Selection of roselle genotypes tolerant/resistant to spots induced by *Corynespora cassiicola* in Guerrero, Mexico

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Data de chegada: 07/02/2021. Aceito para publicação em: 13/01/2022

10.1590/0100-5405/248479

ABSTRACT

Palemón-Alberto, F.; Reyes-García, G.; Ortega-Acosta, S.Á.; Rojas-García, A.R.; Maldonado-Peralta, M.Á.; Toledo-Hernández, E.; Villar-Luna, E.; García-López, E.; Domínguez-Monge, S.; Cruz-Lagunas, B.; Lázaro-Delgado, M.Á. Selection of roselle genotypes tolerant/resistant to spots induced by *Corynespora cassiicola* in Guerrero, Mexico. ***Summa Phytopathologica,*** v.48, n.2, p.55-60, 2022.

Roselle spot caused by *Corynespora cassiicola* affects calyx production in Mexico. The aim of the present study was to evaluate twenty-two roselle genotypes against *C. cassiicola* under greenhouse conditions. The area under the disease progress curve (AUDPC) was used to detect susceptible, tolerant and resistant genotypes. Considering the incidence in leaves, susceptible genotypes were Conpoz, Sudlaz, Morcot and Sudigua (AUDPC values of 7379.2 to 5891.7); tolerant genotypes were Conayu and Chinxal (AUDPC 1341.6 and 1313.2). Based on the severity in leaves, susceptible genotypes were Sudigua, Conpoz and Morena2 (AUDPC from 788.4 to 436.3); tolerant genotypes were Almatech, Crioxal, Rojatec and Conayu (37.7 to 28.76 AUDPC). Assessment of the incidence in calyces indicated that susceptible genotypes were Morena2, Morena, Conpoz, Descop and Chinayu (AUDPC from 6572.1 to 2319.4); for the severity in calices, susceptible genotypes were Morena2, Descop, Morena, Cabxal, Chinayu and Conpoz (AUDPC from 3355.4 to 779.2). Incidence and severity on calyces evidenced resistant genotypes, which were Almatech, Criolaz2, Criocot, Criolaz, Rojatec, Criopoz, Crioxal, Chinxal and Conayu (0.0 AUDPC). In the present study, tolerant and resistant genotypes were detected to leaf and calyx spot caused by *C. cassiicola* in roselle.

Keywords: *Hibiscus sabdariffa*, fungal disease, disease management, screening

RESUMO

Palemón-Alberto, F.; Reyes-García, G.; Ortega-Acosta, S.Á.; Rojas-García, A.R.; Maldonado-Peralta, M.Á.; Toledo-Hernández, E.; Villar-Luna, E.; García-López, E.; Domínguez-Monge, S.; Cruz-Lagunas, B.; Lázaro-Delgado, M.Á. Seleção de genótipos de vinagreira tolerantes/resistentes à mancha foliar induzida por *Corynespora cassiicola* em Guerrero, México. ***Summa Phytopathologica,*** v.48, n.2, p.55-60, 2022.

A doença manchada de vinagreira causada por *Corynespora cassiicola* afeta a produção de cálice no México. O objetivo deste estudo foi avaliar vinte e dois genótipos de vinagreira contra *C. cassiicola* em casa de vegetação. Com base nas área abaixo da curva de progresso da doença (AACPD) foi possível detectar genótipos suscetíveis, tolerantes e resistentes. Dada a incidência nas folhas, os genótipos suscetíveis foram Conpoz, Sudlaz, Morcot e Sudigua (valores de AACPD 7379,2 a 5891,7); tolerantes foram Conayu e Chinxal (AACPD 1341,6 e 1313,2). Enquanto isso, pela severidade nas folhas, os suscetíveis foram Sudigua, Conpoz e Morena2 (AACPD de 788,4 a 436,3); tolerantes foram Almatech, Crioxal, Rojatec e Conayu (37,7 a 28,76 de AACPD). Enquanto, avaliando a incidência em cálices, genótipos suscetíveis foram; Morena2, Morena, Conpoz, Descop e Chinayu (AACPD de 6572,1 a 2319,4); para a gravidade suscetível foram Morena2, Descop, Morena, Cabxal, Chinayu e Conpoz (AACPD de 3355,4 a 779,2). A incidência e severidade nos cálices detectaram genótipos resistentes, que foram Almatech, Criolaz2, Criocot, Criolaz, Rojatec, Criopoz, Crioxal, Chinxal e Conayu (0,0 de AACPD). Neste estudo, foram detectados genótipos tolerantes e resistentes à mancha foliar e cálice causada por *C. cassiicola* em vinagreira.

Palavras-chave: *Hibiscus sabdariffa*, doença fúngica, manejo de doenças, rastreamento.

Roselle (*Hibiscus sabdariffa*) is a plant belonging to the Malvaceae family, which is cultivated in tropical and sub-tropical regions (25). The major countries producing calyces of roselle are Egypt, Sudan, Mexico, Thailand and China (9). Roselle is cultivated principally for its calyces, which are used to prepare various foods and beverages (4), i.e., sweet drinks, jam, syrup, wine, juice, ice cream or tea (1). Additionally, roselle extracts have medicinal properties such as antimicrobial, anti-inflammatory and antioxidant properties (13, 26).

There are diverse plant pathogens that affect roselle, such as *Phytophthora parasitica*, which causes the "black leg" disease; *Fusarium oxysporum*, which induces vascular wilt, and *Ralstonia pseudosolanacearum* and *R. solanacearum*, which lead to bacterial wilt (2, 20, 5).

In Mexico, in the state of Guerrero, the annual production of roselle calyces is affected by roselle spot, a disease caused by the fungus *Corynespora cassiicola* (19); this pathogen is widely distributed in the major roselle-producing municipalities in Guerrero State (15). Calyx and leaf spot in roselle has been especially managed with conventional and alternative chemical substances (17). However, sustainable alternatives should be searched for the management of this disease. Host tolerance could be an option for farmers, as this would reduce production costs and minimize the use of chemical pesticides; papaya and cucumber genotypes tolerant to *C. cassiicola* have been detected (22, 28, 6).

In Mexico, no tolerant/resistant roselle genotypes have been identified for the disease caused by *C. cassiicola*; thus, the aim of the current study was to evaluate the response of twenty-two roselle genotypes against *C. cassiicola*.

MATERIALS AND METHODS

Experimental site

The study was conducted at Facultad de Ciencias Agropecuarias y Ambientales de la Universidad Autónoma de Guerrero, located in Iguala de la Independencia, Guerrero State, Mexico, at an altitude of 730 meters above sea level and at the latitude coordinates: 18°20'59"N and 99°33'00"W. The experimental area has an average temperature of 25.9°C and average rainfall of 1,050.6 mm (8). The evaluation was carried out under greenhouse conditions.

Sampling and sowing roselle genotypes

In March 2018, roselle genotypes were collected from the major roselle producing areas in the state of Guerrero, Mexico, at the municipalities of Ayutla, Tecoanapa, Copalillo and Iguala (Table 1).

The genotypes selected for the experiment (Table 1) were planted in June 2018, in polyethylene (15 x 25 cm) pots. The planting medium was composed of 30% compost and 70% soil as substrate.

Experimental design

Each genotype (Table 1) was considered a treatment; four replicates/treatment were established, and each replicate comprised two plants/pot. The experiment was arranged as completely randomized design under greenhouse conditions.

Inoculum source

For the inoculation of roselle genotypes, the strain CC47GRO of *Corynespora cassiicola* was used (GenBank: MF000875) (16). To obtain sufficient inoculum, the fungus was increased in Petri dishes containing Potato Dextrose Agar (PDA) culture medium (BIOXON®) and subsequently placed in an incubator (NOVATECH®) for 14 days at 28°C (14).

Inoculation of *C. cassiicola*

The fungal inoculum concentration was 2 x 10^5 conidia mL^{-1} (19). Two inoculations of *C. cassiicola* were conducted by means of drip sprays of the inoculum onto the foliage, at 40 and 60 days after sowing (DAS), and onto the calyces, from maturation according to their

Table 1. Roselle (*H. sabdariffa*) genotypes used in the experiment to evaluate tolerance against leaf and calyx spot induced by *C. cassiicola* under greenhouse conditions.

Genotype	Collection site	Municipality	Key of the collection
Sudán	Iguala	Iguala	Sudigua
Criolla	Cotzálzin	Ayutla	Criocot
Morena	Cotzálzin	Ayutla	Morcot
China Negra	Ayutla	Ayutla	Chinayu
Criolla	Xalpatláhuac	Tecoanapa	Crioxal
Coneja	Ayutla	Ayutla	Conayu
Criolla	Lázaro Cárdenas	Tecoanapa	Criolaz
China roja	Xalpatláhuac	Tecoanapa	Chinxal
Criolla	Lázaro Cárdenas	Tecoanapa	Criolaz2
Coneja	Pozolapan	Ayutla	Conpoz
Criolla	Pozolapan	Ayutla	Criopoz
Sudan	Lázaro Cárdenas	Tecoanapa	Sudlaz
Grande de Guerrero	Xalpatláhuac	Tecoanapa	Cabxal
Alma blanca grande	Tecoanapa	Tecoapana	Almatecg
Uvita	Tecoanapa	Tcoanapa	Uvitec
Negro Guerrero	Tecoanapa	Tcoanapa	Negtec
China de Guerrero	Tecoanapa	Tcoanapa	Almatech
Roja abierta	Tecoanapa	Tcoanapa	Rojatec
Roja Tecoanapa	Tecoanapa	Tcoanapa	Tecroja
Morena cora grande	San Francisco Ostutla	Copalillo	Morena
Morena 2	San Francisco Ostutla	Copalillo	Morena2
Unknow	San Francisco Ostutla	Copalillo	Descop

vegetative cycle (early, intermediate and late) (27); the last inoculation in the calyces was performed after 20 days. For each inoculation, plants were covered with transparent plastic bags for 72 h.

Assessment of incidence and severity

The incidence was measured and used to divide each plant into three strata: low, medium, and high (17). For each stratum, three leaves or calyces were recorded, according to the phenological stage of the studied genotypes. Incidence (%) was determined as percentage of symptomatic leaves or calyces in the total observed leaves or calyces.

Disease severity (%) was estimated based on a diagrammatic logarithmic scale for leaves: 0=0, 1= (>0-2 to 4), 2 = (>4-7 to 12), 3 = (>12-19 to 29), 4 = (>29-42 to 57) and 5 = (>57- 70 to ≤100), and for calyces: 0=0, 1 = (>0-3 to 5), 2 = (>5-10 to 18), 3 = (>18-30 to 46), 4 = (>46-63 to 77) and 5 = (>77-87 to ≤100) (18). Nine evaluations were carried out at weekly intervals for leaves and calyces.

Recording of agronomic characteristics

Periodic monitoring was carried out, and the vegetative cycle and calyx coloration were recorded for each roselle genotype (27).

Recording of environmental variables

During the experiment, constant monitoring was carried out with a digital hygrothermograph (Extech Instruments® RHT10), programmed to record relative humidity and temperature at two-hour intervals. Relative humidity in the greenhouse was kept constant by using two humidifiers throughout the experiment.

Statistical analysis

The area under the disease progress curve (AUDPC) was estimated for each roselle genotype (treatment), based on the trapezoidal integration method described by Campbell and Madden (7), using estimated incidence and severity data of roselle leaves and calyces, while analysis of variance and separation of means were conducted according to Tukey's test ($p \leq 0.05$). Statistical analyses were performed using SAS® version 9.4 statistical program.

RESULTS

The evaluated roselle genotypes (Table 1) presented diverse phenological stages and were classified as early, intermediate and late. A high phenotypic diversity was also observed for calyx coloration (27). Under greenhouse conditions, the materials showed four shades of calyx colors, which were pale red, light red, red and intense red (Table 2).

The average temperature and relative humidity recorded during the experiment was 26 °C and 82%, respectively. This environmental condition is within the optimal range for the development of *C. cassiicola* (11, 10).

Analysis of AUDPC for incidence and severity in leaves and calyces

Based on the AUDPC analysis for the disease incidence and severity induced by *C. cassiicola*, the response differed according to the evaluated roselle genotype. The AUDPC analysis was used to describe the plants according to severity and incidence as susceptible,

Table 2. Analysis of the incidence and severity (AUDPC) for leaves and calyces of 22 roselle genotypes before artificial inoculation under greenhouse conditions.

			Leaves		Calyces	
Genotype	**Calyx color[w]**	**Phenological cycle**	**Incidence**	**Severity**	**Incidence**	**Severity**
Sudigua	Intense red	Intermediate[x]	5891.7[y] abcde[Z]	788.4 a	1037.5 fg	547.4 cde
Criocot	Pale red	Late	2819.4 efghi	111.2 de	0.0 h	0.0 f
Morcot	Red	Intermediate	6348.6 abc	347.7 bcd	2208.3 de	506.0 cde
Chinayu	Red	Intermediate	3568.1 efgh	82.6 de	2319.4 d	839.3 c
Crioxal	Pale red	Late	1516.6 hi	34.2 e	0.0 h	0.0 f
Conayu	Light red	Late	1341.6 i	28.8 e	0.0 h	0.0 f
Criolaz	Light red	Intermediate	4063.9 defg	118.1 de	0.0 h	0.0 f
Chinxal	Light red	Late	1313.2 i	53.5 de	0.0 h	0.0 f
Criolaz2	Light red	Late	2265.2 fghi	120.3 de	0.0 h	0.0 f
Conpoz	Light red	Intermediate	7379.2 a	470.1 b	4432.0 c	779.2 c
Criopoz	Pale red	Late	2099.9 fghi	141.4 cde	0.0 h	0.0 f
Sudlaz	Red	Intermediate	6835.3 ab	304.1 bcde	495.3 fgh	685.7 cd
Cabxal	Light red	Early	4851.3 bcde	282.9 bcde	2061.1 de	982.7 c
Almatecg	Light red	Intermediate	2255.5 fghi	141.9 cde	890.2 fgh	279.8 def
Uvitec	Red	Intermediate	4161.1 cdef	331.1 bcde	136.1 gh	78.8 ef
Negtec	Red	Intermediate	3091.7 efghi	87.0 de	1323.5 ef	278.6 def
Almatechi	Red	Late	1886.1 ghi	37.7 e	0.0 h	0.0 f
Rojatec	Pale red	Late	1545.7 hi	30.9 e	0.0 h	0.0 f
Tecroja	Intense red	Intermediate	3957.0 defg	174.0 bcde	547.2 fgh	252.9 def
Morena	Red	Early	2625.0 fghi	201.6 bcde	5516.7 b	1753.2 b
Morena2	Red	Early	3033.3 efghi	436.3 bc	6572.1 a	3355.4 a
Descop	Intense red	Intermediate	2625.0 fghi	172.7 bcde	2950.0 d	2207.9 b

[w]Visual estimation of calyx coloration. [x]Days to the harvest of calyces; early=136 to 160; intermediate=161 to 180; Late=181 to 198. [Y]AUDPC (Area Under the Disease Progress Curve). [Z]Means for each parameter (leaves or calyces) and for each variable (incidence or severity) followed by the same letter are not significantly different, according to the Tukey's test (p=0.05).

intermediate and tolerant for the leaves, and as susceptible, intermediate, tolerant and resistant for the calyces (Table 2).

Regarding incidence in the leaves, the most statistically susceptible material was: Conpoz, followed by Sudlaz, Morcot and Sudigua, the AUDPC values of which were 7379.2, 6835.3, 6348.6 and 5891.7, respectively; the most tolerant genotypes were Conayu and Chinxal, showing AUDPC values of 1341.6 and 1313.2, respectively. The remaining genotypes presented intermediate tolerance according to the recorded AUDPC values (Table 2).

For severity in the leaves, the most susceptible genotype was Sudigua, followed by Conpoz and Morena2 , which had AUDPC values of 788.43, 470.14 and 436.32, respectively; the most tolerant genotypes were Almatech, Crioxal, Rojatec and Conayu, reaching AUDPC values of 37.70, 34.18, 30.94 and 28.76, respectively (Table 2; Figure 1A and B). The remaining genotypes can be considered intermediate based on their AUDPC values (Table 2).

As to calyces, the recorded incidence evidenced the following genotypes as susceptible: Morena2, Morena, Conpoz, Descop and Chinayu, which showed AUDPC values of 6572.1, 5516.7, 4432.0, 2950.0 and 2319.4, respectively. On the other hand, nine genotypes did not present infections and were considered resistant: Almatech, Criolaz2, Criocot, Criolaz, Rojatec, Criopoz, Crioxal, Chinxal and Conayu, presenting values of 0.0 AUDPC. The remaining genotypes were classified as intermediate to the infection induced by *C. cassiicola* (Table 2).

Considering the severity in calyces, the most susceptible genotype was Morena2, followed by Descop, Morena, Cabxal, Chinayu and Conpoz, which had AUDPC values of 3355.4, 2207.9, 1753.2, 982.7, 839.3 and 779.2; again, the genotypes Almatech, Criolaz2, Criocot, Criolaz, Rojatec, Criopoz, Crioxal, Chinxal and Conayu did not present calyx infection (0.0 AUDPC). Seven materials had intermediate severity values and can be considered tolerant based on the AUDPC analysis (Table 1; Figure 1B).

The roselle genotypes evaluated in this study showed wide diversity in terms of phenological characteristics and calyx coloration (Table 2).

Figure 1. Representative symptoms after artificial inoculation with *C. cassiicola* isolate CC47GRO, under greenhouse conditions, in roselle (*H. sabdariffa*) genotypes. For leaves, susceptible: A= Morena2 and tolerant: B = Conayu. For calyces, susceptible: C= Morena2 and resistant: D= Chinxal.

DISCUSSION

The present results based on the AUDPC analysis suggest a wide variation in terms of susceptibility and tolerance for leaves. Susceptible, tolerant and resistant genotypes were detected for calyces (Table 2).

Considering *C. cassiicola*-induced infection in the leaves, susceptible roselle genotypes were Conpoz, Sudlaz, Morcot, Sudigua and Morena2, collected from the municipalities of Ayutla, Tecoanapa, Iguala and Copalillo (Table 1); the most tolerant genotypes were Conayu, Chinxal, Almatech, Crioxal, Rojatec and Conayu, collected from Ayutla and Tecoanapa. This may be justifiable since the disease is endemic to these municipalities and some materials have been under constant natural selection against *C. cassiicola*, the pathogen that causes roselle leaf and calyx spots, resulting in possible development of tolerance (23).

As regards calyces, the genotypes that expressed the highest susceptibility were Morena2, Morena, Conpoz, Descop, Chinayu and Cabxal, collected from Ayutla, Tecoanapa and Copalillo. Of these, three Copalillo genotypes were most affected (Table 2; Figure 1A and C), probably because the disease has not been reported in the said municipality and the said genotypes have not developed defense mechanisms against *C. cassiicola* (23, 12).

Absence of infection by *C. cassiicola* was recorded for nine genotypes (Table 2), which should, however, be continuously evaluated under different inoculum densities so that their resistance can be validated for calices (21); the tolerance of calyces was different from that of leaves, the latter organs showed infection in a potato crop (*Solanum tuberosum*), when resistance to late blight caused by *Phytophthora infestans* in foliage was detected and was poorly correlated with resistance in tubers, suggesting that different genes are involved depending on the plant organ (3).

In Mexico, research was carried out to find resistance sources in roselle cultivars (*H. sabdariffa* L.) against root "black leg" caused by *Phytophthora parasitica*, evaluating genotypes with different tolerance degrees (24); the obtained results were similar to those observed in the present study. In Philippines, similar studies were conducted to determine the resistance level of roselle to bacterial wilt caused by *Ralstonia solanacearum* and *R. pseudosolanacearum* (5).

Studies performed in Brazil have found tolerant *Carica papaya* genotypes against *C. cassiicola*; the authors determined that the genotypes Grampola, Golden Verde Oscuro and Gran Golden, presented tolerance (22). Similarly, other studies have detected genotypes of *Cucumis sativus* (Taisho, Nikkey, Yoshinari and Safira) presenting resistance to leaf spot caused by *C. cassiicola* (28). A study with hybrids of *C. sativus* (Hokuchim, Soudai, Long Green and Natsubayashi), under greenhouse conditions, showed that they may be an alternative to the high damage caused by *C. cassiicola* for presenting low damage levels (6).

The present investigation represents the possibility of incorporating desirable tolerance and resistance traits through genetic improvement. Finally, evaluations should be performed under natural field conditions for *C. cassiicola* inoculum.

Based on the AUDPC analysis, the present study detected that the following genotypes were tolerant to leaf and calyx spot induced by *C. cassiicola*: Conayu, Chinxal, Almatech, Crioxal, Rojatec and Conayu, presenting moderate infection percentages. On the other hand, nine genotypes were detected as resistant: Almatech, Criolaz2, Criocot, Criolaz, Rojatec, Criopoz, Crioxal, Chinxal and Conayu. The latter have the potential to be used under protected greenhouse conditions and represent new cultivation options for farmers.

Disclosure statement

There is no potential conflict of interest in the submitted paper.

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