Pesquisa Agropecuária Brasileira

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Genetics/ Original Article

Reaction of the half-sibling progeny obtained from the BRS Bravo rootstock to citrus tristeza virus

Abstract – The objective of this work was to evaluate the reaction of citrus hybrids to natural infection by local isolates of the citrus tristeza virus (CTV; species: *Closterovirus tristezae*). Half-siblings obtained through open pollination were evaluated, and cultivar BRS Bravo, known for its tolerance to CTV, was used as the female parent. The experiment was conducted at the experimental field of Embrapa Mandioca e Fruticultura and involved 1,329 hybrids. Of these, 130 individuals were selected at 5–6 years of age, based on visual evaluations of vigor to favor plants with a vigorous vegetative growth through the mass selection method. The reaction to CTV was evaluated by the presence and severity of stem pitting symptoms. For this, ten branches per plant analyzed on a scale from 1 (absence) to 5 (entire surface covered by superficial or deep pits). CTV infection was determined by indirect ELISA using a polyclonal antiserum against CTV. Samples that tested negative in the serological assay were further analyzed by RT–PCR using primers F-CN119 (5'-AGATCTACCATGGACGACGAAACAAAG-3') and R-CN120 (5'-GAATTCGCGGCCGCTCAACGTGTGTTAAATTTCC-3'). Data were subjected to the principal component analysis, which showed that 46.9% of the hybrids were classified as tolerant and 53.1% as resistant to CTV, highlighting their potential for the selection of new citrus rootstocks.

Index terms: *Citrus* spp., *Closterovirus tristezae*, stem pitting, tolerance.

Reação da progênie de meios-irmãos obtida do porta-enxerto BRS Bravo ao citrus tristeza virus

Resumo – O objetivo deste trabalho foi avaliar a reação de híbridos de citros à infecção natural por isolados locais do citrus tristeza virus (CTV; espécie: *Closterovirus tristezae*). Foram avaliados meios-irmãos obtidos por polinização aberta, e a cultivar BRS Bravo, conhecida por sua tolerância ao CTV, foi utilizada como genitor feminino. O experimento foi conduzido no campo experimental da Embrapa Mandioca e Fruticultura e envolveu 1,329 híbridos. Desses, 130 indivíduos foram selecionados aos 5–6 anos de idade, com base em avaliações visuais de vigor, para favorecer plantas com crescimento vegetativo vigoroso por meio do método de seleção massal. A reação ao CTV foi avaliada pela presença e severidade de sintomas de caneluras nos ramos. Para tanto, foram analisados dez ramos por planta em uma escala de 1 (ausência) a 5 (toda a superfície coberta por caneluras superficiais ou profundas). A infecção por CTV foi determinada pelo teste ELISA indireto, tendo-se utilizado antissoro policlonal contra o CTV. As amostras com resultado negativo no teste sorológico também foram avaliadas por RT–PCR, tendo-se utilizado os *primers* F-CN119 (5'-AGATCTACCATGGACGACGAAACAAAG-3') e R-CN120 (5'-GAATTCGCGGCCGCTCAACGTGTGTTAAATTT CC-3'). Os dados foram submetidos à análise de componentes principais, que mostrou que 46.9% dos híbridos foram classificados como tolerantes e 53.1% como

Luana Laís de Almeida dos Santos (✉)
Universidade Federal do Recôncavo da Bahia, Cruz das Almas, BA, Brazil.
E-mail: luanalaais@gmail.com

Danilo Pereira Costa
Módulo Rural Ltda., Itabuna, BA, Brazil.
E-mail: danilocosta_1739@hotmail.com

Raquel Moreira Oliveira
Universidade Federal da Bahia, Salvador, BA, Brazil. E-mail: raquel_123123@hotmail.com

Samuel Araújo dos Santos
Universidade Federal da Bahia, Salvador, BA, Brazil. E-mail: samuelaraujo@ufba.br

Sandriele Santos de Araujo
Universidade Federal do Recôncavo da Bahia, Cruz das Almas, BA, Brazil.
E-mail: sandrielessa@gmail.com

Edilane dos Santos Maciel
Universidade Federal do Recôncavo da Bahia, Cruz das Almas, BA, Brazil.
E-mail: edilanemaciel5@gmail.com

Walter dos Santos Soares Filho
Embrapa Mandioca e Fruticultura, Cruz das Almas, BA, Brazil.
E-mail: walter.soares@embrapa.br

Cristiane de Jesus Barbosa
Embrapa Mandioca e Fruticultura, Cruz das Almas, BA, Brazil.
E-mail: cristiane.barbosa@embrapa.br

✉ Corresponding author

Received
July 7, 2025

Accepted
November 3, 2025

How to cite
SANTOS, L.L. de A. dos; COSTA, D.P.; OLIVEIRA, R.M.; SANTOS, S.A. dos; ARAUJO, S.S. de; MACIEL, E. dos S.; SOARES FILHO, W. dos S.; BARBOSA, C. de J. Reaction of the half-sibling progeny obtained from the BRS Bravo rootstock to citrus tristeza virus. **Pesquisa Agropecuária Brasileira**, v.61, e04184, 2026. DOI: https://doi.org/10.1590/S1678-3921.pab2026.v61.04184.

resistentes ao CTV, evidenciando seu potencial para seleção de novos porta-enxertos de citros.

Termos para indexação: *Citrus spp*., *Closterovirus tristezae*, resistência, caneluras, tolerância.

Introduction

Tristeza is one of the most destructive diseases in global citrus cultivation, responsible for the loss of over 100 million trees in the past century (Bar-Joseph et al., 2021; Folimonova & Sun, 2022). The causal agent, *Closterovirus tristezae* (citrus tristeza virus, CTV), family *Closteroviridae*, infects most citrus species. Endemic in Brazil (Giampani et al., 2016), CTV is transmitted via infected propagative material and aphid vectors, mainly the brown citrus aphid (*Toxoptera citricidus* Kirkaldy) (Sun et al., 2024).

The most common symptoms include stem pitting on branches and trunks of sensitive plant varieties. In intolerant plants like sour orange (*Citrus* × *aurantium* L.), severe symptoms, such as quick decline, result in bronze discoloration, phloem necrosis, and reduced root systems (Folimonova & Sun, 2022). Other symptoms include seedling yellowing, which affects oranges, lemons, grapefruits, and rough lemons (*Citrus jambhiri* Lush.) (Sun et al., 2024). The specific CTV reaction of various citrus species and varieties depends on the plant ability to replicate the virus post-infection, the tolerance of phloem tissues to viral presence, and the interaction of these aspects when considering both the scion and the rootstock.

In general, citrus phloem tissues may be tolerant, intolerant, or partially tolerant to viral particles. Resistance, defined as the ability to replicate CTV, also varies: some do not replicate the virus, others do abundantly, and some show partial behavior. As CTV spreads mainly through the phloem, host tolerance influences systemic infection; susceptible species show widespread cell infection, while tolerant ones restrict it. Additionally, antiviral proteins, RNA interference, and secondary metabolites may block further replication. Thus, phloem tolerance and molecular defenses jointly shape the plant ability to limit CTV, highlighting the complexity of virus–host interactions in citrus (Khalilzadeh et al., 2025).

Disease control includes the use of tolerant rootstocks, and in the case of sensitive citrus species used as scions, pre-immunization with less aggressive CTV isolates is also employed (Sharma, 2023). In Brazil, the replacement of CTV-intolerant rootstocks with tolerant alternatives has enabled coexistence with the disease (Sun et al., 2024). Consequently, the success of citrus cultivation in endemic areas depends on breeding programs to develop new CTV tolerant rootstocks in Brazil (Carvalho et al., 2019, 2021). The BRS Bravo rootstock cultivar {*Citrus sunki* (Hayata) hort. ex Tanaka × [*Citrus limonia* Osbeck × *Poncirus trifoliata* (L.) Raf.]}, selected by Embrapa Mandioca e Fruticultura, stands out for its tolerance to CTV and high productivity, representing a promising alternative to 'Rangpur' lime (*C. limonia*) (Rodrigues et al., 2015; Costa et al., 2020).

The objective of this work was to evaluate the reaction of citrus hybrids to natural infection by local isolates of CTV.

Materials and Methods

The experiment was carried out in the citrus orchard of the Embrapa Mandioca e Fruticultura experimental field, located in the municipality of Cruz das Almas, in the Recôncavo Baiano region, state of Bahia, Brazil (12°40'39"S, 39°06'23"W, at 225 m of altitude). It comprised three populations of half-sibling hybrids, established in July of 2016, with a 4.0×1.5 m spacing. These populations grew under rainfed conditions without additional fertilization, ensuring exposure to a rustic field environment. The site featured a Latossolo Amarelo dystrophic, i.e., Ferralsol, typical of the flat relief found in the Northeastern Coastal Tablelands region (Santos et al., 2025). According to the Köppen classification (1931), the local climate is tropical, hot, and humid, ranging from Aw to Am, with a mean annual temperature of 23.9ºC, 81% relative humidity, and 1,130 mm of average rainfall, which concentrates between March and August (Silva et al., 2016).

The plant material consisted of half-siblings obtained via open pollination of the BRS Bravo rootstock cultivar, using four seven-year-old plants as female parents. Potential pollen donors included various *Citrus* accessions, such as the citrandarins 'San Diego' (*C. sunki* × *P. trifoliata* 'Swingle'), 'Indio' (*C. sunki* × *P. trifoliata* 'English'), and 'Riverside' (*C.* sunki × P. trifoliata 'English'); the Rangpur lime 'Cravo Santa Cruz' (*C. limonia*); the hybrids TSKC × CTSW [*C. sunki* common selection × (*Citrus paradisi* Macfad. × *P. trifoliata*)], TSKFL × CTTR {*C. sunki* 'Florida' selection × [*Citrus sinensis* (L.) Osbeck × *P. trifoliata* 'Troyer')]};

DOI: 10.1590/S1678-3921.pab2026.v61.04184

among others. Seeds from 'BRS Bravo' were germinated under protected conditions. After emergence, nucellar individuals, i.e. genetically identical to the mother plant, were discarded, and seedlings of zygotic or hybrid origin were identified based on leaf morphology (Ferreira et al., 2013). The identified hybrids were transplanted to the field as ungrafted plants.

A total of 1,329 hybrids were established in the orchard, and 130 were selected through successive visual evaluations carried out between October of 2021 and August of 2022. At 5 to 6 years of age, these hybrids had been fully exposed to natural infection by local isolates of CTV, since the virus is endemic in Brazilian citrus orchards and transmitted abundantly by aphid vectors (Giampani et al., 2016). Transmission occurs during brief feeding periods and peaks with continuous exposure of 24–48 hours. Since aphids transmit CTV semipersistently and retain inoculativity for up to two days, repeated feeding over time makes infection nearly inevitable. Therefore, evaluations at 5–6 years ensured adequate exposure to local isolates and consistent symptom expression (Frias et al., 2023).

Individuals selected via mass selection (Orton, 2020) showed vigorous growth, demonstrating good adaptation to the Northeastern Coastal Tablelands, an environment marked by a pronounced dry season, a cohesive soil layer that limits root growth, and low fertility (Lira et al., 2016). For comparison, the cultivar BRS Bravo, used as the female parent, was also evaluated, enabling validation of hybrid performance relative to both parent and reference standards (Rodrigues et al., 2014).

To assess the presence and severity of stem pitting symptoms, ten branches measuring approximately 20 cm from different quadrants of every hybrid plant were collected. To facilitate bark removal, the branches were autoclaved at 200°C for 10 min, allowing direct inspection of the phloem. The assessment was conducted by three evaluators using the scale described by Meissner Filho et al. (2002): 1, absence of stem pitting on the branches; 2, sparse presence of stem pitting on the branches; 3, intermediate number of stem pitting on the branches; 4, presence of multiple superficial stem pitting or a few deep stem pitting on the branches; and 5, the entire surface of the branch is covered with stem pitting on the branches. The average score obtained from the ten branches of each plant was recorded.

CTV infection was detected using the indirect ELISA test (Koenig, 1981), conducted between January and March, and between June and July of 2022 and 2023. Samples from young branches were macerated in carbonate buffer and diluted at a 1:100 ratio. The polyclonal antiserum, provided by Instituto Agronômico de Campinas, was diluted at a 1:1,000 ratio, and detection was performed using anti-rabbit IgG conjugated to alkaline phosphatase (Sigma Aldrich, St. Louis, MO, USA), diluted at a 1:10,000 ratio. Positive and negative CTV controls were used to determine the absorbance threshold. To establish the absorbance threshold for distinguishing infected from healthy samples, the mean absorbance value of the negative control plus three standard deviations (x + 3s) was applied. Consequently, samples were considered positive if at least one replicate exhibited an absorbance reading exceeding the mean negative control value plus the standard deviation in each assay.

Samples that tested negative in the serological test were further evaluated using RT-PCR to detect CTV RNA. Total RNA was extracted using Trizol (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions. Reverse transcription was performed using the M-MLV enzyme (Promega, Madison WI, USA), following the manufacturer recommendations. An aliquot of the obtained cDNA was used to amplify the coat protein gene using the specific primers: CN-119 (5’-AGATCTACCATGGACGACGAAACAAAG-3’), and CN-120 (5’-GAATTCGCGGCCGCTCAACGTG TGTTAAATTTCC-3’) in a thermal cycler programmed for 35 cycles of 2 min at 94°C, 30 seconds at 55°C, and 1 min at 72°C. The PCR product was visualized under ultraviolet light after electrophoresis on a 1% agarose gel containing ethidium bromide.

Hybrid classification was defined based on the results of symptom evaluation and on the diagnostic tests results: resistant – absence of both stem pitting symptoms and CTV infection; tolerant – presence of CTV infection, but with either the absence of stem pitting symptoms or the presence of sparse stem pitting symptoms; intolerant – intermediate number of stem pitting symptoms or entire branch surface covered with symptoms and CTV infection (Bordignon et al., 2003).

Principal Component Analysis (PCA) was used to evaluate the results obtained for the presence and severity of stem pitting symptoms, and results from

DOI: 10.1590/S1678-3921.pab2026.v61.04184

the indirect ELISA and RT-PCR diagnostic tests. For this purpose, data were standardized prior to PCA, which was performed using the ‘prcomp’ function from the ‘stats’ package in the R environment (R Core Team, 2025). Hybrid grouping was visualized in a bivariate plot of the first two principal components (PC1 and PC2), with overlaid two-dimensional kernel density plots, and differentiation based on the hybrid classification regarding virus tolerance (resistant or tolerant). The analysis was performed using R and the packages ggplot2, ggrepel, and viridis.

Results and Discussion

Evaluations of stem pitting presence and severity revealed that all hybrids achieved average scores equal to or less than 1.53. Hybrids H051, H058, and H047 displayed the highest severity indices, with values of 1.37, 1.50, and 1.53, respectively (Table 1). According to the Meissner Filho et al. (2002) scale, these scores fall between 1 (absence of stem pitting) and 2 (presence of sparse stem pitting). Additionally, 47.69% of the hybrids analyzed showed no stem pitting as shown in Table 1 and Table 2. These results indicate that most hybrids did not develop significant stem pitting symptoms and therefore are not sensitive to the local CTV isolates.

The indirect ELISA tests detected CTV in 47 (36.15%) of the 130 selected hybrids (Table 1). However, these individuals did not show severe stem pitting symptoms (Meissner Filho et al., 2002) and were classified as tolerant according to the criteria adopted in this study (Bordignon et al., 2003). The 83 (63.85%) hybrids that tested negative in the indirect ELISA test were subsequently evaluated by RT-PCR. Among these, 15 hybrids (11.54% of the total) were confirmed to be infected. This demonstrates the higher sensitivity of RT-PCR compared with indirect ELISA for detecting low concentrations of the virus. The BRS Bravo cultivar, used as reference, showed a mean stem-pitting score of 1.0, testing negative by ELISA but positive by RT-PCR, and was therefore classified as tolerant. This result is consistent with previous reports for this cultivar (Rodrigues et al.,

Table 1. Results of the evaluation of stem pitting symptoms caused by citrus tristeza virus (CTV; species: *Closterovirus tristezae*) and diagnostic tests, conducted on CTV tolerant maternal half-siblings of the BRS Bravo rootstock cultivar.

Hybrids	Stem pitting symptoms[(1)]	CTV diagnosis[(2)]	Hybrids	Stem pitting symptoms	CTV diagnosis	Hybrids	Stem pitting symptoms	CTV diagnosis	Hybrids	Stem pitting symptoms	CTV diagnosis
H017	1.07	+	H053	1.00	+	H095	1.00	+	H166	1.06	+
H018	1.25	+	H054	1.00	+	H096	1.00	+	H002	1.00	+[3]
H021	1.13	+	H055	1.00	+	H097	1.04	+	H011	1.03	+[3]
H022	1.10	+	H058	1.50	+	H106	1.00	+	H012	1.00	+[3]
H027	1.03	+	H060	1.03	+	H111	1.10	+	H009	1.10	+[3]
H028	1.00	+	H061	1.03	+	H115	1.07	+	H025	1.03	+[3]
H030	1.30	+	H067	1.00	+	H124	1.00	+	H029	1.04	+[3]
H038	1.00	+	H075	1.00	+	H126	1.00	+	H035	1.10	+[3]
H039	1.03	+	H076	1.00	+	H127	1.00	+	H036	1.00	+[3]
H040	1.29	+	H085	1.10	+	H128	1.00	+	H133	1.06	+[3]
H042	1.00	+	H086	1.00	+	H130	1.00	+	H137	1.06	+[3]
H045	1.00	+	H088	1.00	+	H144	1.00	+	H151	1.00	+[3]
H047	1.53	+	H089	1.00	+	H149	1.10	+	H186	1.03	+[3]
H049	1.00	+	H092	1.00	+	H153	1.04	+	H204	1.00	+[3]
H051	1.37	+	H093	1.03	+	H160	1.07	+	H210	1.03	+[3]
H052	1.07	+									

[(1)]Evaluation carried out using the 1 to 5 rating scale for the presence and severity of stem pitting described by Meissner Filho et al. (2002). [(2)]The diagnosis of CTV was performed using indirect ELISA; samples that tested negative in the serological assay were subsequently analyzed by RT-PCR. [(3)]Samples diagnosed as positive for CTV only when the RT-PCR technique was used.

2014) and highlights its relevance as a genetic source of resistance and partial CTV tolerance. The comparison with BRS Bravo also demonstrated that part of the progeny displayed equal or superior performance to the original parent, reinforcing the genetic-improvement potential of this population.

Among the 130 half-sibling progenies of 'BRS Bravo' evaluated, 53.08% were classified as resistant, as they exhibited no stem pitting symptoms and no detectable virus (Bordignon et al., 2003). In contrast, the remaining 46.92% were classified as tolerant, with 29 of the 61 individuals evaluated showing no symptoms, while 32 exhibited sparse stem pitting despite allowing virus replication. No hybrids were classified as intolerant, as no severe stem pitting symptoms were observed. The results can be explained by the genetic inheritance of the three parental species of 'BRS Bravo': the Sunki mandarin and the Rangpur lime, which are tolerant to CTV, and *P. trifoliata*, known for its resistance and tolerance to the virus (Sharma et al., 2023). The genetic diversity among the half-siblings highlights the presence of resistance or tolerance gene combinations, following the dominant-recessive epistasis model proposed by Bordignon et al. (2004). This model suggests that a dominant allele at the Az locus, paired with any allele at the t locus, is sufficient to confer tolerance to CTV. Studies by Xie et al. (2023) support dominant-recessive epistasis as the type of genetic interaction in which an epistatic gene suppresses the effect of another gene, resulting in a tolerant or resistant phenotype.

The PCA revealed a clear separation among 'BRS Bravo' hybrids, allowing the identification of distinct response patterns to CTV regarding the presence of stem pitting symptoms and infection. The analysis distinguishes between resistant and tolerant hybrid groups, highlighting differences in their behavior toward the pathogen (Figure 1). The PCA revealed that among the hybrids classified as tolerant, those that were diagnosed via indirect ELISA appeared farther to the left of the graph. The tolerant hybrids that tested negative in the ELISA but were diagnosed only through RT-PCR (H002, H009, H011, H012, H025, H029, H035, H036, H049, H137, H151, H133, H186, H204, and

Table 2. Results of the evaluation of stem pitting symptoms caused by citrus tristeza virus (CTV; species: *Closterovirus tristezae*) and diagnostic tests, conducted on CTV resistant maternal half-siblings of the BRS Bravo rootstock cultivar.

Hybrids	Stem pitting symptoms[(1)]	CTV diagnosis[(2)]	Hybrids	Stem pitting symptoms	CTV diagnosis	Hybrids	Stem pitting symptoms	CTV diagnosis	Hybrids	Stem pitting symptoms	CTV diagnosis
H001	1.00	-	H066	1.03	-	H107	1.00	-	H152	1.00	-
H003	1.03	-	H068	1.00	-	H109	1.00	-	H154	1.00	-
H004	1.00	-	H071	1.00	-	H110	1.08	-	H156	1.00	-
H005	1.00	-	H072	1.03	-	H112	1.00	-	H159	1.08	-
H006	1.17	-	H074	1.00	-	H113	1.00	-	H161	1.00	-
H007	1.03	-	H077	1.00	-	H114	1.00	-	H162	1.00	-
H008	1.13	-	H078	1.00	-	H117	1.04	-	H164	1.00	-
H010	1.30	-	H081	1.00	-	H118	1.03	-	H169	1.07	-
H019	1.07	-	H084	1.10	-	H121	1.07	-	H170	1.03	-
H023	1.00	-	H087	1.00	-	H122	1.00	-	H175	1.06	-
H024	1.00	-	H090	1.00	-	H123	1.03	-	H177	1.00	-
H031	1.07	-	H098	1.00	-	H129	1.00	-	H178	1.07	-
H033	1.27	-	H099	1.00	-	H132	1.00	-	H180	1.00	-
H037	1.00	-	H100	1.00	-	H136	1.03	-	H182	1.03	-
H041	1.00	-	H103	1.03	-	H141	1.26	-	H187	1.00	-
H046	1.00	-	H104	1.00	-	H145	1.00	-	H207	1.05	-
H048	1.00	-	H105	1.00	-	H150	1.10	-	H208	1.00	-

[(1)]Evaluation carried out using the 1 to 5 rating scale for the presence and severity of stem pitting described by Meissner Filho et al. (2002). [(2)]The diagnosis of CTV was performed using indirect ELISA and RT-PCR tests.

H210) were clustered closer to the center of the graph. The PCA results suggest that hybrids diagnosed only by RT-PCR, although classified as tolerant, appear to have limited CTV multiplication in their tissues. Citrus species and varieties may exhibit intermediate behavior in relation to CTV multiplication in their tissues, as well as different levels of CTV tolerance (Khalilzadeh et al., 2025).

Another discriminated group among the tolerant hybrids in the PCA consists of hybrids that showed average stem pitting severity scores higher than the mean obtained for most hybrids classified as tolerant, including H018, H030, H040, H047, H051, and H058 (Table 1). Similarly, among the hybrids classified as resistant, H010, H033, and H141 were also grouped together (Table 2). Despite testing negative for infection, they presented average symptom severity scores ranging from 1.0 to 2.0, slightly higher than the mean for most resistant hybrids, which was 1.0. These average scores are closer to 1 and 2, considered as the absence of stem pitting symptoms, and the sparse presence of stem pitting on the branches, respectively (Meissner Filho et al., 2002). Similar results were reported by Rodrigues et al. (2014) and Santana et al. (2024), who also observed sparse stem pitting symptoms in resistant hybrids.

The hybrids identified as resistant that show agronomic potential for Embrapa Mandioca e Fruticultura citrus breeding program will undergo further evaluation. This process includes indexing on CTV indicator plants, inoculation with viral isolates, and qPCR diagnostics (Sharma et al., 2023). Additionally, further evaluations are needed to assess hybrid tolerance as rootstocks for susceptible scions (Bowman et al., 2021). Since hybrid responses to CTV infections may also vary according to the prevalence of viral strains and local climatic conditions (Gonçalves et al., 2018), the findings of this study require validation through multi-environment trials across different citrus-producing regions of Brazil. These subsequent

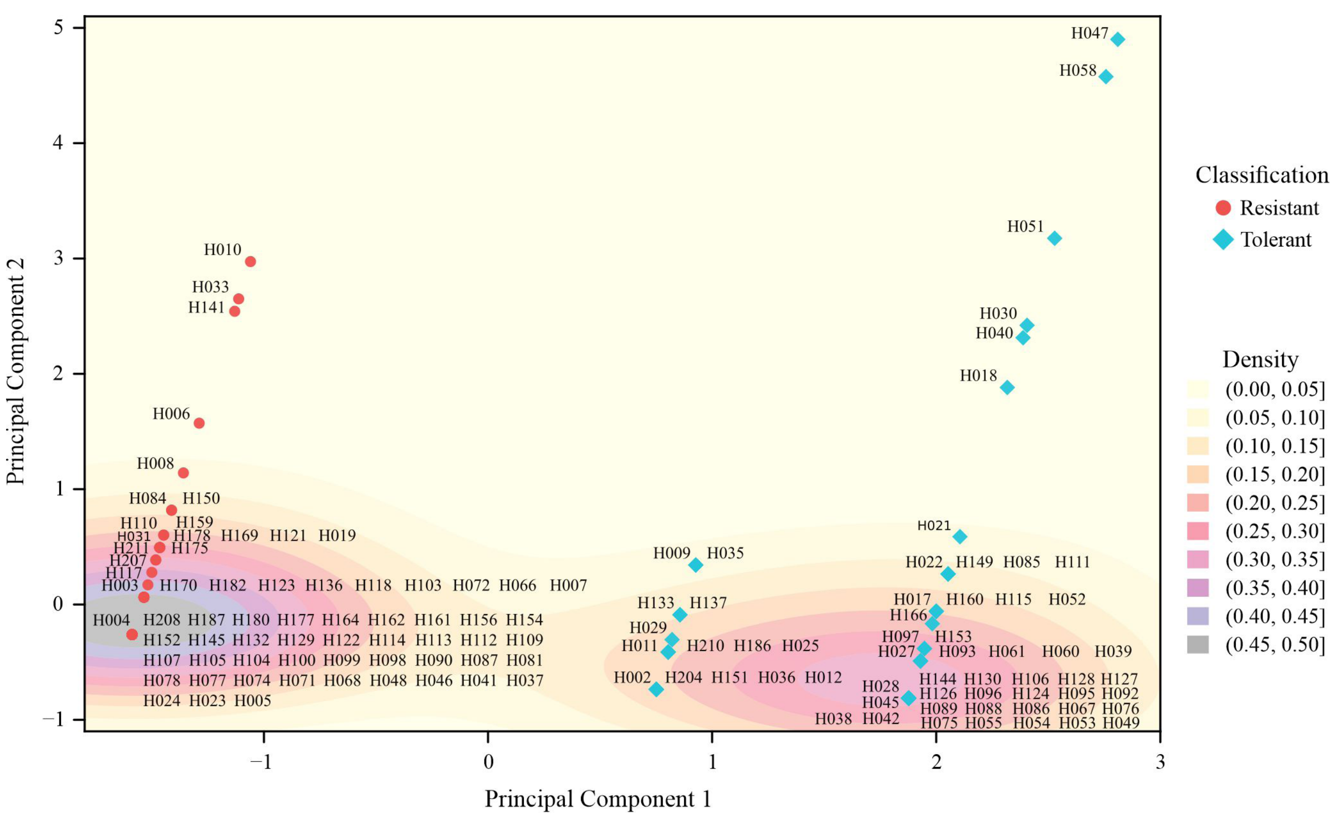

Figure 1. Distribution of resistance and tolerance of maternal half-siblings of the BRS Bravo rootstock cultivar to local isolates of citrus tristeza virus (CTV; species: *Closterovirus tristezae*), based on Principal Component Analysis.

DOI: 10.1590/S1678-3921.pab2026.v61.04184

evaluations will ensure that selected hybrids maintain resistance and agronomic performance under diverse field conditions.

The PCA effectively visualized the variability in hybrid responses to natural infection by local CTV isolates, supporting the selection of resistant genotypes and those with varying tolerance levels (Figure 1). The density layer highlighted clusters of hybrids with similar responses, such as H089, H039, H130, H127, and H106. In contrast H012, H053, and H076 showed more variable or infrequent reactions. Although spatially isolated, these genotypes contribute to genetic diversity and remain valuable for breeding. The density layer enhanced the interpretation of hybrid distribution and reinforced PCA response patterns.

The selection of CTV tolerant clones and the genetic improvement of rootstocks targeting this trait have been long-established strategies for sustaining Brazilian citrus production (Carvalho et al., 2019, 2021; Sun et al., 2024). Given the ongoing expansion into new cultivation areas, and the emergence of aggressive CTV isolates capable of overcoming previously tolerant rootstocks (Gonçalves et al., 2018) continuous breeding efforts focused on tristeza management are increasingly relevant.

Conclusions

1. The BRS Bravo cultivar, used as the female parent in hybridizations, shows potential to generate CTV-resistant or CTV-tolerant progeny, underscoring its value as a key genetic source for the development of new citrus rootstocks.

2. Among the 130 selected and evaluated hybrids, 53.08% were classified as resistant, 46.92% as tolerant to the local of CTV isolates.

References

BAR-JOSEPH, M.; HARPER, S.J.; DAWSON, W.O. Citrus Tristeza Virus (*Closteroviridae*). In: BAMFORD, D.H.; ZUCKERMAN, M. (Ed.). **Encyclopedia of Virology**. 4th ed. Cambridge: Academic Press, 2021. v.3, p.327-335. DOI: https://doi.org/10.1016/B978-0-12-809633-8.21296-X.

BORDIGNON, R.; MEDINA FILHO, H.P.; MULLER, G.W.; SIQUEIRA, W.J. A tristeza dos citros e suas implicações no melhoramento genético de porta-enxertos. **Bragantia**, v.62, p.345-355, 2003. DOI: https://doi.org/10.1590/S0006-87052003000300002.

BORDIGNON, R.; MEDINA-FILHO, H.P.; SIQUEIRA, W.J.; TEÓFILO SOBRINHO, J. The genetics of tolerance to tristeza disease in citrus rootstocks. **Genetics and Molecular Biology**, v.27, p.199-206, 2004. DOI: https://doi.org/10.1590/S1415-47572004000200013.

BOWMAN, K.D.; MCCOLLUM, G.; ALBRECHT, U. SuperSour: a new strategy for breeding superior citrus rootstocks. **Frontiers in Plant Science**, v.12, art.741009, 2021. DOI: https://doi.org/10.3389/fpls.2021.741009.

CARVALHO, H.W.L. de; CARVALHO, L.M. de; TEODORO, A.V.; BARROS, I. de; GIRARDI, E.A.; SOARES FILHO, W. dos S.; PASSOS, O.S. Yield, fruit quality, and survival of 'Pêra' sweet orange on eight rootstocks in tropical cohesive soils. **Pesquisa Agropecuária Brasileira**, v.56, e02151, 2021. DOI: https://doi.org/10.1590/s1678-3921.pab2021.v56.02151.

CARVALHO, S.A. de; GIRARDI, E.A.; MOURÃO FILHO, F. de A.A.; FERRAREZI, R.S.; dela COLETTA FILHO, H.. Advances in citrus propagation in Brazil. **Revista Brasileira de Fruticultura**, v.41, e-422, 2019. DOI: https://doi.org/10.1590/0100-29452019422.

COSTA, D.P.; STUCHI, E.S.; GIRARDI, E.A.; GESTEIRA, A. da S.; COELHO FILHO, M.A.; LEDO, C.A. da S.; FADEL, A.L.; SILVA, A.L.V. da; LEÃO, H.C. de; RAMOS, Y.C.; PASSOS, O.S.; SOARES FILHO, W. dos S. Hybrid rootstocks for valencia sweet orange in rainfed cultivation under tropical savannah climate. **Journal of Agricultural Science**, v.12, p.40-55, 2020. DOI: https://doi.org/10.5539/jas.v12n11p40.

FERREIRA, F.R.; SANTOS, I.R.I.; SOARES FILHO, W. dos S.; CUNHA SOBRINHO, A.P. da; PASSOS, O.S.; SOUZA, A. da S. Recursos genéticos. In: CUNHA SOBRINHO, A.P. da; MAGALHÃES, A.F. de J.; SOUZA, A. da S.; PASSOS, O.S.; SOARES FILHO, W. dos S. (Ed.). **Cultura dos citros**. Brasília: Embrapa, 2013. v.1, p.47-60.

FOLIMONOVA, S.Y.; SUN, Y.-D. *Citrus Tristeza Virus*: From pathogen to panacea. **Annual Review of Virology**, v.9, p.417-435, 2022. DOI: https://doi.org/10.1146/annurev-virology-100520-114412.

FRIAS, A.A.T.; ZANUTTO, C.A.; MENDONÇA, A.C. da S.; IBANEZ-CARRASCO, F.; TAMBORINDEGUY, C.; NUNES, W.M. de C. Transmission of isolates of *Citrus tristeza virus* by single aphids (*Toxoptera citricida*). **Acta Scientiarum. Agronomy**, v.46, e63165, 2023. DOI: https://doi.org/10.4025/actasciagron.v46i1.63165.

GIAMPANI, J.S.; TAZIMA, Z.H.; BABA, V.Y.; YADA, I.F.U.; LEITE JÚNIOR, R.P. Citrus tristeza disease severity and agronomic performance of 'Pêra' sweet orange cultivars and selections in the North and Northwest regions of the State of Paraná, Brazil. **Revista Brasileira de Fruticultura**, v.38, e-073, 2016. DOI: https://doi.org/10.1590/0100-29452016073.

GONÇALVES, A.P.; SANTOS, K.S. dos; SILVA, C. de C.; NOVAES, T.G. de; MOLINA, R. de O. Identificação de isolados de *Citrus tristeza virus* (CTV) protetivos para *Citrus sinensis* (L.) Osbeck. **Summa Phytopathologica**, v.44, p.17-22, 2018. DOI: https://doi.org/10.1590/0100-5405/172270.

KHALILZADEH, M.; ALDRICH, D.J.; MAREE, H.J.; LEVY, A. Complex interplay: the interactions between citrus tristeza virus and its host. **Virology**, v.603, art.110388, 2025. DOI: https://doi.org/10.1016/j.virol.2024.110388.

KOENIG, R. Indirect ELISA methods for the broad specificity detection of plant viruses. **Journal of General Virology**, v.55, p.53-62, 1981. DOI: https://doi.org/10.1099/0022-1317-55-1-53.

KÖPPEN, W. **Grundriss der Klimakunde**. Berlin: Walter de Gruyter, 1931. 390p. DOI: https://doi.org/10.1515/9783111667751.

LIRA, R. de A.; ARAÚJO, M. do S.B. de; ALMEIDA, B.G. de; MARCELO, V.F. Uso agrícola e atributos físico-hídricos de solo coeso. **Revista Brasileira de Geografia Física**, v.9, p.2277-2289, 2016. DOI: https://doi.org/10.5935/1984-2295.20160162.

MEISSNER FILHO, P.E.; SOARES FILHO, W. dos S.; VELAME, K.V.C.; DIAMANTINO, E.P.; DIAMANTINO, M.S.A.S. Reação de porta-enxertos híbridos ao *Citrus tristeza virus*. **Fitopatologia Brasileira**, v.27, p.312-315, 2002. DOI: https://doi.org/10.1590/S0100-41582002000300014.

ORTON, T.J. Mass selection and the basic plant breeding algorithm. In: ORTON, T.J. **Horticultural Plant Breeding**. London: Academic Press, 2020. p.85-95. DOI: https://doi.org/10.1016/B978-0-12-815396-3.00005-6.

R CORE TEAM. **R**: a language and environment for statistical computing. Available at: <https://www.r-project.org/>. Accessed on: 29 May 2025.

RODRIGUES, A.S.; BARBOSA, C. de J.; SOARES FILHO, W. dos S.; FREITAS-ASTÚA, J. Comportamento de híbridos de citros em relação à infecção natural pelo *Citrus tristeza virus* e à presença de sintomas de descamamento eruptivo. **Revista Brasileira de Fruticultura**, v.36, p.735-741, 2014. DOI: https://doi.org/10.1590/0100-2945-179/13.

RODRIGUES, M.J. da S.; LEDO, C.A. da S.; GIRARDI, E.A.; ALMEIDA, L.A. da H.; SOARES FILHO, W. dos S. Caracterização de frutos e propagação de porta-enxertos híbridos de citros em ambiente protegido. **Revista Brasileira de Fruticultura**, v.37, p.457-470, 2015. DOI: https://doi.org/10.1590/0100-2945-068/14.

SANTANA, L.G.L.; SOARES FILHO, W. dos S.; LEDO, C.A. da S.; BARBOSA, C. de J.; SANTOS, M.G. Reação de progênies de citros ao vírus-da-tristeza-dos-citros (Citrus tristeza virus – CTV). **Revista Contemporânea**, v.4, p.1-14, 2024. DOI: https://doi.org/10.56083/RCV4N4-022.

SANTOS, H.G. dos; JACOMINE, P.K.T.; ANJOS, L.H.C. dos; OLIVEIRA, V.Á. de; LUMBRERAS, J.F.; COELHO, M.R.; ALMEIDA, J.A. de; ARAÚJO FILHO, J.C. de; LIMA, H.N.; MARQUES, F.A.; OLIVEIRA, J.B. de; CUNHA, T.J.F. **Sistema Brasileiro de Classificação de Solos**. 6.ed. rev. e ampl. Brasília: Embrapa, 2025. 393p.

SHARMA, S. A review on Citrus tristeza virus (CTV) and its management approaches. **Turkish Journal of Agriculture - Food Science and Technology**, v.11, p.803-810, 2023. DOI: https://doi.org/10.24925/turjaf.v11i4.803-810.5273.

SHARMA, S.K.; PATHAW, N.; WANGKHEM, B.; JACKSON, K.S.; DEVI, K.S.; ROY, S.S.; SINGH, A.R.; SINGH, R.; BANERJEE, A.; KUMAR, S.; NINGOMBAM, A.; FIRAKE, D.M.; SINGH, T.S. Simple template-based reverse transcription-recombinase polymerase amplification assay for routine diagnosis of citrus tristeza virus. **Letters in Applied Microbiology**, v.76, art.ovac060, 2023. DOI: https://doi.org/10.1093/lambio/ovac060.

SILVA, T.S.M. da; COELHO FILHO, M.A.; COELHO, E.F. **Boletim meteorológico da estação convencional de Cruz das Almas, BA**: variabilidade e tendências climáticas. Cruz das Almas: Embrapa Mandioca e Fruticultura, 2016. 72p. (Embrapa Mandioca e Fruticultura. Documentos, 216).

SUN, Y.; YOKOMI, R.K.; FOLIMONOVA, S.Y. Citrus tristeza virus: a century-long challenge for the world's citrus industries. **Annals of Applied Biology**, v.185, p.304-322, 2024. DOI: https://doi.org/10.1111/aab.12939.

XIE, X.; SUN, X.; WANG, Y.; LEHNER, B.; LI, X. Dominance vs epistasis: the biophysical origins and plasticity of genetic interactions within and between alleles. **Nature Communications**, v.14, art.5551, 2023. DOI: https://doi.org/10.1038/s41467-023-41188-8.

Author contributions

Luana Laís de Almeida dos Santos: conceptualization, formal analysis, investigation, methodology, writing – original draft, writing – review & editing; **Danilo Pereira Costa**: formal analysis, investigation, methodology, supervision, visualization; **Raquel Moreira Lima**: data curation, methodology; **Samuel Araújo dos Santos**: methodology; **Sandriele Santos de Araujo**: methodology; **Edilane dos Santos Maciel**: data curation, methodology; **Walter dos Santos Soares Filho**: conceptualization, formal analysis, project administration, supervision, validation, writing – review & editing; **Cristiane de Jesus Barbosa**: conceptualization, formal analysis, investigation, project administration, supervision, validation, visualization, writing – original draft, writing – review & editing.

Chief editor: Edemar Corazza
Edited by: Madalena Rinaldi

Data availability statement

Data available upon request: research data are only available upon reasonable request to the corresponding author.

Declaration of use of AI technologies

During the preparation of this work, the author(s) used ChatGPT, by OpenAI, to assist with language revision and organization of editorial responses. After this use, the author(s) reviewed and edited the content as needed and take full responsibility for it.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgments

To Embrapa Mandioca e Fruticultura and to Centro Tecnológico Agropecuário da Bahia, for the technical support and infrastructure provided for this study; to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for financing, in part, this study (Finance Code 001).