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Genetic diversity of *Xanthomonas phaseoli* pv. *manihotis* populations using rep-PCR and VNTR molecular markers

Abstract – The objective of this work was to evaluate the genetic diversity of *Xanthomonas phaseoli* pv. *manihotis* (*Xpm*) from eight populations from five cassava producing states in Brazil, through the rep-PCR (BOX-PCR and ERIC-PCR) and variable number of tandem repeat (VNTR) markers. Cassava leaves with symptoms of cassava bacterial blight were collected in eight municipalities, and the *Xpm* isolates were identified by amplification with primers specific for these isolates. The identity of the *Xpm* isolates was confirmed with the BOX-PCR, ERIC-PCR, and VNTR markers. The observed selection pressure, together with the mode of reproduction and the mechanisms that increase genetic variability, allows of the pathogen populations to adapt according to microclimate variation, contributing to a differentiated reproductive success. ERIC-PCR and VNTRs are the best markers for evaluating the genetic variability in the eight studied *Xpm* populations. However, ERIC-PCR is the marker that best separated the groups by population and presented a higher similarity between the isolates of the same population. The study of the genetic diversity of *Xpm* is key to improve disease monitoring and management strategies in cassava crops.

Index terms: *Manihot esculenta*, bacterial blight, DNA markers, genetic diversity.

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Diversidade genética de populações de *Xanthomonas phaseoli* pv. *manihotis* em mandioca por meio de marcadores rep-PCR e VNTRs

Resumo – O objetivo deste trabalho foi avaliar a diversidade genética de *Xanthomonas phaseoli* pv. *manihotis* (*Xpm*) de oito populações de cinco estados produtores de mandioca no Brasil, por meio de marcadores rep-PCR (BOX-PCR e ERIC-PCR) e *variable number of tandem repeats* (VNTRs). Folhas de mandioca com sintomas de crestamento bacteriano foram coletadas em oito municípios, e os isolados *Xpm* foram identificados por amplificação com iniciadores específicos para esses isolados. A identidade dos isolados *Xmp* foi confirmada com os marcadores BOX-PCR, ERIC-PCR e VNTRs. A pressão de seleção observada, junto com o modo de reprodução e os mecanismos que aumentam a variabilidade genética, permite que as populações do patógeno se adaptem de acordo com a variação dos microclimas, o que contribui para o sucesso reprodutivo diferenciado. ERIC-PCR e VNTRs são os melhores marcadores para avaliar a variabilidade genética das oito populações *Xpm* estudadas. No entanto, ERIC-PCR é o marcador que melhor separou os grupos por população e apresentou maior similaridade entre os isolados de uma mesma população. O estudo da diversidade genética de *Xpm* é fundamental

para delinear estratégias de manejo e monitoramento de doenças na cultura da mandioca.

Termos para indexação: *Manihot esculenta*, crestamento bacteriano, marcadores de DNA, diversidade genética.

Introduction

Cassava (*Manihot esculenta* Crantz) is a staple food that provides a source of income for millions of people in tropical countries (Bayata, 2019; Sonnewald et al., 2020). Brazil is the sixth largest producer in the world, with approximately 18.2 million tons produced in 2021 on 1.2 million hectare, generating R$ 10.9 billion for the cassava agribusiness (FAO, 2023). However, this crop is threatened by several pests and diseases that significantly affect its yield and commercialization, such as cassava bacterial blight (CBB), widely distributed in the different areas where the species is grown (Rache et al., 2019, 2023; Zárate-Chaves et al., 2021a).

CBB is caused by *Xanthomonas phaseoli* pv. *manihotis* (*Xpm*), a vascular pathogen that is generally found on the surface of leaves, but may enter the plants through wounds and/or natural openings such as stomata. In the leaves, the main symptoms are dark-green and angular spots and water-soaked lesions (Veley et al., 2023). Vascular colonization can be associated with gummosis (exudation), vascular necrosis of the stem, and plant wilting and dieback, and one of the main means of pathogen dispersion is the movement of infected but asymptomatic stems (Medina, 2018). In the case of severe infection, premature dehydration and defoliation are common, as well as the death of the non-lignified soft tissue at the top of the growing shoot (Teixeira et al., 2021).

Since, to date, there is no evidence of effective methods to prevent and treat CBB, the use of cultural practices and of resistant varieties is considered the best management strategy against the disease (Bart et al., 2012; Teixeira et al., 2021; Zárate-Chaves et al., 2021b; Ye et al., 2023). Therefore, a good characterization of the *Xpm* pathogen is important for the development of resistant varieties.

Molecular markers are indispensable tools for studies of plant pathogenic bacteria diversity. The most used are microsatellites or simple sequence repeats (SSRs) and rep-PCR (BOX-PCR and ERIC-PCR) markers (Kapantaidaki et al., 2021; Díaz-Tatis et al., 2022). However, variable number of tandem repeat (VNTR) markers are considered as the most informative for the evaluation of diversity analysis in *Xpm* populations (Trujillo et al., 2014; Zárate-Chaves et al., 2021a, 2021b).

In this scenario, the lack of information on *Xpm* population dynamics and the broad range of different edaphoclimatic conditions represented by the different locations where cassava is cultivated still represent a potential risk for crop growers in Brazil.

The objective of this work was to evaluate the genetic diversity of *Xanthomonas phaseoli* pv. *manihotis* (*Xpm*) from eight populations from five cassava producing states in Brazil, through the rep-PCR (BOX-PCR and ERIC-PCR) and VNTR markers.

Materials and Methods

Cassava leaves with symptoms of CBB were collected from varieties that represent the genetic variability in Brazil or that were the most planted in properties located in the main producing regions of the country, precisely in: the municipalities of Londrina (23°08'S, 50°52'W) and Paranavaí (23°4'S 52° 27'W) in the state of Paraná, the municipality of Ocauçu (22°26'S, 49°55'W) in the state of São Paulo, the municipalities of Laje (13°9'S, 39°25'W) and Guanambi (14º13'S, 42º46'W) in the state of Bahia, the municipality of Russas (5º37'S, 38º07'W) in the state of Ceará, and the municipalities of Dourados (22º13'S, 54°48'W) and Naviraí (23°3'55"S, 54°11'26"W) in the state of Mato Grosso do Sul (Figure 1).

According to Köppen-Geiger's classification, the climates of the municipalities are: Cfa, humid subtropical, in Londrina and Paranavaí in the state of Paraná; Aw, tropical savanna, in Ocauçu in the state of São Paulo; BWh, hot desert, in Laje and Guanambi in the state of Bahia and Russas in the state of Ceará; and Am, tropical monsoon, in Dourados and Naviraí in the state of Mato Grosso do Sul.

For isolation procedures, sampling was systematically performed using diagonal transects across three to four fields in each location. Specifically, leaves exhibiting distinctive symptoms of CBB or stems with bacterial exudation were carefully selected for bacterial isolation. The quantity of samples collected varied based on the incidence of the disease within each field. Given the extensive survey conducted on

a wide range of cassava cultivars and landraces across multiple fields and municipalities, the study did not assign a primary emphasis on evaluating the influence of cassava genotype.

For analyses, the plant samples were taken to the Phytopathology Laboratory at Embrapa Mandioca e Fruticultura. First, the leaf samples were washed in running water, cut into fragments of approximately 1.0 cm^2, and subjected to a disinfection process in 70% alcohol for 30 s, 50% sodium hypochlorite for 1 min, and, again, in 70% alcohol for 30 s, followed by rinsing three times in sterilized water.

The tissue fragments were macerated using a mortar and pestle in 1.0 mL sterilized distilled water (SDW). A serial dilution (10^{-1}, 10^{-2}, and 10^{-3}) was performed for each isolate in the suspension obtained in 1.5 µL microtubes containing 450 µL SDW. From this dilution, the tissues were plated in three Petri dishes (one for each dilution), containing the YPG medium with 5.0 g yeast extract, 5.0 g protease (peptone), 5.0 g glucose, 15 g agar, and 1.0 L SDW (Restrepo et al., 2000).

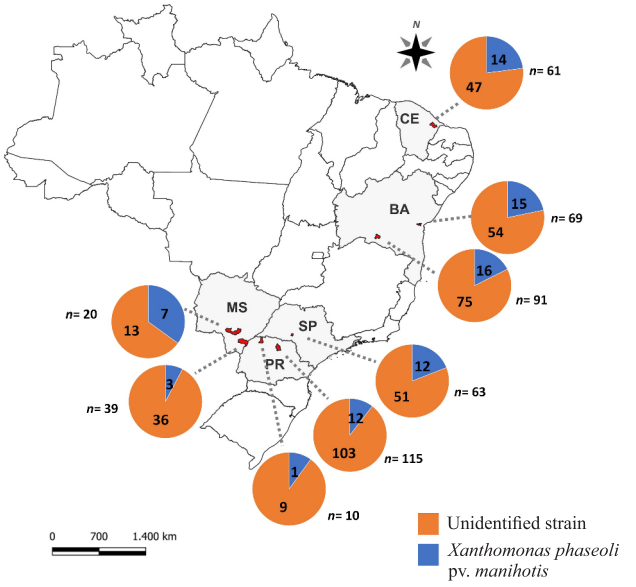

Figure 1. Map of Brazil showing the geographical locations (municipalities) where the cassava (*Manihot esculenta*) leaf samples with cassava bacterial blight symptoms were originally collected and the number of total isolates obtained per location (n). The locations were: the municipality of Russas in the state of Ceará (CE), the municipalities of Laje and Guanambi in the state of Bahia (BA), the municipality of Ocauçu in the state of São Paulo (SP), the municipalities of Naviraí and Dourados in the state of Mato Grosso do Sul (MS), and the municipalities of Paranavaí and Londrina in the state of Paraná (PR). Orange, negative samples for primer XV and Xkmod amplification; and blue, positive samples for XV and Xkmod amplification.

Using a sterilized wooden toothpick, two colonies were scraped from each plate and placed in PCR microtubes containing 100 µL SDW and, then, taken to a thermocycler for 10 min at 95°C; this process was used for cell lysis and subsequent DNA extraction. The samples were kept at 4°C for use in the polymerase chain reactions (PCRs). The molecular characterization of the bacterial isolates was conducted via amplification with specific primers for pathogenic *Xpm* isolates (Verdier et al., 1998; Melo et al., 2019).

PCR was performed using the following final concentrations: 3.0 µL denatured DNA at 95°C, 1X Taq buffer, 2.5 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} dNTPs, 0.4 µmol L^{-1} primers XV (5'-TTC-GGC-AAC-GGC-AGT-GAC-CAC-C-3'), and XK_MOD (3'-AAT-CGG-AGA-TTA-CCT-GAG-CG-5') specific for *Xpm* (Verdier et al., 1998; Melo et al., 2019), with a final volume adjusted to 15 µL. The programming conditions were: initial denaturation at 95°C for 5 min, followed by 36 cycles of denaturation for 30 s at 95°C, primer annealing at 60°C for 30 s, and extension at 72°C for 1 min, ending with a 7 min cycle at 72°C. The amplified product was visualized on 1.0% agarose gel stained with ethidium bromide, in 0.5% TBE buffer, for 4 hours at 80 V. The marker used was 1 Kb DNA Ladder (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA).

Only the isolates identified as *Xpm*, based on the presence of a characteristic band for the pathovar obtained through PCR (XV/XK_MOD), were considered. To prepare the inoculum, bacteria were streaked on YPG medium, at 28°C, for 24 hours. A single colony of each *Xpm* isolate was grown in YPG, at 28°C, with shaking at 230 rpm for 24 hours. Cells were harvested by centrifugation at 3,000 *g* and re-suspended in 10 mmol L^{-1} $MgCl_2$; the suspension was adjusted to obtain 1×10^6 CFU mL^{-1}, measured by 0.01 absorbance on a spectrophotometer, as described by Mora et al. (2019).

For inoculation procedures, three wounds were made on the abaxial side of the cassava leaf, and the bacterial suspension was deposited by infiltration using a needleless syringe. During this process, the leaf

was pushed against the syringe by the lab technician, who used a finger from their free hand to apply enough pressure for the inoculum to penetrate the intercellular spaces of the leaf blade. Five replicates were carried out per isolate, per cassava cultivar. The cultivars used here were BRS Formosa, BRS Kiriris, and BRS Aramaris, previously described in the literature as moderately susceptible, susceptible, and moderately resistant to CBB, respectively (Teixeira et al., 2021).

After inoculation, the plants were bagged for 24 hours in order to facilitate pathogen penetration and colonization. The experimental design was three randomized complete blocks with 55 plants per block of each variety, totaling 165 plants. At the beginning of the experiment, the plants were 12 weeks old and about 40 cm in height. Assessments were performed every five days. After eight days, symptoms appeared in some plants within the replicates. Subsequently, every three days, new evaluations were conducted from the absence of symptoms until plant death.

For the BOX-PCR reactions, the BOX A_1R primer was used (5' CTA CGG CAA GGC GAC GCT GAC G 3'). The reaction was composed of the following reagents: 10X buffer, 50 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} dNTPs, 0.2 primer, 1U Taq DNA polymerase, 3.0 µL DNA, and ultrapure water to a final volume of 18 µL. The samples were amplified in a thermocycler, with the following amplification program: 95°C for 2 min, followed by 39 cycles of 94°C for 1 min for denaturation, 51°C for 1 min for annealing, and 72°C for 1 min for extension, ending at a final extension at 72°C for 10 min. PCR products were analyzed on 1% agarose gel in 0.5X TBE buffer by electrophoresis conducted at 70–80V for approximately 4 hours.

For the analysis of the ERIC-PCR primers (ERIC1R and ERIC2F), the same abovementioned composition of BOX-PCR reagents and amplification program, but at an annealing temperature of 48°C, were used. For the PCR reactions of the VNTRs, the final concentrations of the reagents used were: 3.0 µL DNA (5.0 ng μL^{-1}), 1X Taq buffer, 2.0 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} dNTPs, and 0.005 mmol L^{-1} primers for five additional *Xpm*-specific VNTR loci (XaG1_02, XaG1_29, XaG1_67, XaG1_52, and XaG1_73) (Nakamura et al., 1987). The program used for amplification was 95°C for 4 min, followed by 39 cycles of 94°C for 50 s for denaturation, Δ°C (according to each primer pair, which ranged from 52 to 55°C) for 50 s for annealing, and 72°C for 1 min for extension. Final extension was performed at 72°C for 10 min. PCR products were also analyzed on 1% agarose gel, in 0.5X TBE buffer, by electrophoresis carried out at 70–80V for approximately 4 hours. The primer sequences used in the molecular study of the isolates are presented in Table 1.

The band profiles generated from the BOX-PCR, ERIC-PCR, and VNTR markers were used to construct binary matrices, in which 0 = absence of bands and 1 = presence of bands, and were converted into dissimilarity matrices. Dendrograms for BOX-PCR and ERIC-PCR were constructed based on the Jaccard coefficient, using the unweighted-pair group method with arithmetic mean clustering analysis.

Data from the binary matrix were imported into the POPGENE 1.31 software (Yeh et al., 1999) to perform the combined analysis, where the following diversity parameters were estimated: number of effective alleles, number of observed alleles, Nei's gene diversity (Nei 1973), Shannon diversity index (Lewontin, 1972), number of polymorphic loci, percentage of polymorphic loci (PPL%), allelic diversity, total genetic diversity, genetic diversity of the subpopulation in relation to the total population (G_{ST}) as in Nei (1973), and gene flow.

Table 1. Markers used for the molecular analysis.

Marker	Primer	Primer sequence (5′-3′)	Reference
rep-PCR	BOX 1AR	CTACGGCAAGGCGACGCTGACG	Koeuth et al. (1995)
	ERIC1R	ATGTAAGCTCCTGGGGATTCAC	Versalovic et al. (1991)
	ERIC2F	AAGTAAGTGACTGGGGTGAGCG	
VNTR	G1_02	TCCCCAT	Nakamura et al. (1987)
	G1_29	ATCCCGA	Nakamura et al. (1987)
	G1_52	CCGCCACAACGCA	Nakamura et al. (1987)
	G1_67	CGACAC	Nakamura et al. (1987)
	G1_73	GGTCAT	Nakamura et al. (1987)

Genetic similarity and genetic distance between populations were also computed using the model presented in Nei (1978). The analysis of molecular variance (AMOVA) was used to reveal the distribution of genetic diversity within and between populations. In this analysis, the genetic diversity level (F_{ST}) for the estimate of the genetic structure was calculated, as well as total genetic diversity, which was divided into two distinct hierarchical levels: difference between populations and between individuals within a population. The AMOVA was performed according to Excoffier et al. (2005), using the Arlequin software, version 3.5.2.2. The significance of differentiation was tested with 1,000 permutations, where p denotes the probability of obtaining a random value equal to or greater than the observed value.

The minimum spanning network analysis to represent the genetic distances, as well as the connectivity among haplotypes, was also calculated using the vegan and poppr packages of the R software (R Core Team, 2018). Correlations between the distance matrices generated by markers BOX-PCR + ERIC-PCR, BOX-PCR + VNTR, and ERIC-PCR + VNTR were performed using the Mantel test in the ade4 package, also of the R software.

Results and Discussion

A total of 468 isolates collected in the different producing regions in the Brazilian states were associated with CBB (Figure 1). The expected target fragment of 900 bp was detected in the DNA from 17% (80) of these isolates when subjected to amplification with primers XV/XKmod, confirming the identity of the pathogen (Table 2). The distribution of the presence and absence of symptoms, as well as leaf drop, differed significantly between the varieties by the chi-square test, at 5% probability. After 8 days after inoculation, angular lesions became evident, with necrosis 13 days later, followed by leaf drying and drop.

According to the dendrogram analysis of the *Xpm* isolates differentiated with the BOX-PCR, ERIC-PCR, and VNTR markers, the isolates clustered by the BOX-PCR and ERIC-PCR markers were divided into seven groups (G1–G7), as shown in Figure 2 A. The isolates from the municipalities of Laje and Ocau**çu**, in the states of Bahia and São Paulo, respectively, were genetically close according to this marker, which may be due to the shared alleles, indicating a same origin, with migration caused by the exchange of contaminated cuttings by farmers. The data obtained with the ERIC-PCR marker also formed seven groups (G1–G7) (Figure 2 B). However, the isolates from the same population that were separated with BOX-PCR were now grouped in this dendrogram. An intra-group discriminatory capacity was also observed, as the isolates from Guanambi, in the state of Bahia, were separated into two distinct groups despite being in the same node. The VNTR markers also showed a similar pattern to those of the BOX-PCR and ERIC-PCR markers (Figure 2 C), even though they are found in less conserved regions of the genome. In addition, the isolates from Ocauçu and Laje, in the states of São Paulo and Bahia, respectively, analyzed using the VNTR markers showed some type of grouping by the BOX-PCR method. This is an indicative that, despite the geographic separation, the isolates have some genetic similarities that remained unchanged throughout their evolutionary trajectory. Overall, the clusters were similar considering the BOX-PCR, ERIC-PCR, and VNTR molecular techniques (Figure 2). However, the ERIC-PCR primers best separated the groups by population, showing the highest similarity between the isolates from the same populations (Figure 2 B). In general, according to the dendrograms, ERIC-PCR was the marker that best separated the groups by population and presented a higher similarity between the isolates of the same population.

The minimum spanning network (MSN) for the population of the studied isolates according to the BOX-PCR, ERIC-PCR, and VNTR markers and their combination is shown in Figure 3. Regarding the MSN, for the BOX-PCR marker, isolates from Russas, Paranavaí, and Laje in the states of Ceará, Paraná, and Bahia, respectively, share the same genetic profile, with smaller genetic distances (Figure 3 A). Since the isolates were basically distributed according to their own populations, the minimal genetic distance between them was smaller, showing that, for this marker, diversity was lower between and within the populations due to the similar genetic profiles, with less unique haplotypes.

For the ERIC-PCR markers, the MSN revealed that the most frequent haplotypes were from the states of Bahia, São Paulo, and Paraná, forming the largest

Table 2. Identified isolates associated with cassava bacterial blight, origin of the cassava (*Manihot esculenta*) variety, and respective collection sites used in the pathogenicity test and in the analysis of genetic diversity[1].

ID#	Isolates/cassava variety	Location[2]	XKMOD	BOX-PCR	ERIC-PCR	VNTR	Test	Pathogenicity
13	Caipira.3111	Londrina-PR	+	-	-	-	No	NR
14	Caipira.3112	Londrina-PR	+	-	-	+	No	NR
18	Caipira.3412	Londrina-PR	+	+	-	+	No	NR
21	Caipira.3521	Londrina-PR	+	-	-	+	No	NR
25	Naviraí.1111	Naviraí-MS	+	+	-	+	No	NR
26	Naviraí.1112	Naviraí-MS	+	+	-	+	No	NR
55	Corrente.11	Londrina-PR	+	+	-	+	Yes	+
56	Corrente.12	Londrina-PR	+	+	-	+	Yes	+
83	Bernardo.111	Laje-BA	+	+	-	+	Yes	+
93	Cigana.1.1	Laje-BA	+	+	-	+	Yes	+
101	Cigana.3.1	Laje-BA	+	-	-	+	Yes	+
133	BGM 1502.41	Londrina-PR	+	-	-	+	No	NR
134	BGM 1502.42	Londrina-PR	+	-	-	+	No	NR
167	Novo Rumo.22	Laje-BA	+	+	-	+	Yes	+
168	Novo Rumo.31	Laje-BA	+	-	-	+	Yes	+
169	Novo Rumo.32	Laje-BA	+	+	-	+	Yes	+
171	NS 86-B12	Laje-BA	+	+	-	-	Yes	+
172	'IAC 90'.111	Ocauçu-SP	+	+	-	+	Yes	+
173	'IAC 90'.112	Ocauçu-SP	+	+	-	+	No	NR
174	'IAC 90'.121	Ocauçu-SP	+	+	-	+	Yes	+
175	'IAC 90'.122	Ocauçu-SP	+	-	+	+	Yes	+
176	'IAC 90'.211	Ocauçu-SP	+	-	+	+	Yes	+
177	'IAC 90'.212	Ocauçu-SP	+	+	+	+	No	NR
178	'IAC 90'.221	Ocauçu- SP	+	+	+	+	Yes	+
179	'IAC 90'.222	Ocauçu- SP	+	-	+	+	No	NR
180	Novo Rumo.21	Laje-BA	+	+	+	+	Yes	+
184	Corrente11	Laje-BA	+	+	+	+	Yes	+
186	Caipira.1111	Laje-BA	+	-	-	+	Yes	+
187	Caipira.1112	Laje-BA	+	+	+	+	Yes	+
188	Caipira.1121	Laje-BA	+	-	-	+	Yes	+
192	Caipira.221	Laje-BA	+	+	+	+	Yes	+
196	Caipira.4111	Laje-BA	+	-	+	-	Yes	+
209	Caipira.6111	Laje-BA	+	+	+	+	Yes	+
245	Tapioqueira.3122	Londrina-PR	+	+	+	+	Yes	+
249	Tapioqueira.4212	Londrina-PR	+	+	+	+	No	NR
251	Tapioqueira.4222	Londrina-PR	+	+	+	+	Yes	+
260	Caipira.4112	Londrina-PR	+	+	+	-	Yes	+
261	261PRA.11	Paranavaí-PR	+	+	+	+	Yes	+
296	Guanambi.1111	Guanambi-BA	+	+	+	+	No	NR
297	Guanambi.1112	Guanambi-BA	+	+	+	+	No	NR
311	Guanambi.4112	Guanambi-BA	+	+	+	-	No	NR
312	Guanambi.4121	Guanambi-BA	+	+	+	+	No	NR
313	Guanambi.4122	Guanambi-BA	+	+	+	-	No	NR
315	Guanambi.5112	Guanambi-BA	+	+	+	-	No	NR
316	Guanambi.5121	Guanambi-BA	+	+	+	+	No	NR
317	Guanambi.5122	Guanambi-BA	+	+	-	+	Yes	+
318	Guanambi.521	Guanambi-BA	+	+	+	+	Yes	+
322	Guanambi.6121	Guanambi-BA	+	-	+	-	No	NR
323	Guanambi.6122	Guanambi-BA	+	-	+	-	No	NR
332	Ocauçu.1111	Ocauçu-SP	+	-	+	+	Yes	+
333	Ocauçu.1112	Ocauçu-SP	+	-	+	+	Yes	+

Continuation...

Table 2. Continuation...

ID#	Isolates/cassava variety	Location[2]	XKMOD	BOX-PCR	ERIC-PCR	VNTR	Test	Pathogenicity
357	Corrente.41	Dourados-MS	+	+	+	-	Yes	+
361	Corrente.10	Dourados-MS	+	-	+	+	Yes	+
370	Russas.121	Russas-CE	+	+	-	-	No	NR
389	Russas.711	Russas-CE	+	+	-	+	No	NR
390	Russas.712	Russas-CE	+	+	-	+	Yes	+
391	Russas.731	Russas-CE	+	+	+	+	No	NR
392	Russas.732	Russas-CE	+	+	+	+	Yes	+
409	Russas.09B22	Russas-CE	+	+	+	+	Yes	+
413	'BRS 399'.2	Dourados-MS	+	-	+	+	Yes	+
414	'BRS 399'.3	Dourados-MS	+	-	+	+	No	NR
429	Cigana.2	Guanambi-BA	+	-	+	+	Yes	+
434	BGM 0934.2	Guanambi-BA	+	+	+	+	Yes	+
440	Ocauçu.2	Ocauçu-SP	+	-	-	+	Yes	+
441	Ocauçu.3	Ocauçu- SP	+	+	+	+	Yes	+
443	Naviraí.032	Naviraí-MS	+	+	+	+	Yes	+
450	NS 81B	Dourados-MS	+	+	+	+	Yes	+
451	NS 80B	Dourados-MS	+	+	+	+	Yes	+
452	NS 83A	Dourados-MS	+	+	+	+	Yes	+
453	Russas CE021	Russas-CE	+	-	+	+	Yes	+
456	Russas CE024	Russas-CE	+	-	+	+	Yes	+
458	Russas CE026	Russas-CE	+	-	+	+	Yes	+
459	Russas CE061	Russas-CE	+	+	+	+	Yes	+
460	Russas CE062	Russas-CE	+	-	+	+	Yes	+
461	Russas CE063	Russas-CE	+	-	+	-	Yes	+
462	Russas CE064	Russas-CE	+	+	-	-	Yes	+
463	Russas CE065	Russas-CE	+	+	+	+	Yes	+
465	IF-GBI-032	Guanambi-BA	+	-	+	+	Yes	+
466	IF-GBI-033	Guanambi-BA	+	-	+	-	No	NR
467	IF-GBI-034	Guanambi-BA	+	-	+	-	No	NR

[1]ID#, identification number; +, presence of amplification for the primer/marker; -, absence of amplification for the primer/marker; and NR, not carried out. [2]Municipalities in the Brazilian states of: PR, Paraná; MS, Mato Grosso do Sul; BA, Bahia; SP, São Paulo; and CE, Ceará.

groups, probably ancestral haplotypes (Figure 3 B). The minimal genetic distance between the isolates was larger between two isolates from Bahia (180 and 184), collected in the same location.

The MSN from the VNTRs had most ramifications (Figure 3 C); however, the distance between the genotypes was low compared with the network generated by the ERIC-PCR primer (Figure 3 B). Although the isolates were different (unique haplotypes), they were closer within each specific population. Moreover, the VNTR markers best discriminated the isolates (Figure 3 C), also showing the variability between these and the ERIC-PCR markers that best distanced the populations (Figure 3 B).

When the combined data of the BOX-PCR, ERIC-PCR, and VNTRs markers was analyzed (Figure 3 D), the populations became very distant and isolates were not shown, since the data used in minimum spanning was selected from the common isolate among the three markers. In this case, the BOX-PCR primer showed the lowest similarity between the groups.

The Mantel test revealed a low positive correlation of 0.19 between the BOX-PCR and VNTR molecular markers. In contrast, there was a positive and relatively high correlation of 0.52 between the ERIC-PCR and VNTR markers and of 0.68 between BOX-PCR and ERIC-PCR.

Considering the analysis of genetic diversity in the populations of *Xpm*, those with the highest contrast in PPL and N_a were observed in the states of Bahia (PPL = 93.33 % and N_a = 1.9333) and Ceará (PPL = 62.22% and N_a =1.6222) (Table 3). These two populations also presented the highest values of 0.2263 and 0.3686 in Bahia and 0.1826 and 0.2857 in Ceará, respectively,

for Nei's gene diversity and the Shannon index. The low values found for the Shannon index suggest that the studied populations were possibly influenced by the founder effect, leading to a low genetic variability, since the population structure is influenced by the processes of dispersion (gradual/natural vs. stochastic/interposed by man), considering the geographic scale.

Nei's genetic similarities ranged from 0.845 to 0.977, and genetic distance, from 0.024 to 0.069 (Table 4). The maximum estimated value of genetic

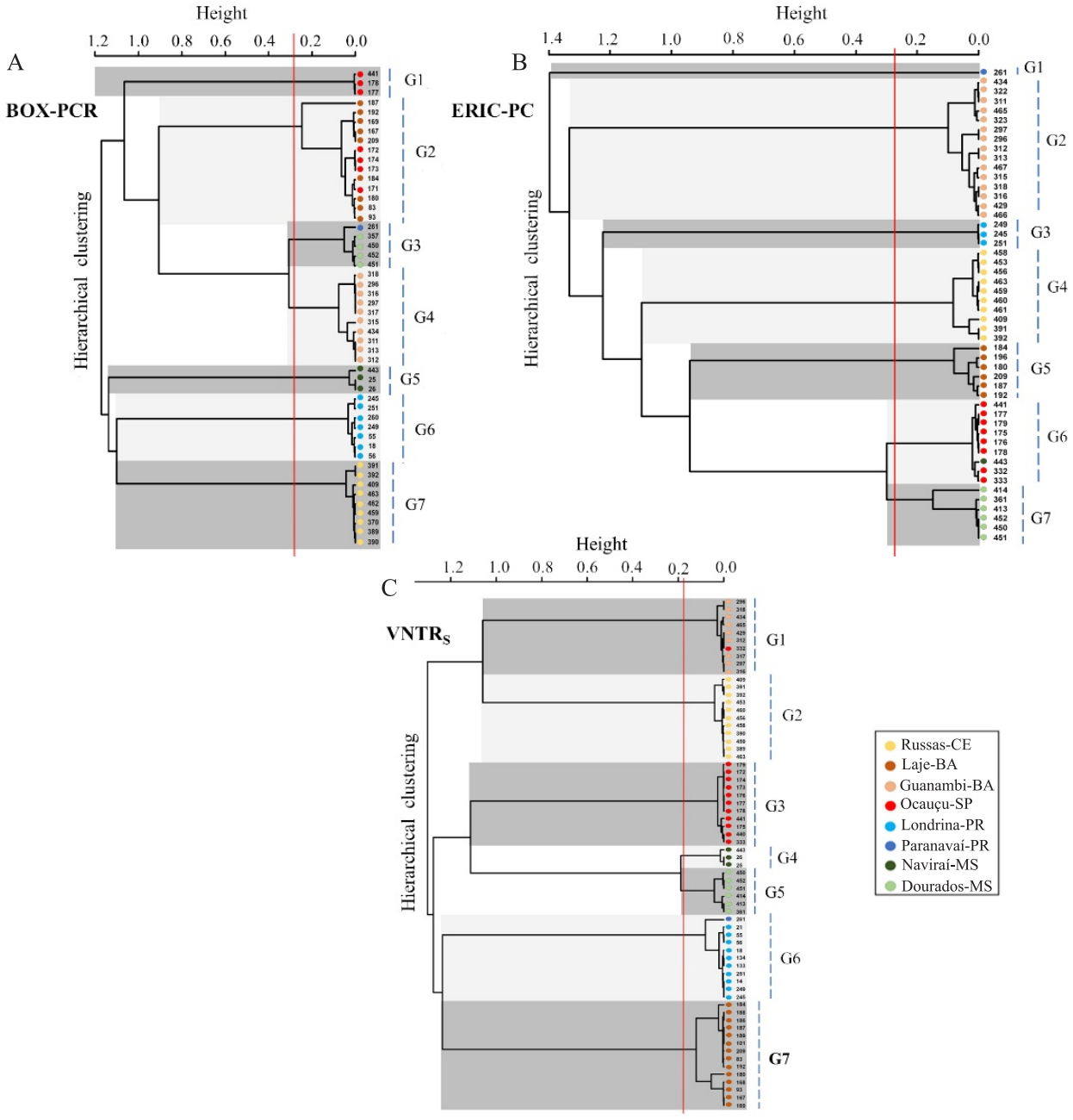

Figure 2. Cluster analysis of 50 isolates of *Xanthomonas phaseoli* pv. *manihotis* using the BOX-PCR (A), ERIC-PCR (B), and VNTR (C) markers, based on the Jaccard similarity coefficient and dendrogram construction by the unweighted pair group method using arithmetic means. The cut-off threshold is based on Mingoti (2005). G1–G7, seven formed groups. The isolates were obtained from populations from the following Brazilian states: BA, Bahia; SP, São Paulo; PR, Paraná; MS, Mato Grosso do Sul; and CE, Ceará.

similarity was 0.977 between the populations of Ceará and Bahia, and the minimum estimated value was 0.845 between the populations of Mato Grosso do Sul and São Paulo. In contrast, the maximum observed value of genetic distance was 0.069 between the populations of São Paulo and Ceará, whereas the lowest was 0.024 between the populations of Bahia and Ceará.

Genetic variation was estimated among all populations by the G_{ST} parameter, which was 0.1468 for the total population, indicating a moderate genetic differentiation between the subpopulations. The G_{ST} value was used to calculate the number of migrants (N_m), which was equal to 2.9068 for the total population (Table 5). This value could explain the low

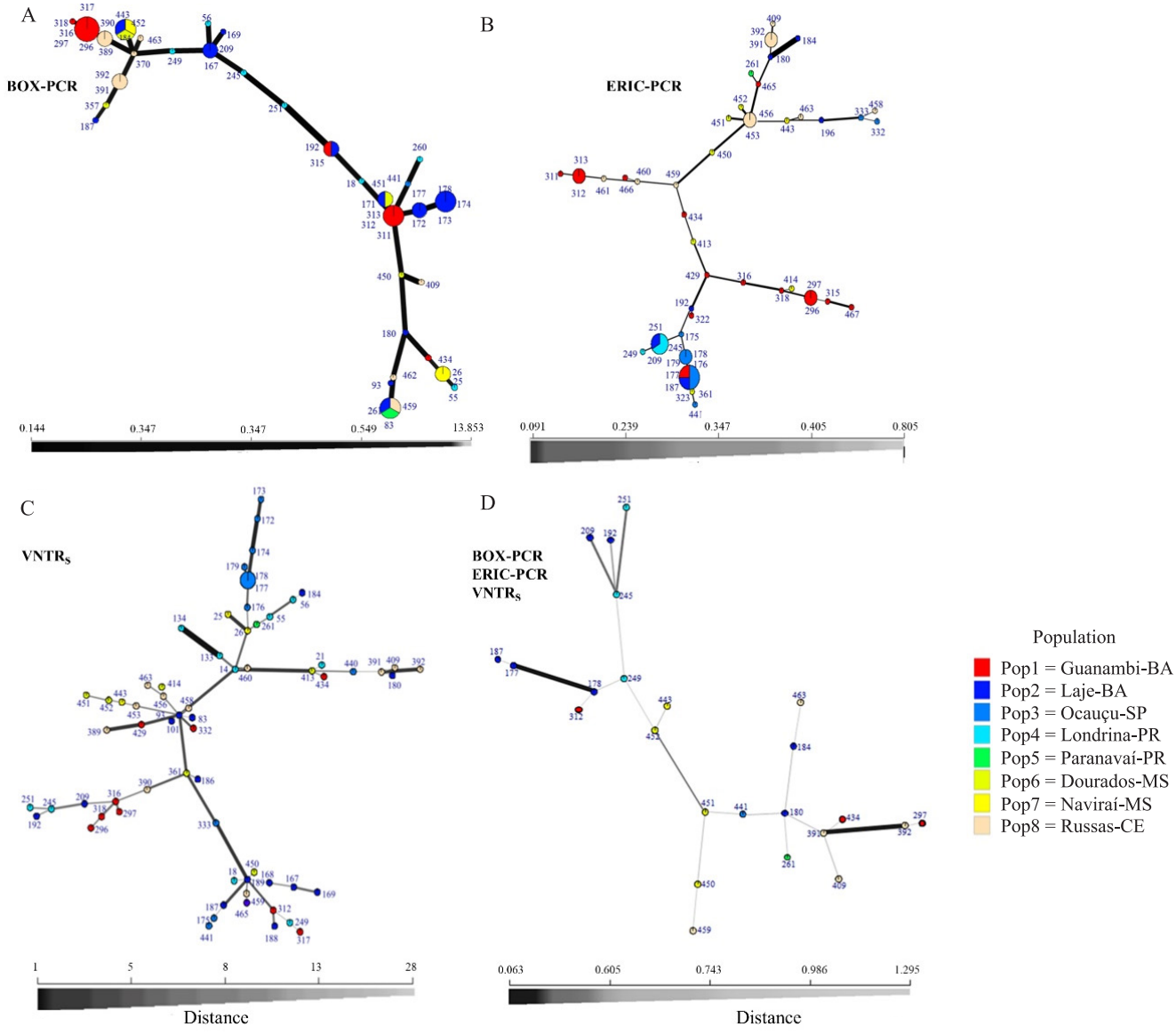

Figure 3. Minimum spanning network formed by markers BOX-PCR (A), ERIC-PCR (B), VNTRs (C), and their combination (D), showing the relationship between individual genotypes with multiple loci (MLGs) observed in the *Xanthomonas phaseoli* pv. *manihotis* populations. Each node represents a different MLG. The size and colors of the nodes correspond to the number of individuals and members of the population, respectively. The thickness and color of the border are proportional to the absolute genetic distance. The lengths of the edges are arbitrary. Brazilian states where the populations were obtained from: BA, Bahia; SP, São Paulo; PR, Paraná; MS, Mato Grosso do Sul; and CE, Ceará.

diversity between populations, in alignment with the results obtained with the highest diversity within and not between populations, as also found by Trujillo et al. (2014) among isolates from the Caribbean region of Colombian. This result may be related to the genetic composition in common between the primary inoculum of the sampled areas where the low levels of interpopulation genetic diversity occur, which suggests a continuous gene flow between populations (Goodwin et al., 1993), an important factor in the structure of populations. According to Wright (1949), when $N_m > 1.0$, that is, when one or more individuals migrate per generation, the effects of migration are sufficient to counteract the effects of drift, meaning that the number of migrants per generation prevents divergence among populations.

The AMOVA indicated that 8.83% of total variance is due to differences between populations and 91.17% within populations, showing that the greater genetic differentiation is in the intrapopulation component rather than in the interpopulation component (Table 6).

Therefore, the obtained results show how diversity is distributed in space, how quickly a population of *Xpm* can evolve, and which factors contribute to change. In the literature, *Xpm* populations have been characterized using many different DNA markers (Restrepo et al., 2000; Zárate-Chaves et al., 2021b; Díaz-Tatis et al., 2022; Rache et al., 2023). From 1995 to 2000, several prospection studies were conducted resulting in the collection of 906 isolates from Colombia, Venezuela, and Brazil. The characterization of these isolates resulted in the description of 111 haplotypes (Restrepo & Verdier, 1997; Restrepo et al., 2000, 2004). In addition, researches involving molecular characterization have shown that a new population structure can be established in a single harvest cycle (Restrepo et al., 2000, 2004; Verdier et al., 2004). In the present study, genetic diversity may be associated with rapid changes and the local adaptation of the pathogen since *Xpm* populations are unstable and can change rapidly in less than a year (Verdier et al., 2004).

High levels of genetic variability in populations of phytopathogens may allow them to adapt to different environments and genotypes of newly introduced resistant hosts (Churchill, 2011). Trujillo et al. (2014), characterizing *Xpm* populations from the Colombian Caribbean coast using AFLP and VNTRs markers, reported that 80% of genetic variation occurred within populations, which was attributed to the geographic characteristics of the origin of the isolates from each sampled population. Rache et al. (2019), using an improved multiple loci variable number of tandem repeat scheme that targeted 15 VNTR loci (MLVA-15), were able to distinguish 88.9% of haplotypes within the *Xpm* strain from the Caribbean region of Colombia.

Bart et al. (2012) carried out a high-throughput sequencing and identification of effectors to target durable resistance to CBB of temporarily diverse *Xam* strains from 65 different geographic locations. The authors verified the phylogeny of Brazilian *Xam* strains, showing a distinct clade that shares a common ancestor with both Colombia and African clades,

Table 3. Diversity parameters in five populations of *Xanthomonas phaseoli* pv. *manihotis*[(1)].

Population[(2)]	n	N_a	N_e	H	I	NPL	PPL (%)
BA	24	1.9333 (σ±0.2508)	1.3335 (σ±0.2467)	0.2263 (σ±0.1324)	0.3686 (σ±0.1801)	84	93.33
SP	11	1.5222 (σ±0.5023)	1.2446 (σ±0.3118)	0.1543 (σ±0.1737)	0.2413 (σ±0.2554)	47	52.22
PR	11	1.5222 (σ±0.5023)	1.2769 (σ±0.3437)	0.1679 (σ±0.1869)	0.2570 (σ±0.2707)	47	52.22
MS	9	1.5556 (σ±0.4997)	1.2916 (σ±0.3349)	0.1794 (σ±0.1838)	0.2755 (σ±0.2679)	50	55.56
CE	11	1.6222 (σ±0.4875)	1.2888 (σ±0.3154)	0.1826 (σ±0.1744)	0.2857 (σ±0.2533)	56	62.22

[(1)]n, number of isolates; Na, number of observed alleles; Ne, effective number of alleles; H, Nei's gene diversity; I, Shannon diversity index; NPL, number of polymorphic loci; and PPL, percentage of polymorphic loci. [(2)]Brazilian states: BA, Bahia; SP, São Paulo; PR, Paraná; MS, Mato Grosso do Sul; and CE, Ceará. Number between parentheses represents standard deviation.

indicating the occurrence of a global movement and the need for more globally effective resistance strategies (Bart et al., 2012).

Restrepo et al. (2004), when evaluating strains of *Xpm* from six locations spanning four different edaphoclimatic zones in Colombia from 1995 to 1999, identified 45 different *Xam* RFLP types or haplotypes. Additionally, the authors concluded that the presence of identical RFLP patterns in several fields of the same or different edaphoclimatic zone indicated pathogen migration.

In the present study, the populations of *Xpm* from different regions of Brazil resulted in a clonal population structure for each region according to the host and edaphoclimatic conditions regardless of the region. Therefore, it can be assumed that *Xpm* populations are structurally organized at the regional level of the country, which could be because producers use endemic cassava species in each region, which added to the edaphoclimatic conditions and selection pressure of *Xpm* populations (Teixeira et al., 2021). Contrastingly, Lelis et al. (2014), evaluating the diversity of 85 *Xpm* isolates collected in the main producing regions using the AFLP technique, found no correlation between geographic origin and pathogenicity.

Therefore, resistant cassava varieties should be introduced in each region according to the degree of diversity, structure, and aggressiveness of the pathogen. Despite the difference in the number of samples, it is evident that the VNTR markers provide congruent results for populations of *Xpm*. It is worth noting that this is the first study using VNTR markers in *Xmp* populations from Brazil. The greater intrapopulation diversity observed indicates the urgent need for adopting resistant varieties and measures to minimize the advancement of the pathogen, as, for example, the use of pathogen-free cuttings.

All this information is of paramount importance for establishing disease management strategies. For this, germplasm with a specific set of *Xpm* strains that represents the range of genetic diversity within a production area should be evaluated. Furthermore, the variability of *Xpm* populations and migrations should be considered to improve quarantine measures in order to prevent the importation of infected planting material to areas with a low incidence of the disease.

Table 4. Estimates of genetic similarity (upper diagonal) and genetic distance (lower diagonal) according to Nei (1972) among the five populations of *Xanthomonas phaseoli* pv. *manihotis*.

Population[(1)]	BA	SP	PR	MS	CE
BA	****	0.952	0.960	0.962	0.977
SP	0.049	****	0.950	0.845	0.934
PR	0.041	0.052	****	0.952	0.940
MS	0.038	0.056	0.049	****	0.955
CE	0.024	0.069	0.062	0.047	****

[(1)]Obtained in the following Brazilian states: BA, Bahia; SP, São Paulo; PR, Paraná; MS, Mato Grosso do Sul; and CE, Ceará.

Table 5. Estimate of genetic diversity, population differentiation (G_{ST}), and gene flow estimate for populations of *Xanthomonas phaseoli* pv. *manihotis*[(1)].

	H_T	H_S	G_{ST}	Gene flow
Total population	0.2134	0.1821	0.1468	2.9068
(average)	(σ±0.0204)	(σ±0.0140)		

[(1)]H_T, total diversity; and H_S, expected diversity. Number between parentheses represents standard deviation.

Table 6. Analysis of molecular variance in populations (pop.) of *Xanthomonas phaseoli* pv. *manihotis*[(1)].

Source of variation	DF	Sum of squares	Estimated variance	Percentage	p-value (<0.000)
Between pop.	4	82.561	0.899	8.830	
Within pop.	61	565.985	9.278	91.170	
Total	65	648.546	10.177		
F_{ST} = 0.088					

[(1)]DF, degrees of freedom, with probabilities calculated by 1,023 random permutations; and F_{ST}, level of genetic diversity.

Conclusions

1. ERIC-PCR and VNTRs are the best markers for assessing genetic variability in eight *Xanthomonas phaseoli* pv. *manihotis* (*Xpm*) populations from five cassava (*Manihot esculenta*) producing states in Brazil, and ERIC-PCR is the marker that best separated the groups by population and presented a higher similarity between the isolates of the same population.

2. The observed selection pressure, together with the mode of reproduction and mechanisms that increase genetic variability, allows of the pathogen populations to adapt according to the variation of microclimates, contributing to a differentiated reproductive success.

3. The genetic diversity of X*pm* is key for implementing efficient management strategies and for improving the monitoring of cassava bacterial blight in cassava crops.

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References

BART, R.; COHN, M.; KASSEN, A.; MCCALLUM, E.J.; SHYBUT, M.; PETRIELLO, A.; KRASILEVA, K.; DAHLBECK, D.; MEDINA, C.; ALICAI, T.; KUMAR, L.; MOREIRA, L.M.; RODRIGUES NETO, J.; VERDIER, V.; SANTANA, M.A.; KOSITCHAROENKUL, N.; VANDERSCHUREN, H.; GRUISSEM, W.; BERNAL, A.; STASKAWICZ, B.J. High-throughput genomic sequencing of cassava bacterial blight strains identifies conserved effectors to target for durable resistance. **Proceedings of the National Academy of Sciences of the United States of America**, v.109, p.E1972-E1979, 2012. DOI: https://doi.org/10.1073/pnas.1208003109.

BAYATA, A. Review on nutritional value of cassava for use as a staple food. **Science Journal of Analytical Chemistry**, v.7, p.83-91, 2019. DOI: https://doi.org/10.11648/j.sjac.20190704.12.

CHURCHILL, A.C.L. *Mycosphaerella fijiensis*, the black leaf streak pathogen of banana: progress towards understanding pathogen biology and detection, disease development, and the challenges of control. **Molecular Plant Pathology**, v.12, p.307-328, 2011. DOI: https://doi.org/10.1111/j.1364-3703.2010.00672.x.

DÍAZTATIS, P.A.; OCHOA, J.C.; RICO, E.M.; RODRÍGUEZ, C.; MEDINA, A.; SZUREK B.; CHAVARRIAGA, P.; LÓPEZ, C.E. RXam2, a NLR from cassava (*Manihot esculenta*) contributes partially to the quantitative resistance to *Xanthomonas phaseoli* pv. manihotis. **Plant Molecular Biology**, v.109, p.313-324, 2022. DOI: https://doi.org/10.1007/s11103-021-01211-2.

EXCOFFIER, L.; LAVAL, G.; SCHNEIDER, S. Arlequin (version 3.0): an integrated software package for population genetics data analysis. **Evolutionary Bioinformatics Online**, v.1, p.47-50, 2005. DOI: https://doi.org/10.1177/117693430500100003.

FAO. Food and Agriculture Organization of the United Nations. **Faostat**. Available at: <https://www.fao.org/faostat/en/#data/QCL/visualize>. Accessed on: Feb. 12 2023.

GOODWIN, S.B.; SAGHAI MAROOF, M.A.; ALLARD, R.W.; WEBSTER, R.K. Isozyme variation within and among populations of *Rhynchosporium secalis* in Europe, Australia and the United States. **Mycological Research**, v.97, p.49-58, 1993. DOI: https://doi.org/10.1016/S0953-7562(09)81112-X.

KAPANTAIDAKI, D.E.; ANTONATOS, S.; EVANGELOU, V.; PAPACHRISTOS, D.P.; MILONAS, P. Genetic and endosymbiotic diversity of Greek populations of *Philaenus spumarius*, *Philaenus signatus* and *Neophilaenus campestris*, vectors of *Xylella fastidiosa*. **Scientific Reports**, v.11, art.3752, 2021. DOI: https://doi.org/10.1038/s41598-021-83109-z.

KOEUTH, T.; VERSALOVIC, J.; LUPSKI, J.R. Differential subsequence conservation of interspersed repetitive *Streptococcus pneumoniae* BOX elements in diverse bacteria. **Genome Research**, v.5, p.408-418, 1995. DOI: https://doi.org/10.1101/gr.5.4.408.

LELIS, T. de P. **Variabilidade genética de X*anthomonas axonopodis* pv. *manihotis* nas regiões Nordeste e Centro Sul do Brasil**. 2014. 434p. Dissertação (Mestrado) – Universidade Federal de Viçosa, Viçosa.

LEWONTIN, R.C. The apportionment of human diversity. In: DOBZHANSLY, T.; HECHT, M.K.; STEERE, W.C (Ed.). **Evolutionary biology**. New York: Appleton-Century-Crofts, 1972. p.381-398. DOI: https://doi.org/10.1007/978-1-4684-9063-3_14.

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.E.; BERNAL, A. The role of type III effectors from *Xanthomonas axonopodis* pv. *manihotis* in virulence and suppression of plant immunity. **Molecular Plant Pathology**, v.19, p.593-606, 2018. DOI: https://doi.org/10.1111/mpp.12545.

MELO, R.de C.C.; BRAGANÇA, C.A.D.; PESTANA, K.N.; SILVA, H.S.A. da; FERREIRA, C.F.; OLIVEIRA, S.A.S. de. Improvement of the specific detection of *Xanthomonas phaseoli* pv. *manihotis* based on the *pthB* gene. **Acta Scientiarum. Agronomy**, v.41, e42708, 2019. DOI: https://doi.org/10.4025/actasciagron.v41i1.42708.

MINGOTI, S.A. **Análise de dados através de métodos de estatística multivariada**: uma abordagem aplicada. Belo Horizonte: UFMG, 2005. 295p.

MORA, R.E.; RODRIGUEZ, M.A.; GAYOSSO, L.Y.; LÓPEZ, C.E. Using *in vitro* plants to study the cassava response to *Xanthomonas phaseoli* pv. *manihotis* infection. **Tropical Plant Pathology**, v.44, p.423-429, 2019. DOI: https://doi.org/10.1007/s40858-019-00296-x.

NAKAMURA, Y.; LEPPERT, M.; O'CONNELL, P.; WOLFF, R.; HOLM, T.; CULVER, M.; MARTIN, C.; FUJIMOTO, E.; HOFF, M.; KUMLIN, E.; WHITE, R. Variable number of tandem repeat (VNTR) markers for human gene mapping. **Science**, v.235, p.1616-1622, 1987. DOI: https://doi.org/10.1126/science.3029872.

NEI, M. Analysis of gene diversity in subdivided populations. **Proceedings of the National Academy of Sciences of the United States of America**, v.70, p.3321-3323, 1973. DOI: https://doi.org/10.1073/pnas.70.12.3321.

NEI, M. Estimation of average heterozygosity and genetic distance from a small number of individuals. **Genetics**, v.89, p.583-590, 1978. DOI: https://doi.org/10.1093/genetics/89.3.583.

NEI, M. Genetic distance between populations. **American Naturalist**, v.106, p.283-292, 1972. DOI: https://doi.org/10.1086/282771.

R CORE TEAM. **R**: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2018.

RACHE, L.; BLONDIN, L.; FLORES, C.; TRUJILLO, C.; SZUREK, B.; RESTREPO, S.; KOEBNIK, R.; BERNAL, A.; VERNIÈRE, C. An optimized microsatellite scheme for assessing populations of *Xanthomonas phaseoli* pv. *manihotis*. **Phytopathology**, v.109, p.859-869, 2019. DOI: https://doi.org/10.1094/PHYTO-06-18-0210-R.

RACHE, L.; BLONDIN, L.; TATIS, P.D.; FLORES, C.; CAMARGO, A.; KANTE, M.; WONNI, I.; LÓPEZ, C.; SZUREK, B.; DUPAS, S.; PRUVOST, O.; KOEBNIK, R.; RESTREPO, S.; BERNAL, A.; VERNIÈRE, C. A minisatellite-based MLVA for deciphering the global epidemiology of the bacterial cassava pathogen *Xanthomonas phaseoli* pv. *manihotis*. **PLoS One**, v.18, e0285491, 2023. DOI: https://doi.org/10.1371/journal.pone.0285491.

RESTREPO, S.; VELEZ, C.M.; DUQUE, M.C.; VERDIER, V. Genetic structure and population dynamics of *Xanthomonas axonopodis* pv. *manihotis* in Colombia from 1995 to 1999. **Applied and Environmental Microbiology**, v.70, p.255-261, 2004. DOI: https://doi.org/10.1128/AEM.70.1.255-261.2004.

RESTREPO, S.; VÉLEZ, C.M.; VERDIER, V. Measuring the genetic diversity of *Xanthomonas axonopodis* pv. *manihotis* within different fields in Colombia. **Phytopathology**, v.90, p.683-690, 2000. DOI: https://doi.org/10.1094/PHYTO.2000.90.7.683.

RESTREPO, S.; VERDIER, V. Geographical differentiation of the population of *Xanthomonas axonopodis* pv. *manihotis* in Colombia. **Applied and Environmental Microbiology**, v.63, p.4427-4434, 1997. DOI: https://doi.org/10.1128/aem.63.11.4427-4434.1997.

SONNEWALD, U.; FERNIE, A.R.; GRUISSEM, W.; SCHLÄPFER, P.; ANJANAPPA, R.B.; CHANG, S.-H.; LUDEWIG, F.; RASCHER, U.; MULLER, O.; VAN DOORN, A.M.; RABBI, I.Y.; ZIERER, W. The cassava source–sink project: opportunities and challenges for crop improvement by metabolic engineering. **The Plant Journal**, v.103, p.1655-1665, 2020. DOI: https://doi.org/10.1111/tpj.14865.

TEIXEIRA, J.H. dos S.; GUIMARÃES, M.A.S.; CARDOSO, S.C.; BRITO, A. dos S.; DINIZ, R.P.; OLIVEIRA, E.J. de; OLIVEIRA, S.A.S. de. Evaluation of resistance to bacterial blight in Brazilian cassava germoplasm and disease-yield relationships. **Tropical Plant Pathology**, v.46, p.324-335, 2021. DOI: https://doi.org/10.1007/s40858-021-00419-3.

TRUJILLO, C.A.; ARIAS-ROJAS, N.; POULIN, L.; MEDINA, C.A.; TAPIERO, A.; RESTREPO, S.; KOEBNIK, R.; BERNAL, A.J. Population typing of the causal agent of cassava bacterial blight in the Eastern Plains of Colombia using two types of molecular markers. **BMC Microbiology**, v.14, art.161, 2014. DOI: https://doi.org/10.1186/1471-2180-14-161.

VELEY, K.M.; ELLIOTT, K.; JENSEN, G.; ZHONG, Z.; FENG, S.; YODER, M.; GILBERT, K.B.; BERRY, J.C.; LIN, Z.-J.D.; GHOSHAL, B.; GALLEGO-BARTOLOMÉ, J.; NORTON, J.; MOTOMURA-WAGES, S.; CARRINGTON, J.C.; JACOBSEN, S.E.; BART, R.S. Improving cassava bacterial blight resistance by editing the epigenome. **Nature Communications**, v.14, art.85, 2023. DOI: https://doi.org/10.1038/s41467-022-35675-7.

VERDIER, V.; MOSQUERA, G.; ASSIGBÉTSÉ, K. Detection of the cassava bacterial blight pathogen, *Xanthomonas axonopodis* pv. *manihotis*, by polymerase chain reaction. **Plant Disease**, v.82, p.79-83, 1998. DOI: https://doi.org/10.1094/PDIS.1998.82.1.79.

VERDIER, V.; RESTREPO, S.; MOSQUERA, G.; JORGE, V.; LOPEZ, C. Recent progress in the characterization of molecular determinants in the *Xanthomonas axonopodis* pv. *Manihotis*-cassava interaction. **Plant Molecular Biology**, v.56, p.573-584, 2004. DOI: https://doi.org/10.1007/s11103-004-5044-8.

VERSALOVIC, J.; KOEUTH, T.; LUPSKI, R.J. Distribution of repetitive DNA sequences in eubacteria and application to finerprinting of bacterial genomes. **Nucleic Acids Research**, v.19, p.6823-6831, 1991. DOI: https://doi.org/10.1093/nar/19.24.6823.

WRIGHT, S. The genetical structure of populations. **Annals of Eugenics**, v.15, p.323-354, 1949. DOI: https://doi.org/10.1111/j.1469-1809.1949.tb02451.x.

YE, Y.; OUYANG, Z.; GUO, C.; WU, Y.; LI, J.; ABDOULAYE, A.H.; TANG, L.; XIA, W.; CHEN, Y. Identification of two cassava receptor-like cytoplasmic kinase genes related to disease resistance via genome-wide and functional analysis. **Genomics**, v.115, art.110626, 2023. DOI: https://doi.org/10.1016/j.ygeno.2023.110626.

YEH, F.C.; YANG, R.; BOYLE, T. **Popgene version 1.31**: Microsoft Windows-based freeware for population genetic analysis: quick user guide. Edmonton: University of Alberta, 1999.

ZÁRATE-CHAVES, C.A.; GÓMEZ de la CRUZ, D.; VERDIER, V.; LÓPEZ, C.E.; BERNAL, A.; SZUREK, B. Cassava diseases caused by *Xanthomonas phaseoli* pv. *manihotis* and *Xanthomonas cassavae*. **Molecular Plant Pathology**, v.22, p.1520-1537, 2021a. DOI: https://doi.org/10.1111/mpp.13094.

ZÁRATE-CHAVES, C.A.; OSORIO-RODRÍGUEZ, D.; MORA, R.E.; PÉREZ-QUINTERO, A.L.; DEREEPER, A.; RESTREPO, S.; LÓPEZ, C.E.; SZUREK, B.; BERNAL, A. TAL effector repertoires of strains of *Xanthomonas phaseoli* pv. *manihotis* in commercial cassava crops reveal high diversity at the country scale. **Microorganisms**, v.9, art.315, 2021b. DOI: https://doi.org/10.3390/microorganisms9020315.