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SHORT COMMUNICATION

Pathogenicity and aggressiveness of *Macrophomina phaseolina* isolates to castor (*Ricinus communis*)

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ABSTRACT
Charcoal rot (*Macrophomina phaseolina*) is one of the most important diseases of castor (*Ricinus communis)* in Northeastern Brazil, particularly in the State of Bahia, where approximately 65% of the Brazilian yield of castor originates. A study was made on the variability of the pathogenicity and aggressiveness of the charcoal rot pathogen to castor. Twenty-seven isolates of *M. phaseolina* were obtained from six plant species: *Ricinus communis* (n=1), *Gossypium hirsutum* (n=2), *Sesamum indicum* (n=1), *Helianthus annuus* (n=1), *Jatropha gossypifolia* (n=1) and *Arachis hypogaea* (n=1). All were inoculated on plants belonging to the castor genotype BRS Energia. All isolates were pathogenic to castor independently from their original host species. A high variability in their aggressiveness was observed.

Key words: charcoal rot, sclerotial wilt, variability.

Charcoal rot is one of the most important diseases of castor (*Ricinus communis* L.) in the state of Bahia, the main region of castor production in Brazil, where approximately 65% of the national yield originates (Severino et al. 2012). Nevertheless, there is no published information on the yield losses due to charcoal rot on castor in Brazil. *Macrophomina phaseolina* (Tassi) Goid., the fungus causing charcoal rot infects more than 500 plant species, including some of the world's most important crops such as soybean, cotton and corn (Su et al. 2001; Gupta et al. 2012). In Brazil, charcoal rot is common and it is considered one of the most prevalent disease problems of soybean (Reis et al. 2014).

The fungal infection and disease development are favored by high temperatures and drought (Dhingra and Sinclair 1978; Grezes-Besset et al. 1996; Gupta et al. 2012) and in Northeastern Brazil, castor is usually cultivated under such conditions (Severino et al. 2012). Moreover, castor is frequently cultivated in association or in rotation schemes with crops that are also susceptible to *M. phaseolina*, such as cowpea, corn and cotton, allowing the maintainance of high populations of the fungus in the soil.

Recent initiatives to incorporate castor in the crop rotation system in areas of the Brazilian Cerrado, mainly after soybean (Evogene, 2011, 2013), can further increase the importance of this pathogen in those areas. Therefore, this study had the objective of assessing the pathogenicity and aggressiveness of *M. phaseolina* isolates obtained from different hosts and locations to castor.

Twenty-seven isolates of *M. phaseolina* were obtained from plants of castor (*Ricinus communis*, n=21), sesame (*Sesamum indicum*, n=1), sunflower (*Helianthus annuus*, n=1), bellyache bush (*Jatropha gossypifolia*, n=1), cotton (*Gossypium hirsutum*, n=2) and peanut (*Arachis hypogaea* n=1), showing typical symptoms of charcoal rot, collected in the states of Bahia (BA), Maranhão (MA), Paraíba (PB) and Rio Grande do Norte (RN) (Table 1).

The inoculum production and inoculation technique were adapted from Mihail (1992), Grezes-Besset et al. (1996), and Amusa et al. (2007). Parboiled rice grains (100 g) and water (20 mL) were added to 200 mL Erlenmeyer flasks and autoclaved at 120 °C for 30 min. After cooling, five mycelial plugs of each *M. phaseolina* isolate, obtained from 5-day-old cultures grown on potato-dextrose-agar (PDA) were placed in the flasks, which were then maintained at 30 °C, in continuous dark, until complete colonization of the rice grains. The flasks were shaken daily to allow a fast and even colonization. After 15 to 20 days, when full colonization was reached, the colonized rice grains were dried overnight in a laminar flow cabinet and then stored in paper bags at room temperature until inoculation.

Castor seeds of BRS Energia were treated with carboxin+thiram (Coutinho et al., 2012) and sown in 280 mL nursery tubes filled with an autoclaved substrate consisting of peat and vermiculite (3:1 v:v). Inoculation was performed by inserting a colonized rice grain into the stem of each 30-day-old castor plant 2 cm above the soil surface. The stems were previously wounded with a metal rod in order to facilitate the insertion of the rice grain. Plants having non-colonized rice grains inserted in the stems as described above were used as control. The experiment was performed in a greenhouse in a randomized block scheme with three replications of 10 plants each, and the experiment was performed twice.

Evaluations consisted on the determination of the percentage of dead plants 10 days after inoculation. Data was found not to be normally distributed and variance was not homogeneous, hence the data was analyzed with a generalized linear model, assuming a Poisson response and a logit link function, using the R software (R Core Team 2013) with the "multcomp" package (Hothorn et al. 2008). Contrast analysis of treatments were run by means of *z* test (Tukey-contrasts, p<0.01).

All the isolates were pathogenic and able to induce necrotic symptoms on castor stems. Additionally a high variability on the aggressiveness of the isolates was observed and this was independent from the original host of the isolate or its geographical procedence (Table 1).

Seventeen isolates were able to induce more than 75% of plant mortality and among them 10 isolates caused a mortality rate higher than 90%. From the 21 isolates originally obtained from castor, 13 provoked more than 75% of plant mortality and nine caused more than 90% of plant mortality. Among the six isolates originally obtained from hosts other than castor, four provoked a mortality rate higher than 75%, but only isolate CCMF-CNPA 278 was able to induce a mortality rate higher than 90%.

A contrasting result was observed among isolates CCMF-CNPA 293 and 294, obtained from a single cotton plant: Isolate CCMF-CNPA 293 caused 80% plant mortality whereas isolate CCMF-CNPA 294 killed only 3% of the plants.

The isolates of *M. phaseolina* from Bahia (n=13) were obtained from distinct areas where castor had been regularly grown for several years. Notwithstanding, a high variability was observed among them. Six isolates led to a mortality rate higher than 75%, whereas six others led to less than 30% of plant mortality. Additionally, one isolate (CCMF-CNPA 282) caused 56.70% mortality (Table 1). A high degree of variability in aggressiveness was also found for isolates from an area where castor was being grown for the first time - Balsas/MA. A single locality which was previously utilized for the cultivation of soybean, corn and cowpea yielded four isolates, three of which (CCMF-CNPA 652, 653 and 654) caused more than 75% of plant mortality, whereas one isolate (CCMF-CNPA 651) was not able to induce plant death (Table 1). Additionally to isolate CCMF-CNPA 651, only one other isolate included in this study (CCMF-CNPA 290) did not induce plant death.

The results of this study demonstrated that a high degree of variation in the aggressiveness of *M. phaseolina*

TABLE 1 - Pathogenicity and aggressiveness to castor (*Ricinus communis*) of 27 Brazilian isolates of *Macrophomina phaseolina* obtained from different hosts and geographical locations.

Isolate	Original Host		Geographical origin (municipality/state)	Pathogenicity[a]	Percent of Plant Death[b]	
	Species	Cultivar				
CCMF-CNPA 287	*R. communis*	CNPAM 2001-42	Patos/PB	+	100.00	a[c]
CCMF-CNPA 291	*R. communis*	BRS Nordestina	Central/BA	+	100.00	a
CCMF-CNPA 295	*R. communis*	BRS Energia	Campina Grande/PB	+	100.00	a
CCMF-CNPA 278	*Jatropha gossypifolia*	-	Lagoa Seca/PB	+	96.70	a
CCMF-CNPA 279	*R. communis*	-	Central/BA	+	96.70	a
CCMF-CNPA 288	*R. communis*	IAC 2028	Irecê/BA	+	96.70	a
CCMF-CNPA 296	*R. communis*	BRS Energia	Campina Grande/PB	+	96.70	a
CCMF-CNPA 653	*R. communis*	*	Balsas/MA	+	93.30	a
CCMF-CNPA 280	*R. communis*	-	Central/BA	+	90.00	a
CCMF-CNPA 292	*R. communis*	BRS Nordestina	Irecê/BA	+	90.00	a
CCMF-CNPA 669	*Arachis hypogaea*	BRS 1	Apodí/RN	+	86.70	a
CCMF-CNPA 277	*Helianthus annuus*	-	Campina Grande/PB	+	83.30	ab
CCMF-CNPA 283	*R. communis*	BRS Energia	Irecê/BA	+	83.30	ab
CCMF-CNPA 293	*Gossypium hirsutum*	BRS 286	Apodí/RN	+	80.00	ab
CCMF-CNPA 617	*R. communis*	-	Campina Grande/PB	+	80.00	ab
CCMF-CNPA 652	*R. communis*	*	Balsas/MA	+	76.70	ab
CCMF-CNPA 654	*R. communis*	*	Balsas/MA	+	76.70	ab
CCMF-CNPA 282	*R. communis*	BRS Energia	Irecê/BA	+	56.70	b
CCMF-CNPA 274	*Sesamum indicum*	BRS Seda	Campina Grande/PB	+	30.00	c
CCMF-CNPA 281	*R. communis*	-	Central/BA	+	26.70	c
CCMF-CNPA 285	*R. communis*	BRS Energia	Irecê/BA	+	23.30	c
CCMF-CNPA 284	*R. communis*	BRS Energia	Irecê/BA	+	16.70	cd
CCMF-CNPA 286	*R. communis*	BRS Energia	Irecê/BA	+	6.70	de
CCMF-CNPA 289	*R. communis*	BRS Nordestina	Irecê/BA	+	6.70	de
CCMF-CNPA 294	*G. hirsutum*	BRS 286	Apodí/RN	+	3.30	e
CCMF-CNPA 290	*R. communis*	BRS Paraguaçu	Irecê/BA	+	0.00	e
CCMF-CNPA 651	*R. communis*	*	Balsas/MA	+	0.00	e

*Advanced lines of Evofuel company; [a]Ability to induce necrotic symptoms on the castor genotype inoculated in the present study; [b]Percentage of plant death as mean from both assays; [c] Means followed by the same letter, in the column, do not differs by *z* test (Tukey-contrasts, p<0.01).

isolates from distinct hosts and from different geographical origins to castor exists (Table 1). This should be regarded as of major concern, particularly where castor is intended to be used as an alternative crop for rotation systems in the Brazilian Cerrado. Charcoal rot is likely to become a major problem in such situations since castor, together with soybean and corn are susceptible to this pathogen and, consequently, a progressive increase in the pathogen population is likely to occur through successive crops in areas where this scheme is adopted.

The findings for the *M. phaseolina* isolates included in the present study were equivalent to those of several other studies (Pearson et al. 1987; Mayek-Perez et al. 2001; Su et al. 2001; Almeida et al. 2008; Rayatpanah et al. 2012) and in agreement with their conclusions that *M. phaseolina* has no host-specificity and is highly variable for virulence or aggressiveness, even among isolates obtained from a single plant (Dhingra and Sinclair 1973).

It could also be argued that different cryptic taxa exist under the name *M. phaseolina* which might be associated with charcoal rot in Brazil. This hypothesis is strengthened by a recent work where a new species (*Macrophomina pseudophaseolina*) was described causing charcoal rot of *Arachis hypogaea* L., *Hibiscus sabdariffa* L. and *Vigna unguiculata* (L.) Walp. in Senegal (Sarr et al. 2014). According to the authors, although only isolates originated from Senegal grouped in the clade of *M. pseudophaseolina*, the fungus may have a much wider distribution. Additionally, the authors also highlighted that isolates used in their study could not be allocated to specific groups according to host or geographic origins. According to them, some isolates from the same host or location tended to group together, while, some isolates from the same host or location belonged to two species (e.g. *M. phaseolina* and *M. pseudophaseolina*). Therefore, it is possible that the so-called *M. phaseolina*, in fact includes other yet undescribed cryptic species. This might explain the variability of the results obtained in the present study and throughout the literature. Thus, further studies involving the pathogenicity and aggressiveness of *Macrophomina*, independently from their host species, should previously consider the possibility of elucidating the correct identity of the pathogen through molecular methods, including larger number and wider distribution of isolates as well as including the performance of cross inoculation test.

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