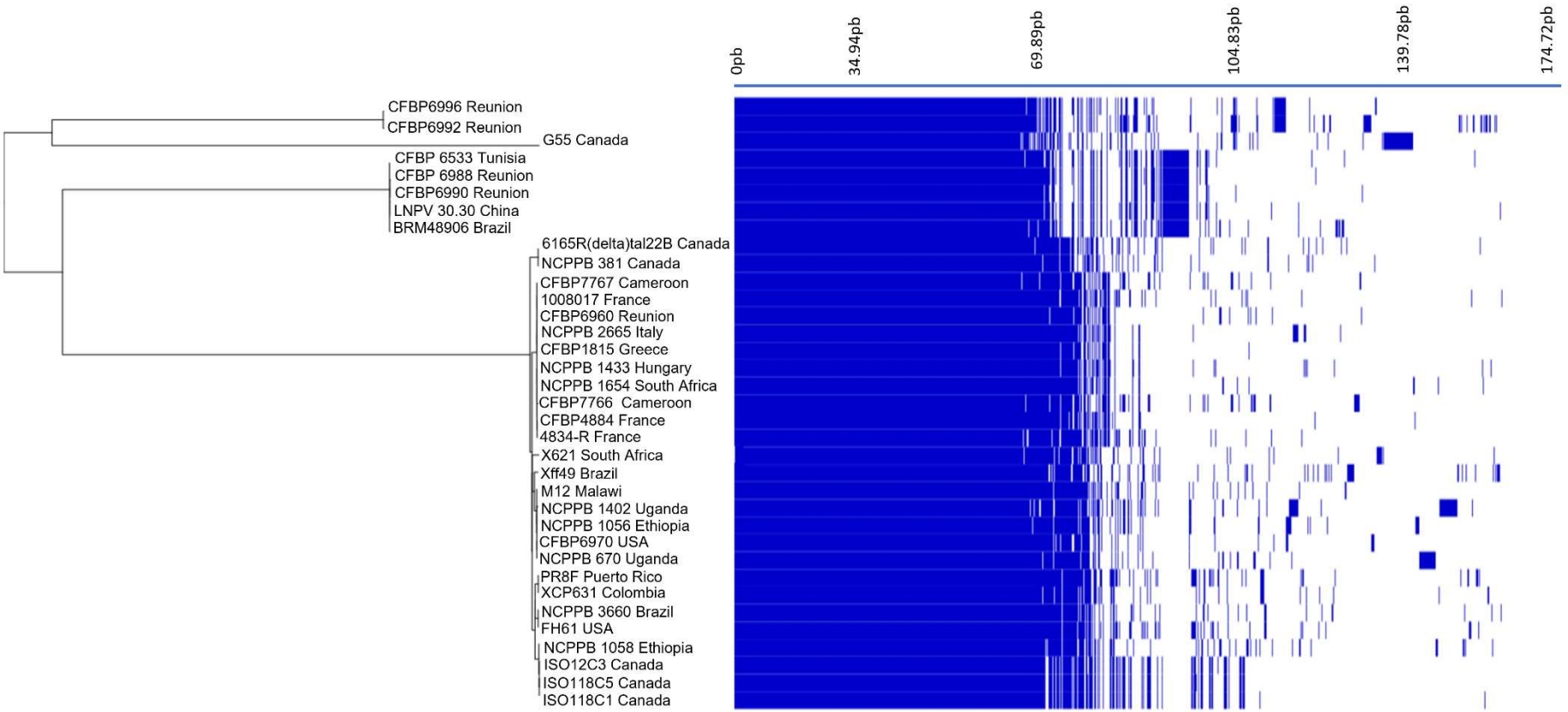

Figure 3. Clustering of 35 *Xanthomonas citri* pv. *fuscans* isolates based on gene presence/absence in their genome sequences. Isolate names followed by their geographic origins are indicated in the dendrogram. Blue bars indicate genes shared among the analyzed genome sequences.

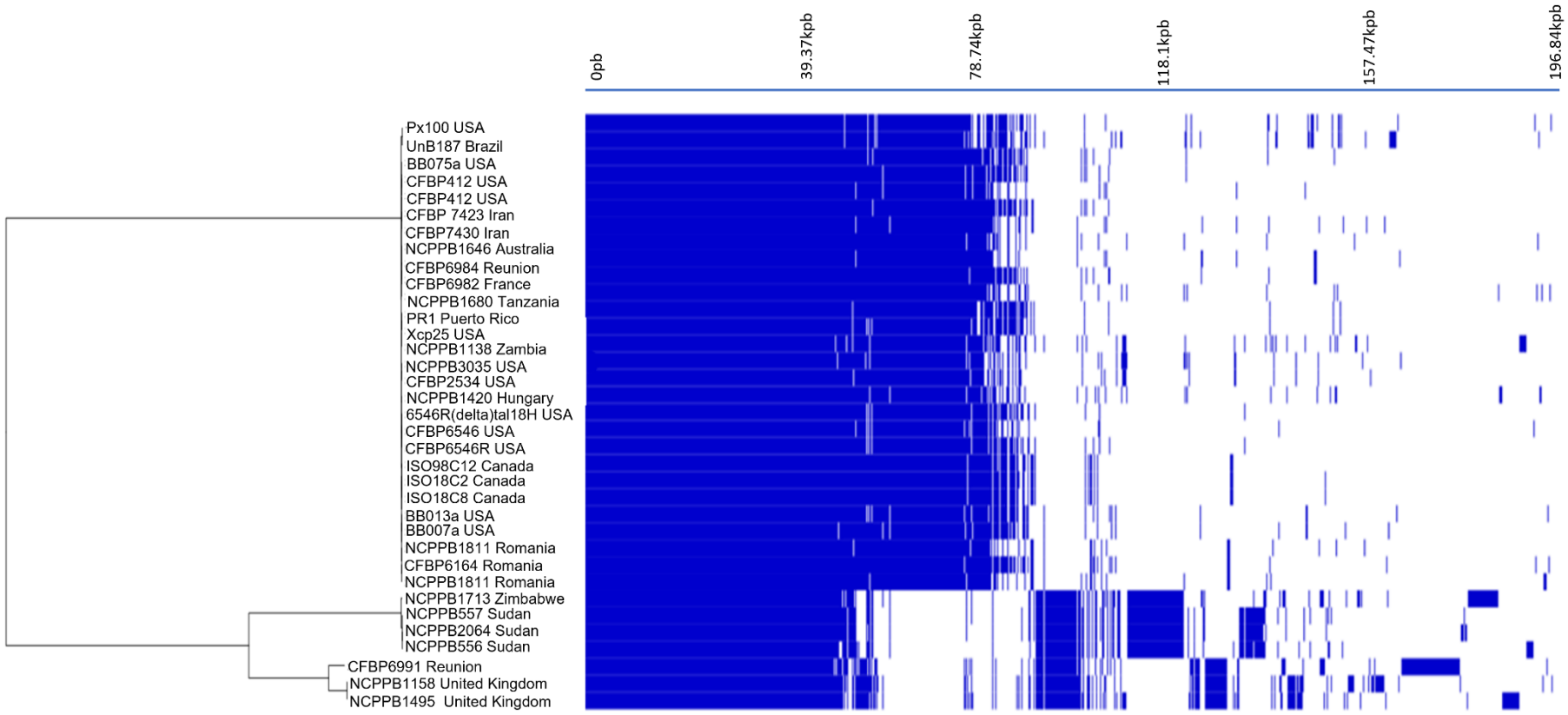

Figure 4. Clustering of 36 *Xanthomonas phaseoli* pv. *phaseoli* isolates based on gene presence/absence in their genome sequences. Isolate names followed by their geographic origins are indicated in the dendrogram. Blue bars indicate genes shared among the analyzed genome sequences.

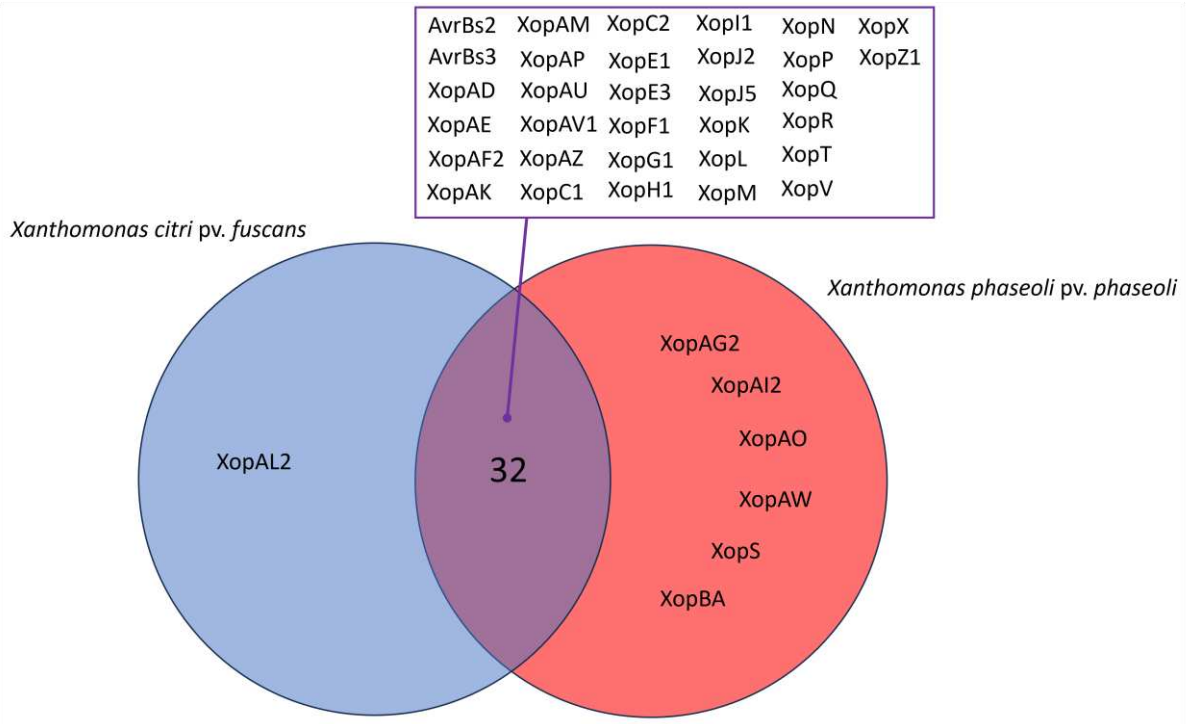

Figure 5. Venn diagram depicting shared and unique type III secretion effectors (T3SEs) between *Xanthomonas citri* pv. *fuscans* and *Xanthomonas phaseoli* pv. *phaseoli*. Distinct colors are used to group the count of effectors for each pathovar, with the number within the intersection representing T3SEs shared between the two pathovars.

4.8. SUPPLEMENTARY MATERIAL

Table S1. Genome sequences of *Xanthomonas citri* pv. *fuscans* isolated from *Phaseolus vulgaris* obtained from the NCBI database.

Isolate	NCBI assembly	Geographic origin	Assembly level	N50 (kb)	L50	Depth of sequencing
ISO118C5	ASM400047v1	Canada	Complete Genome			
ISO118C1	ASM399948v1	Canada	Complete Genome			
ISO12C3	ASM399950v1	Canada	Complete Genome			
PR8F	ASM1883138v1	Puerto Rico	Complete Genome			
FH61	ASM1883132v1	USA	Complete Genome			
BRM48906	ASM2598766v1	Brazil	Complete Genome			
LNPV 30.30	ASM2598764v1	China	Complete Genome			
CFBP 6533	ASM2598770v1	Tunisia	Complete Genome			
M12	ASM1883128v1	Malawi	Complete Genome			
6165R(delta)tal22B	ASM1774281v1	Canada	Complete Genome			
4834-R	ASM96968v1	France	Complete Genome			
Xff49	ASM230951v1	Brazil	Chromosome	112.211	15	40.0x
CFBP 6988	ASM290667v1	Réunion	Chromosome	1.199.983	2	1800.0x

CFBP7766	XFF7766_PRJEB23080_v1	Cameroon	Scaffold	79.558	20	101x
CFBP7767	XFF7767_PRJEB23080_v1	Cameroon	Scaffold	81.069	19	101x
CFBP6960	XFF6960_PRJEB23080_v1	Réunion	Scaffold	93.163	18	101x
CFBP1815	XFF1815_PRJEB23080_v1	Greece	Scaffold	89.106	18	101x
CFBP6990	XFF6990_PRJEB23080_v1	Réunion	Scaffold	373.703	4	101x
CFBP6992	XFF6992_PRJEB23080_v1	Réunion	Scaffold	155.216	13	101x
CFBP6970	XFF6970_PRJEB23080_v1	USA	Scaffold	99.739	18	101x
NCPPB 381	NCPPB381v2	Canada	Contig	74.010	21	40.0x
CFBP6996	ASM59023v1	Réunion	Contig	1.179.078	2	1200x
1008017	ASM2598748v1	France	Contig	4.984.178	1	211x
X621	X621v3	South Africa	Contig	99.856	16	68x
G55	ASM2087947v1	Canada	Contig	93.986	18	85x
NCPPB 1654	NCPPB1654v2	South Africa	Contig	81.53	16	93x
CFBP4884	ASM74188v1	France	Contig	79.889	18	238x
NCPPB 1056	NCPPB1056v2	Ethiopia	Contig	75.549	20	44x
NCPPB 1433	NCPPB1433v2	Hungary	Contig	73.844	20	51x
NCPPB 3660	NCPPB3660v2	Brazil	Contig	73.151	23	63x
NCPPB 670	NCPPB670v2	Uganda	Contig	71.533	22	32x

NCPPB 1058	NCPPB1058v2	Ethiopia	Contig	71.334	25	150x
NCPPB 2665	NCPPB2665v2	Italy	Contig	71.115	19	70x
XCP631	X631v2	Colombia	Contig	61.459	26	33x
NCPPB 1402	NCPPB1402v2	Uganda	Contig	39.404	43	17x

Table S2. Genome sequences of *Xanthomonas phaseoli* pv. *phaseoli* isolated from *Phaseolus vulgaris* obtained from the NCBI database.

Isolate	NCBI assembly	Geographic origin	Assembly level	N50 (kb)	L50	Depth of sequencing
CFBP6164	ASM275911v2	Romania	Complete Genome			
ISO18C2	ASM399956v1	Canada	Complete Genome			
ISO98C12	ASM399944v1	Canada	Complete Genome			
ISO18C8	ASM399954v1	Canada	Complete Genome			
BB007a	ASM2598774v1	USA	Complete Genome			
UnB187	ASM2598776v1	Brazil	Complete Genome			
BB013a	ASM2598768v1	USA	Complete Genome			

CFBP6982	ASM275915v2	France	Complete Genome			
PR1	ASM1883140v1	Puerto Rico	Complete Genome			
CFBP 7423	ASM2598778v1	Iran	Complete Genome			
CFBP412	ASM275909v2	USA	Complete Genome			
CFBP6546R	ASM275913v3	USA	Complete Genome			
6546R(delta)tal18H	ASM1774279v1	USA	Complete Genome			
Px100	ASM1883142v1	USA	Complete Genome			
BB075a	ASM2598772v1	USA	Complete Genome			
Xcp25	ASM1883144v1	USA	Complete Genome			
CFBP2534	ASM1774534v1	USA	Scaffold	90.958	22	467x
CFBP7430	XAP7430_PRJEB23080_v1	Iran	Scaffold	94.219	19	101x
CFBP6984	XAP6984_PRJEB23080_v1	Réunion	Scaffold	92.145	20	101x
CFBP412	XAP412_PRJEB23080_v1	USA	Scaffold	85.907	22	101x
NCPPB1811	ASM1774531v1	Romania	Scaffold	83.271	23	929x
NCPPB3035	NCPPB3035v2	USA	Contig	59.899	28	27x
CFBP6991	XFF6991_PRJEB23080_v1	Réunion	Contig	202.510	8	101x
NCPPB2064	NCPPB2064v2	Sudan	Contig	123.606	15	108x
NCPPB557	NCPPB557v2	Sudan	Contig	116.632	16	57x

NCPPB1713	NCPPB1713v2	Zimbabwe	Contig	96.285	19	30x
NCPPB1680	NCPPB1680v2	Tanzania	Contig	77.618	24	66x
NCPPB1420	NCPPB1420v2	Hungary	Contig	76.066	23	92x
NCPPB1158	NCPPB1158v2	United Kingdom	Contig	73.833	22	40x
NCPPB1495	NCPPB1495v2	United Kingdom	Contig	70.760	24	30x
NCPPB1646	NCPPB1646v2	Australia	Contig	69.783	24	48x
NCPPB1811	NCPPB1811v2	Romania	Contig	68.458	26	54x
XCP123	XCP123v2	Colombia	Contig	66.622	25	30x
NCPPB1138	NCPPB1138v2	Zambia	Contig	66.168	26	45x
NCPPB556	NCPPB556v2	Sudan	Contig	41.494	38	15x
CFBP6546	ASM58885v1	USA	Contig	399.888	5	2100x

Table S3. Specific *hrp* box sequences searched for by the Python script to identify *Xanthomonas* genes activated by HrpX and HrpG.

Sequence	Reference
TTCGC-N_{15}-TTCGC	Fenselay and Bonas, 1995
TTCGC-N_{8}-TTCGT	Huguet and Bonas, 1997
TTCG-N_{16}-TTCG	Cunnac *et al.,* 2004
TTCGB-N_{15}-TTCGB*	Koebnik *et al.,* 2006
TTCGB-N_{15}-TTCGB-N_{30-32}-YAN_3T*	Koebnik *et al.,* 2006

***** B represents C, G or T; Y represents C or T

5. GENERAL CONCLUSIONS

Based on the findings derived from this research, the following conclusions can be made:

1. Three carioca and three black common bean cultivars with high levels of horizontal resistance towards *Xpp* were identified.
2. Five SNPs that may be linked to resistance against *Xpp* were identified. These SNPs reside within genes encoding proteins with diverse biochemical functions which had not previously been associated with plant resistance to bacterial pathogens.
3. Pangenomic analyses revealed that the *Xcf* and *Xpp* genomes exhibit significant diversity and plasticity.
4. Clustering based on the pangenomes of *Xcf* and *Xpp* did not reveal a clear grouping by geographic origin.
5. Predictions of T3SEs revealed a significant diversity of effectors shared between *Xcf* and *Xpp*, as well as the presence of effectors shared between these common bean pathogens and phytopathogenic *Pseudomonas.*