

IDENTIFICATION OF COWPEA GENOTYPES RESISTANT TO FUSARIUM WILT¹

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ABSTRACT - Fusarium wilt, caused by soil-borne *Fusarium oxysporum* f. sp. *tracheiphilum* (Fot), can reduce cowpea bean yield. Considering that genetic control through resistant genotypes is pivotal for Fusarium wilt control, the aim of the present study was to identify cowpea genotypes that are resistant to *Fot* from the Germplasm Bank of the Pernambuco Agronomic Institute, Embrapa Middle North, and other producing areas in the North and Northeast regions of Brazil. The cultivar BR-17 Gurguéia was used as a susceptible control and MNCO1-649F-2-1 was used as a resistant control. Two experiments were carried out in a completely randomized design in the greenhouse of the Department of Agronomy of the University Federal Rural of Pernambuco. Plants with the first pair of expanded leaves were inoculated with a conidial suspension (10^6 conidia/mL) using the conidia root-immersion methodology. Fusarium wilt severity in plants was assessed 21 d after inoculation, with the aid of a descriptive scale. In the first experiment, we evaluated the resistance of 38 cowpea genotypes, of which 19 (5 moderately resistant and 14 resistant) were selected for the second experiment. In the first experiment, four genotypes (Canapu PE, Miranda IPA 207, Esperança, and BRS Pujante) did not show any symptoms of Fusarium wilt. In the second, 15 genotypes showed high resistance to *Fot*, including Canapu PE and Miranda IPA 207, which again had no symptoms of Fusarium wilt. In conclusion, this study identified that the genotypes, Canapu PE, Miranda IPA 207, Esperança, and BRS Pujante have greater resistance to Fusarium wilt.

Keywords: *Fusarium oxysporum* f. sp. *tracheiphilum*. Cowpea. Genetic Control. *Vigna unguiculata*. Resistant genotype.

IDENTIFICAÇÃO DE GENÓTIPOS DE FEIJÃO-CAUPI RESISTENTES À MURCHA DE FUSARIUM

RESUMO - A murcha de Fusarium, causada por *Fusarium oxysporum* f. sp. *tracheiphilum* (Fot), reduz a produtividade de feijão-caupi. Este estudo teve como objetivo identificar genótipos com resistência à Fot oriundos do banco germoplasma do Instituto agrônomo de Pernambuco, Embrapa Meio Norte e outras regiões produtoras da região Nordeste e Norte do Brasil. A cultivar BR-17 Gurguéia foi utilizada como controle suscetível e a MNCO1-649F-2-1 como controle resistente. Dois experimentos foram conduzidos em um delineamento inteiramente casualizado em casa de vegetação pertencente ao Departamento de Agronomia da Universidade Federal Rural de Pernambuco. Plantas apresentando o primeiro par de folhas desenvolvidas foram inoculadas com uma suspensão de conídios (10^6 conídios/mL) utilizando a metodologia de imersão de raízes. A severidade da murcha de Fusarium foi determinada aos 21 dias após a inoculação através de uma escala descritiva. No primeiro experimento foi avaliado a resistência de 38 genótipos de feijão-caupi, sendo que 19 genótipos (5 moderadamente resistentes e 14 resistentes) foram selecionados para o segundo experimento. No primeiro experimento 4 genótipos (Canapu PE, Miranda IPA 207, Esperança e BRS Pujante) apresentaram ausência de sintomas. Para o segundo experimento, 15 genótipos apresentaram alto nível de resistência à infecção por Fot, com destaque para Canapu PE e Miranda IPA 207, aos quais novamente apresentaram ausência de sintomas de murcha. Em conclusão, este estudo identificou que os genótipos Canapu PE, Miranda IPA 207, Esperança e BRS Pujante apresentam maior resistência à murcha de Fusarium.

Palavras-chave: *Fusarium oxysporum* f. sp. *tracheiphilum*. Caupi. Controle genético. *Vigna unguiculata*. Genótipo resistente.

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¹Received for publication in 06/15/2020; accepted in 03/11/2021.

Paper extracted from the master degree thesis of the first author.

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INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is cultivated in many countries of tropical and subtropical regions (BERGER et al., 2016). The production of cowpea in Brazil is approximately 713,000 tons (CONAB, 2020). Fusarium wilt, caused by soil-borne *Fusarium oxysporum* f. sp. *tracheiphilum* (*Fot*), is one of the most important problems in cowpea (ASSUNÇÃO et al., 2003). The pathogen invades the vascular tissue via the root system, causing wilting and chlorosis of the leaves and sometimes stunting of the plant. Outward symptoms typically become evident at the seedling stage until flowering and early pod development, resulting in high mortality in the affected areas with significant overall yield loss (ARMSTRONG; ARMSTRONG, 1980). Chlorosis, dwarfism, leaf fall, death, and premature death of plants and damage caused by crop yield are commonly observed (ARMSTRONG; ARMSTRONG, 1980).

Currently, the ability to control Fusarium wilt on cowpea depends mainly on genetic resistance (POTTORFF et al., 2012; PORTTORFF et al., 2014; OMOIGUI et al., 2018). Indeed, some genotypes resistant to Fusarium wilt have already been identified, including Magnolia, Iron PI 293520, Iron TVu1 990, Iron TVu 1072, Iron TVu 1611 (ARMSTRONG; ARMSTRONG, 1980), MNC01-649F-2-1 and MNC01-649F-2-11 (NORONHA et al., 2013), TVu 347 (FAWOLE, 1989), TVu 984 and TVu 109 (ARAUJO, 2017), California Blackeye 27, California Blackeye 46, California Blackeye 50, and IT93K-503-1 (EHLERS et al., 2000; EHLERS et al., 2009; POTTORFF et al., 2012; POTTORFF et al., 2014). As genetic control is the most efficient method for the management of Fusarium wilt, it is very important that genotypes are resistant to this disease, mainly because of the different races of the pathogen that can be distributed in planting fields (POTTORFF et al., 2014). Therefore, the identification of cowpea genotypes resistant to Fusarium wilt, which are more adapted to the North and Northeast regions of Brazil, is necessary for farmers and breeders.

Thus, the goal of this study was to identify additional cowpea genotypes resistant to Fusarium with the objective of recommending it for commercial planting and future breeding studies.

MATERIALS AND METHODS

Cowpea seeds (*Vigna unguiculata*) from 38 genotypes were derived from the Germplasm Bank of the Pernambuco, Embrapa Middle North, and other producing areas in the North and Northeast regions of Brazil (Table 1). The cultivars, BR-17

Gurguéia and MNC01-649F-2-1, were used as standards for susceptibility and resistance, respectively (NORONHA et al., 2013). Two experiments were carried out in a completely randomized design under greenhouse conditions at the Department of Agronomy of the Federal Rural University of Pernambuco (latitude 8° 1' 1.16" S and longitude 34° 56' 38.70" W). In the first experiment, the genetic resistance of 38 genotypes to *Fot* was assessed. From that study, 19 genotypes that showed greater resistance were used in the second experiment. For both experiments, seeds were surface sterilized in 10% (vol/vol) NaOCl for 2 min and sown in plastic pots (10 cm diameter) (Ecovaso, Jaguariúna, São Paulo, Brazil) made from a 1:1:1 mixture of pine bark, peat, and expanded vermiculite (Basaplant[®], Artur Nogueira, São Paulo, Brazil). Two seeds were sown per pot. Five days after seedling emergence, each pot was thinned to one seedling. In both experiments, three repetitions were performed for each genotype tested. Each repetition represented one plastic pot (0.3 kg of substrate) with three plants. The substrate in each pot was fertilized weekly with a nutrient solution containing 6.4 g/L KCl, 3.48 g/L K₂SO₄, 5.01 g/L MgSO₄·7H₂O, 2.03 g/L (NH₂)₂CO, 0.009 g/L NH₄MO₇O₂₄·4H₂O, 0.054 g/L H₃BO₃, 0.222 g/L ZnSO₄·7H₂O, 0.058 g/L CuSO₄·5H₂O, 0.137 g/L MnCl₂·4H₂O, 0.27 g/L FeSO₄·7H₂O, and 0.37 g/L ácido etilenodiamino tetra-acético (EDTA bisódico) (XAVIER FILHA et al., 2011). A nutrient solution (15 mL) was applied to each pot after seedling emergence. The first experiment was carried out from March to April 2017 with mean temperatures ranging from 31.0 to 36.6 °C and mean relative humidity between 57% and 74%. The second experiment was conducted between May and June 2017, when mean temperatures ranged from 29 to 31 °C and mean relative humidity ranged from 51% to 78.6%.

The pathogenic isolate of *Fot* (CMM-732) was obtained from the Phytopathogenic Fungi Culture Collection "Prof. Maria Menezes" from the Federal Rural University of Pernambuco. The isolate was preserved on strips of filter paper placed in glass tubes containing silica gel at 4 °C. Pieces of filter paper with fungal mycelia were transferred to Petri dishes containing potato dextrose agar (PDA) medium. The Petri dishes were kept in a growth chamber at 25 °C with a 12 h photoperiod for 7 d. After this period, mycelia-producing conidia were carefully removed from the Petri dishes with a rubber policeman to obtain a conidial suspension. The roots were carefully removed from the pots and washed with distilled water until substrate was removed. The final third of each root was removed with stainless steel scissors and immersed for 5 min in a plastic beaker with 200 mL of conidial

suspension (10^6 conidia/mL) (PASTOR-CORRALES; ABAWI, 1987). Immediately after inoculation, the plants were transplanted into pots

with capacity for 0.3 kg of substrate. Plants with cut roots, which had been immersed in distilled water for 5 min, were used as controls.

Table 1. Cowpea genotypes and its origin.

Cowpea Genotypes	Origins
383 Mosqueado	São José do Norte RS
Pele de Moça	Feira livre - Cabrobó PE
Rasga Letra	Feira livre Araripina PE
Pitiúba	Embrapa Meio Norte
Manteiga	Feira livre Ibimirim PE
MNCO1-649F-2-1	Embrapa Meio Norte
Boca Negra	Embrapa Cenargen
BR-17 Gurguéia	Embrapa Meio Norte
Canapu PE	Feira livre Salgueiro PE
Paulistinha	Embrapa Meio Norte
Epace 10	Estação Experimental do IPA Goiana PE
Maravilha	Feira livre Arara PB
Miranda IPA 207	Instituto Agrônomo de Pernambuco – IPA
BRS Xiquexique	Embrapa Meio Norte
Sempre Verde Verdadeiro	Feira livre Lagedo PE
Bastião	Feira livre Serra Talhada PE
Sempre Verde Salgueiro.	Feira livre Salgueiro PE
Bastãozinho	Feira livre Ibimirim PE
Bajão	Feira livre Igarapu PE
Patativa	Embrapa Meio Norte
Chico Modesto 2	Feira livre Araripina PE
Juazeiro 7	Feira livre BA
Vitória 2	Feira livre Vitoria de Santo Antão PE
BRS Novaera	Embrapa Meio Norte
Rouxinol	Cruzamento entre duas cultivares UFRPE
Esperança	Cruzamento entre duas cultivares UFRPE
Encruzilhada1	Feira Livre Encruzilhada Recife PE
Macaibo	Embrapa Meio Norte Teresina PI
Portalegre RN 1	Feira Livre Porta alegre RN
Portalegre2	Crioula Porta alegre RN
Canapuzinho	Feira livre Salgueiro PE
Cavaleiro 8	Feira livre Cavaleiro Jaboatão dos Guararapes PE
BRS Cauamé	Embrapa Meio Norte
Costela de Vaca	Feira livre Serra Talhada
IPA 206	IPA PE
CNC 0434	Embrapa Meio Norte
BR 10 Piauí	Embrapa Meio Norte
BRS Pujante	Embrapa Semiárido Petrolina PE

The severity of Fusarium wilt in plants was evaluated at 21 d after inoculation using a scale modified from Schoonhoven and Pastor-Corrales (1987), in which: 0 = plants without external symptoms; 1 = less than 10% of foliage with chlorosis and/or wilting; 2 = approximately 25% leaves with chlorosis and/or wilting; 3 = approximately 50% of leaves and branches with chlorosis and/or wilting, along with plants showing dwarfism; 4 = approximately 75% or more of withered leaves and branches, severe dwarfism, and premature defoliation, often resulting in plant death. For each genotype, the average grade of three repetitions was used to group the genotypes into the following reaction classes: 0 to 1.0 = resistant (R); 1.1 to 2.0 = moderately resistant (MR); 2.1 to 4.0 =

susceptible (S).

RESULTS AND DISCUSSION

In the experiment, genotypes 19, 5, and 14 were ranked as susceptible, moderately resistant, and resistant, respectively (Table 2). Susceptible genotypes exhibited symptoms such as chlorotic leaves and branches, wilting, dwarfism, defoliation, and plant death. The moderately resistant genotypes showed 20% of the leaves with symptoms of chlorosis and/or wilt. The mean values ranged from 0 to 0.88, indicating that the genotypes were resistant. The genotypes, Canapu PE, Miranda IPA 207, Esperança, and BRS Pujante, had no symptoms of Fusarium wilt (Table 2).

Table 2. Reaction of 38 cowpea genotypes to Fusarium wilt (R - resistant; MR - moderately resistant; S - susceptible).

Genotypes	Values*	Reaction	Genotypes	Values	Reaction
383Mosqueado	4.00	S	Portalegre 1	1.66	MR
Rasga Letra	4.00	S	Epace 10	1.33	MR
Bastião	4.00	S	Sempre Verde Salgueiro	1.33	MR
Barjão	4.00	S	Canapuzinho	1.33	MR
Rouxinol	4.00	S	Chico Modesto 2	1.22	MR
Boca Negra	4.00	S	Juazeiro 7	0.88	R
BR-17 Gurguéia	4.00	S	Vitória 2	0.88	R
BRS Xiquexique	3.55	S	Sempre verde	0.88	R
CNC 0434	3.55	S	Pitiúba	0.44	R
IPA 206	3.55	S	Manteiga	0.44	R
BR 10 Piauí	3.44	S	Maravilha	0.44	R
BRS Cauamé	3.11	S	Patativa	0.44	R
Paulistinha	3.11	S	BRS Novaera	0.44	R
Bastiãozinho	3.11	S	Costela de Vaca	0.44	R
Cavaleiro 8	3.00	S	MNCO1-649F-2-1	0.44	R
Encruzilhada 1	2.88	S	Canapu PE	0.00	R
Macaibo	2.66	S	Miranda IPA 207	0.00	R
Pele de Moça	2.44	S	Esperança	0.00	R
Portalegre 2	2.22	S	BRS pujante	0.00	R

*The values mean: 0 = plant without external symptoms; 1 = less than 10% of foliage with chlorosis and / or wilting; 2 = approximately 25% leaves with chlorosis and / or wilting; 3 = approximately 50% of leaves and branches with chlorosis and / or wilting, with plants showing dwarfism; 4 = approximately 75% or more of withered leaves and branches, severe dwarfism and premature defoliation, often resulting in plant death.

In the second experiment, 19 genotypes selected in the first study for greater resistance against *Fot* were assessed. These were submitted for re-evaluation to confirm their resistance. Four genotypes, with values ranging from 1.33 to 1.66, were identified as moderately resistant. The remaining 15 genotypes which were characterized as

resistant, had values ranging from 0 to 0.88 and included the genotypes, Sempre verde, Manteiga, Canapu PE, Maravilha, Chico Modesto 2, BRS Nova era, Canapuzinho, Costela de Vaca, and Miranda IPA 207, which were free of Fusarium wilt symptoms (mean 0) (Table 3).

Table 3. Reaction of selected cowpea genotypes to Fusarium wilt (resistant (R); moderately resistant (MR); susceptible (S)).

Genotypes	Values (mean)*	Reaction to Fusarium wilt
R-17 Gurguéia	4.00	S
Portalegre 1	1.66	MR
Sempre verde salgueiro	1.44	MR
Vitória 2	1.33	MR
BRS pujante	1.33	MR
Epace 10	0.88	R
MNCO1-649F-2-1	0.88	R
Juazeiro 7	0.44	R
Pitiúba	0.44	R
Patativa	0.44	R
Esperança	0.33	R
Sempre verde	0.00	R
Manteiga	0.00	R
Canapu PE	0.00	R
Maravilha	0.00	R
Chico Modesto 2	0.00	R
BRS Novaera	0.00	R
Canapuzinho	0.00	R
Costela de Vaca	0.00	R
Miranda IPA 207	0.00	R

*The values mean: 0 = plant without external symptoms; 1 = less than 10% of foliage with chlorosis and / or wilting; 2 = approximately 25% leaves with chlorosis and / or wilting; 3 = approximately 50% of leaves and branches with chlorosis and / or wilting, with plants showing dwarfism; 4 = approximately 75% or more of withered leaves and branches, severe dwarfism and premature defoliation, often resulting in plant death.

In this study, we identified novel genotypes that display resistance to *Fot* infection. The cultivation of resistant cultivars provides an environmentally friendly and low-cost method for disease control and remarkably reduces the incidence of Fusarium wilt in cowpea. Thus, this work provides new possibilities for cowpea genotypes with greater resistance to Fusarium wilt than those already available in the literature. These findings are supported by previous studies that also involved the genetic resistance of plants against wilt pathogens (POTTORFF et al., 2014; MAHMOUD; ABD EL-FATAH, 2020). Considering that genetic control through resistant cultivars is the most efficient control method for Fusarium wilt, this study shows

the importance of exploring genetic variability in cowpea germplasm banks in management practices and for future application in cowpea breeding programs.

The susceptibility of the cultivar, BR-17 Gurguéia, as reported in previous studies (ASSUNÇÃO et al., 2003; RODRIGUES; COELHO, 2004; RODRIGUES et al., 2006; NORONHA et al., 2013), was confirmed, as all the plants were already dead at 15 d after inoculation in both experiments. This effect of disease on BR-17 Gurguéia was important in establishing a susceptibility pattern for 50% of the genotypes in the first experiment. These genotypes showed high levels of stem necrosis and wilt symptoms on the

leaves. The susceptibility of the BRS Xiquexique, BRS Cauamé, and IPA 206 has also been reported in previous studies (ASSUNÇÃO et al., 2003; RODRIGUES et al., 2006; NORONHA et al., 2013).

The genotype, MNCO1-649F-2-1, was maintained as a resistance standard in both experiments. Previous studies have demonstrated its high resistance to Fusarium wilt, displaying no disease symptoms or with less than 10% of the foliage showing chlorosis and/or wilt (NORONHA et al., 2013). MNCO1-649F-2-1 is a semi-commercialized cowpea genotype with tolerance to abiotic stresses, such as water deficit and salinity (SOUZA et al., 2013). For both experiments, 12 genotypes maintained the highest level of resistance highlighted by the genotypes, Miranda IPA, and Canapu PE, which were free of disease symptoms in both experiments. The genotypes, Epace 10, Chico Modesto 2, Canapuzinho BRS Pujante, and Vitória 2 were moderately resistant or resistant in at least one of the experiments performed.

Resistance to various pathogens and insects, abiotic stress tolerance, and high yield should be considered when choosing resistant genotypes (SANTOS et al., 2009). For instance, the cultivar, Epace 10, has a high yield potential, virus resistance, and drought tolerance (BARRETO et al., 1988; SANTOS et al., 2009), while Canapuzinho is considered resistant to *Thanatephorus cucumeris* infection (NECHET; HALFELD-VIEIRA, 2007). The cultivar, BRS Novaera, has high productivity and agronomic characteristics (FREIRE FILHO et al., 2008), and Costela de Vaca produces high yields in dryland cultivation (DANIEL; LUCAS, 2015). The results for Pitiúba, in the present study, were also promising, corroborating the results of Araújo (2017). The cultivars, Miranda IPA 207 and Patativa, which were resistant to Fusarium wilt in the present study, also showed excellent cowpea yield in Pernambuco (COSTA et al., 2013). In particular, the genotype, Miranda IPA 207, has several agronomic characteristics to be considered for cowpea cultivation (COSTA et al., 2013). Santos (2013) proved that Miranda IPA 207 stood out with a grain yield higher than 1,000 kg/ha.

Previous genetic studies on cowpea genotypes and *Fot* have also been reported. According to Pottorff et al. (2012), two resistant loci were identified in the cultivars, California Blackeye 27 and IT93K-503-1, for resistance to race 3 (*Fot3-1*) and race 4 (*Fot4-2*). The identification of these loci will help in the development of molecular markers for use in assisted selection to incorporate resistance into cowpea cultivars (POTTORFF et al., 2014). Therefore, the resistant genotypes identified in this study can be used for resistance inheritance studies, genetic mapping, and identification of molecular markers associated with resistance for use in assisted selection, and for the incorporation of resistance into

cultivars in breeding programs.

Further studies are needed to determine the biochemical resistance mechanisms associated with the activity of defense enzymes, such as phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, chitinase, glucanase, and lipoxygenase (MANDAL; MITRA, 2007; MANDAL et al., 2008; XUE et al., 2014; XUE et al., 2015; FORTUNATO et al., 2015). The enzyme, peroxidase, increases in root responses to attack by pathogens such as *F. oxysporum* f. sp. *phaseoli* (YE; NG, 2002; MANDAL et al., 2008; XUE et al., 2014; XUE et al., 2015) and has accumulated antifungal activity in xylem during colonization of plant tissues by vascular pathogens (YADETA; THOMMA, 2013).

CONCLUSION

In conclusion, the genotypes, Canapu PE, Miranda IPA 207, Esperança, and BRS Pujante, have been shown to have greater resistance to Fusarium wilt. Therefore, these resistant genotypes can be recommended for commercial planting and for future application in cowpea breeding programs.

ACKNOWLEDGMENT

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for granting a Master's scholarship. We also thank the Pernambuco Agronomic Institute (IPA), Antonio Felix da Costa, for the supply of cowpea genotypes. We appreciate the researchers from Embrapa Middle North (Kaesel Jackson Damasceno e Silva and Maurício de Moura Rocha) for providing the MNCO1-649F-2-1 genotype, and Prof. Sami Jorge Michereff for providing the *F. oxysporum* f. sp. *tracheiphilum* (CMM-732).

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