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Identification of pathogenic fungi and preliminary screening for resistance in *Jatropha curcas* L. germplasm

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Abstract *Jatropha curcas* L. (jatropha) is a perennial undomesticated plant with a high untapped potential for sustainable production of food, biofuels and biomass-based products. The identification of resistance sources in the germplasm is important to develop improved varieties. In order to characterize presence of pathogens in multiple production sites, leaf samples showing symptoms of diseases were collected in Argentina, India, and Cameroon. Samples were used for DNA extraction and species identification through PCR, sequencing and BLAST searches. The identified fungal isolates were inoculated onto healthy plants using the agar block method. Re-isolation from symptomatic plants with subsequent DNA extraction and molecular identification confirmed pathogenicity of six species: *Phoma herbarum*, *Diaporthe phaseolorum*, *Nigrospora sphaerica*, *Gibberella moniliformis*, *Alternaria alternata*, and *Fusarium* sp. The identified pathogens were used to screen for resistance in a set of 20 jatropha genotypes. One genotype showed resistance to *P. herbarum*, seven to *N. sphaerica*, five to *D. phaseolorum* and one to *G. moniliformis*. Only three genotypes showed resistance to more than one pathogen. The sources of resistance and their respective level need to be validated in field experiments.

Keywords Jatropha · Pathogens · Resistance · Breeding

Introduction

Jatropha or physic nut is a perennial oilseed plant from the family *Euphorbiaceae*. It is native to Mexico and Central America, although it is also widely distributed in wild or semi-cultivated areas in Central and South America, Africa and Asia (Brittaine and Lutaladio 2010). Jatropha has caught the attention of many researchers and investors and has become a popular crop worldwide, due to the need of energy alternatives, such as the production of renewable energy sources and the growth of energy crops (Contran et al. 2013). With suitable agronomical management, jatropha can be grown on poor quality soils, reducing the risk of competition for land use for food production, although yield might not achieve its full potential (Contran et al. 2013).

Most jatropha genotypes are known to have toxins like phorbol esters, curcins and trypsin inhibitors, which confer special characteristics to the plant for different uses such as medicinal applications and bio-pesticides (Brittaine and Lutaladio 2010; Contran et al. 2013). Moreover, non-toxic genotypes from Mexico have also been studied and described and have potential in further value chains for food and feed (Francis et al. 2013; Vera Castillo et al. 2014). Additionally, with increasing

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energy consumption and the growing need to find sustainable alternatives, jatropha represents an option as a source of vegetable oil for biofuel production (Contran et al. 2013). Nonetheless, jatropha is still an undomesticated plant with great genetic diversity hindering the ability to make sustainable use of its overall potential, therefore improved cultivars with distinctive and uniform characteristics are needed for an efficient agricultural production of this crop (Martin and Montes 2014).

Given the previously mentioned toxic characteristics, jatropha seems not to be greatly threatened by diseases (Brittaine and Lutaladio 2010; Nithiyanantham et al. 2012). However, other studies disprove the claims about the resistance of jatropha to diseases and several groups of pathogens seem to have overcome disease resistance of jatropha (Kumar et al. 2009). Genetic variations might have also played a role in the differential levels of resistance or susceptibility to diseases, especially in monoculture (Gohil and Pandya 2008).

Studies about pathogens affecting jatropha have been carried out in different regions of the world, like Mexico and Korea (Espinoza-Verduzco et al. 2012; Kwon et al. 2012). In these studies research was focused on one particular organism present in a specific location. Nonetheless there are also studies that aim to develop inventories of diseases from particular countries, such as those from Ginting and Maryono (2009) in Indonesia and de Campos Dianese et al. (2010) in Brazil. Various pathogens have been identified in jatropha in countries like Mexico, Brazil, India, China and Nigeria, such as *Lasiodiplodia theobromae*, *Alternaria alternata*, *Fusarium* sp., *Cercospora* sp., *Oidium* sp. and *Colletotrichum* sp., among others (de Campos Dianese et al. 2010; Hudge and Datar 2010; Kwon et al. 2012; Machado and Pereira 2012; Pereira et al. 2009; Wu et al. 2011; Zarafi and Abdulkadir 2012, 2013). In those previous studies, genotype resistance for the mentioned pathogens was not further tested. Nonetheless, it is important to study the potential sources of disease resistance of jatropha germplasm, considering the various disease reports under monoculture conditions (Brittaine and Lutaladio 2010; Contran et al. 2013; Gohil and Pandya 2008).

Taking into consideration that jatropha is produced in various tropical and subtropical countries around the world (Brittaine and Lutaladio 2010), this research aimed to identify fungal pathogens and potential sources of resistance in jatropha germplasm from three worldwide locations (Argentina, India and Cameroon). Our objectives were to: (i) identify through genomic sequencing and confirm pathogenicity of fungi in jatropha, and (ii) assess the level of resistance in a set of 20 diverse genotypes. This research appears to be the first approach of a multiple production site analysis for fungal identification in physic nut in regions of three different continents, as well as the first study of resistance of jatropha genotypes to various pathogens.

Materials and methods

Identification of fungal pathogens from various locations and confirmation of their pathogenicity

A total of twenty-nine symptomatic jatropha leaf samples from all sites, i.e., Formosa, Argentina; Chaswad, Lakkihalli and Pune, India, and Batchenga, Cameroon (Table 1) were used for the identification of pathogens. Each sample was assigned a unique sample code. Leaf samples were collected in the field, placed in plastic bags and stored at 4 °C. Samples were taken according to visual symptoms present on the leaves, which were possibly related to fungal or bacterial infection.

For each sample, small pieces (5 × 5 mm) of leaves were cut with a sterile scalpel, placed into petri dishes with ME-medium (2% malt extract, 1.6% agar, 10 μg ml^{-1} Ampicillin), and incubated for 7 days at

Table 1 Identification of fungi isolated from jatropha leaf samples obtained from various regions in different countries on three continents

Origin of Samples	Isolate Code	Fungus Identified as
Formosa, Argentina	1bAR2	*Phoma herbarum*
Chaswad, India	12IC	*Alternaria alternata*
Lakkihalli, India	4IL	*Nigrospora sphaerica*
Lakkihalli, India	6IL	*Phoma herbarum*
Lakkihalli, India	10IL	*Alternaria alternata*
Lakkihalli, India	13IL	*Phoma herbarum*
Pune, India	19IP	*Alternaria alternata*
Pune, India	24IP	*Gibberella moniliformis*
Batchenga, Cameroon	4CB	*Diaporthe phaseolorum*
Batchenga, Cameroon	6CB	*Phoma herbarum*
Batchenga, Cameroon	13CB	*Thielavia* sp.
Batchenga, Cameroon	18CB	*Fusarium* sp.
Batchenga, Cameroon	21CB	*Muscodor albus*
Batchenga, Cameroon	28CB	*Neurospora sitophila*

28 °C. Each fungus growing from a sample was transferred individually to ME-medium. Each isolate was sequentially transferred until obtaining an apparently axenic culture. Axenic cultures were observed in the microscope to confirm purity of the isolates, and were first identified to the genus level using morphological criteria (Bridge 2013; Narayanasamy 2011).

DNA extraction of fungal cultures was done using the PeqGold Fungal DNA Mini Kit (PeqLab Biotechnologie GmbH, Erlangen) following the manufacturer's instructions. To amplify the extracted DNA, a PCR reaction was performed. The reaction mix consisted of 1x *Taq*-buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 μM forward primer ITS1 (TCCGTAGGTGAACC TGCGG), 0.5 μM reverse primer ITS4 (TCCTCCGC TTATTGATATGC), and 1 U *Taq*-polymerase (Thermo Scientific GmbH, Braunschweig), using 0.2 μg DNA as template, in a final volume of 40 μl (modified from Cordier et al. 2012; Espinoza-Verduzco et al. 2012; Voegele and Schmid 2011; White et al. 1990). The PCR-program used was: (i) initial denaturation at 95 °C for 3 min; (ii) denaturation at 95 °C for 30 s; (iii) annealing at 55 °C for 30 s; (iv) elongation at 72 °C for 1 min; (v) steps 2 to 4 were repeated 34 times; (vi) final elongation at 72 °C for 5 min (modified from Cordier et al. 2012; Voegele and Schmid 2011; White et al. 1990).

PCR products were analyzed on 1% Agarose gels (Biozym LE agarose, Biozym Scientific GmbH, Hessisch Oldendorf) prepared with 1 x TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA), using Gene Ruler 1 kb ladder as marker (Thermo Scientific GmbH). PCR products were visualized with ethidium bromide and documented using the Quantum-Capt ST4 Gel Doc system (PeqLab Biotechnologie GmbH). PCR products with fragment sizes ≥ 450 based pairs (bp) were purified using the PeqGold Cycle-Pure Protocol Kit (PeqLab Biotechnologie GmbH) and sequenced by Microsynth AG (Balgach) using primer ITS1.

Sequences were manually trimmed (sequences below 350 bp were not considered) using the program DNA Star. Nucleotide queries were done at the NCBI BLAST GenBank database. Species were designated based on an E value of 0.0 and a percentage of identity ≥ 99%. Fungal identification was based on sequence and morphological features.

The seeds from genotype JAT000032 provided by JatroSolutions GmbH were sown 2 cm deep in "Profi Substrat" (Einheitserdewerke Gebr. Patzer GmbH & Co. KG, Sinntal-Altengronau) using pots with a 2 liter capacity. Pots were maintained in the greenhouse with a 12 h daylight and 12 h night regime, at temperatures of 24 °C and 22 °C, respectively. Pots were watered every other day.

Eight weeks after sowing, a total of 30 plants were inoculated with previously identified fungi (Table 1). Inoculation was done in the greenhouse using the agar block method (Yang et al. 1991), each plug containing a pure fungal culture. Each leaf had two inoculations with the same isolate, on one side of the central leaf vein the inoculation was done directly on the leaf surface and on the other side, the leaf was punctured 10 times with an insulin needle of 9.525 mm length, and the agar plug was placed over the punctured area, as shown in the figure (Online Resource 1). Inoculation was done on the first three pairs of true leaves, and isolates (Table 1) were used three times for inoculation in three different plants. After inoculation, a plastic frame was positioned covering the jatropha plants and a humidifier (Condair 3001, Axair GmbH, Garching) at 90% humidity was installed for a period of 5 days. Each leaf was labeled according to the fungal sample used for the inoculation. One and two weeks after inoculation (WAI), plants were assessed for the presence of infection and symptoms.

In order to fulfill Koch's postulates and confirm their pathogenicity on jatropha plants, fungi were re-isolated from diseased tissue onto ME-medium and fresh healthy jatropha plants were again inoculated as described above (Pereira et al. 2009). Each fungal isolate was inoculated 3 times.

Three WAI, leaves from each repetition were collected. The inoculated leaf area was cut into 4 small pieces, which were disinfected with 1% sodium hypochlorite amended with 1% Tween 20 (Sigma-Aldrich, Steinheim) for 1 min. Tissue was placed on ME-medium and incubated at 28 °C for a period of 7 days. To proceed with DNA extraction, one isolate each from one repetition was selected. For selection of the isolate, the presence and type of symptoms, i.e., how symptoms manifested in previous tests were considered. The amount of fungal growth was also considered for selection of isolates. Identification of the selected isolates was verified as described above by sequencing.

Screening of resistance to pathogens on 20 different jatropha genotypes

The panel of genotypes used in this study was from the breeding program of JatroSolutions GmbH. The group of genotypes (edible and non-edible) comprises a

representative sample of the genetic diversity available in the germplasm of jatropha. The level of homozygosity in the panel of inbred lines is very high (Montes et al. 2014). Healthy jatropha plants from 20 different genotypes were sown as described above using pots of one gallon capacity. Table 2 shows characteristics of the 20 genotypes.

To evaluate the susceptibility to different fungal isolates, plants were placed in the greenhouse with conditions of 12 h daylight and 12 h night, a day temperature of 27 °C and a night temperature of 18 °C. Inoculation was done 4 weeks after sowing on 220 plants following the previously described method, where one plant per isolate was used to test the preliminary resistance of the evaluated genotypes. Two non-cotyledonal leaves of each plant were inoculated with a single fungal isolate in both punctured and non-punctured areas. Inoculation was repeated on the second leaf of the same plant. The humidifier was set to 85% during the whole experiment. Inoculated plants were covered with transparent foils to promote proper conditions for fungal infection. Observations for presence of symptoms on inoculated plants were done in 5 day intervals during a period of 20 days after the inoculation and were registered for each evaluated genotype.

Table 2 Description of germplasm used to evaluate resistance to pathogens

Genotype	Type	Phorbol Ester *
G1	Line	Present
G2	Hybrid	Present
G3	Line	Present
G4	Hybrid	Present
G5	Line	Present
G6	Line	Present
G7	Hybrid	Present
G8	Line	Absent
G9	Hybrid	Absent
G10	Line	Absent
G11	Hybrid	Absent
G12	Line	Absent
G13	Hybrid	Present
G14	Hybrid	Absent
G15	Line	Absent
G16	Hybrid	Present
G17	Hybrid	Absent
G18	Line	Absent
G19	Hybrid	Present
G20	Line	Absent

* Presence/absence of phorbol esters was measured using the HPLC method as described by Makkar et al. 1997

Results

Identification of fungal pathogens from various locations and confirmation of their pathogenicity

Fungal isolates were identified by morphological criteria and confirmed by sequencing (Table 1), and then used to inoculate healthy jatropha plants. Two WAI, several of the isolates produced symptoms on leaves and were considered as pathogens. The observed symptoms varied according to the identified pathogen.

Figures 1, 2 and 3 illustrate symptoms observed 2 WAI of different isolates identified as *Phoma herbarum* and *Alternaria alternata*. *Phoma herbarum* produced a semi-circular light-brown spot with a dark-brown circumference and slight chlorosis around the spot in the wounded area (Fig. 1a and b, Fig. 2a and b); the adaxial surface of the leaf presented some sporogenic tissue (Figs. 1a and 2a). Symptoms of *A. alternata* were more visible on the abaxial surface of the leaf as irregular brown spots within or close to the punctured area, with slight chlorosis in the surroundings (Fig. 1c and d, Fig. 3a and b). Initial growth of white mycelia was observed in both sides of the leaf.

Plants inoculated with *N. sphaerica* (Fig. 4a and b) showed a necrotic halo around each puncture and chlorosis surrounding the entire punctured area. Meanwhile, inoculations with *G. moniliformis* developed as a necrotic irregular spot within the punctured area with a defined chlorotic surrounding (Fig. 3c and d), whereas *D. phaseolorum* produced a general chlorosis of the punctured region Fig. 4c and d). No sporulation was observed on the leaf surface of these three fungi.

Considering the observed symptoms of the fungal isolates from Table 1, as well as the characterization and identification of the evaluated fungi, ten isolates were selected to re-inoculate healthy jatropha plants (Table 3). After observing the presence of symptoms on the inoculated plants, fungi from diseased tissue were re-isolated and identified by sequence analyses. Pathogenicity on jatropha was confirmed for all ten isolates (Table 3), according to the presence of symptoms in the inoculated areas. Four novel jatropha pathogens were also identified: *P. herbarum*, *N. sphaerica*, *G. moniliformis* and *D. phaseolorum* (Figs. 1, 2, 3 and 4).

Fig. 1 Symptoms caused by two fungal isolates from India to jatropha leaves of a single genotype. Symptoms resulting from *Phoma herbarum* (isolate 13IL) inoculation observed from the adaxial surface of the leaf (**a**), and the abaxial surface of the leaf (**b**). Symptoms caused by *Alternaria alternata* (isolate 19IP) seen from the adaxial surface of the leaf (**c**), and the abaxial surface of the leaf (**d**)

Screening of resistance to pathogens on 20 different jatropha genotypes

Six of the identified isolates from Table 3 were selected to test their pathogenicity on 20 jatropha genotypes (Table 4). Isolate 18CB (*Fusarium* sp.) was not considered for the inoculation since it could not be identified to the species level.

Two of the selected isolates were proven pathogenic for all 20 genotypes (1bAR2, identified as *P. herbarum* and 10IL, identified as *A. alternata*), whereas some isolates were non-pathogenic on specific genotypes. From the isolates with confirmed *P. herbarum* pathogenicity (Table 4), the isolate from Cameroon (6CB) showed symptoms in 19 of the tested 20 genotypes.

Isolates identified as *P. herbarum* initially showed symptoms of yellowish spots on the leaves after 5 to 7 days after inoculation, which later turned into dark brown irregular spots surrounded by chlorotic tissue on both sides of the inoculated leaves. Presence of white mycelium was also observed in some of the genotypes, such as in genotypes 5 and 14 (Fig. 2). *Alternaria alternata* isolated from samples from India (10IL) was seen as an irregular dark brown spot, more visible on the abaxial surface of the leaf, and showed irregular coverage of white mycelia, as seen in genotype 7 (Fig. 3).

Gibberella moniliformis (teleomorph: *Fusarium verticillioides*) isolated from samples from India (24IP) also showed pathogenicity on 19 of the 20 genotypes tested. Symptoms showed small-sized gray/light brown spots with a dark brown halo only visible on the abaxial surface of the leaf, such as in genotype 10 (Fig. 3). Five genotypes were resistant to *Diaporthe phaseolorum*, isolated from Cameroon samples (4CB) and seven genotypes showed resistance to *Nigrospora sphaerica*, the isolate from India (4IL) (Table 4). *Diaporthe phaseolorum* produced symptoms as brownish irregular spots visible on both sides of the leaf; when colonization was more extensive, white mycelium above the spots was also visible (Fig. 4). Inoculation with *N. sphaerica*

Fig. 2 Symptoms on fungal-inoculated jatropha leaves. Symptoms caused by *Phoma herbarum* isolate from Argentina F1b-AR2 on plant genotype 5 observed from the adaxial surface of the leaf (**a**), and the abaxial surface of the leaf (**b**). Symptoms caused by *Phoma herbarum* isolate from Cameroon F6-CB on plant genotype 14 seen from the adaxial surface of the leaf (**c**), and the abaxial surface of the leaf (**d**)

resulted in irregularly shaped dark brown- grayish spots surrounded by chlorotic margins (Fig. 4).

Discussion

Identification of fungal pathogens from various locations and confirmation of their pathogenicity

Previous reports by de Campos Dianese et al. (2010) in Brazil and Ginting and Maryono (2009) in Indonesia, exemplify studies of pathogens affecting jatropha in specific regions. The present research is the first analysis of intercontinental production sites for pathogen identification in physic nut, as well as the first study of jatropha -associated fungi in the specific region of Cameroon.

From the results in Table 3, the identification of the isolate 18CB, limited to the genus, indicates this isolate should be further analyzed in order to determine the species of *Fusarium* that is pathogenic to jatropha. With proven pathogenicity, it is important to identify this *Fusarium* species, perhaps with the help of phylogenetic trees based on a larger dataset of sequence information. So far, the main *Fusarium* species reported as a disease of physic nut is *Fusarium solani*, reported in Brazil (Machado and Pereira 2012) and in China (Wu et al. 2011). Considering these reports, it might be possible that the isolate identified as pathogenic *Fusarium* sp. (isolate 18CB) belongs to the species *F. solani*. In addition, once specific species of pathogenic *Fusarium* have been identified, the symptoms expressed in further inoculation assays should be correlated to symptoms described in the literature, such as those observed in Nigeria (Zarafi and Abdulkadir 2012, 2013) and China (Wu et al. 2011).

According to previous reports on pathogens in jatropha, *Alternaria alternata* causing diseases in physic nut has been reported in Mexico, Indonesia and India (Espinoza-Verduzco et al. 2012; Ginting and Maryono 2009; Hudge and Datar 2010). *Alternaria alternata* was also identified as pathogenic in the current research from

Fig. 3 Symptoms on fungal-inoculated jatropha leaves. Symptoms resulting from *Alternaria alternata* (isolate 10IL) inoculation on plant genotype 7 observed from the adaxial surface of the leaf (**a**), and the abaxial surface of the leaf (**b**). Symptoms caused by *Gibberella moniliformis* (isolate 24IP) on plant genotype 10 seen from the adaxial surface of the leaf (**c**), and the abaxial surface of the leaf (**d**)

samples collected in India. As far as it is known, *A. alternata* was the only fungus from those identified to the species level in this research that has been previously reported as a pathogen of jatropha. It is important to keep this pathogen in mind for the establishment of jatropha plantations, since it has a wide range of plant hosts causing leaf spots, blossom rots, blights and fruit rots (Nasim et al. 2012).

Other pathogenic fungi were also identified which have not been previously reported in jatropha. Firstly, the fungus *Phoma herbarum* was identified in all three studied locations (Argentina, India, and Cameroon) (Table 3). This fungus has been identified as a pathogen in dandelion (*Taraxacum officinale*) (Neumann and Boland 1999), and therefore has also been studied as an alternative to control this weed (Stewart-Wade and Boland 2005). Additionally, *P. herbarum* had been shown to be associated with blister rust on white pine (*Pinus monticola*) (Hunt 1997) and has been reported to produce leaf spot disease on cherry palm (Kumla et al. 2016). The genus *Phoma* sp. is also known to be found in alfalfa developing several dark brown to black irregular spots on the lower leaves (Wang et al. 2003). Another pathogen identified in the current study, so far not reported in jatropha, was *Diaporthe phaseolorum*, which is a pathogen of soybean (Grijalba and Ridao 2014; Sun et al. 2012) and has also been associated with grapevine in China (Dissanayake et al. 2015).

Fusarium verticillioides (teleomorph: *Gibberella moniliformis*), as well as *Nigrospora sphaerica*, were identified isolates from India. These pathogens have not yet been reported to be associated with jatropha. Nonetheless, they have been identified as causal agents of various diseases in other plants. *Fusarium verticillioides* (including its teleomorph *G. moniliformis*) is pathogenic on sugarcane in Iran (Mohammadi et al. 2012) and a destructive disease in maize known as ear rot (Covarelli et al. 2012; Yuan et al. 2013). *Nigrospora sphaerica* causes leaf blight in tea plants (*Camellia sinensis*), as well as leaf spots in mango, the ornamental vine Chinese wisteria (*Wisteria sinensis*), blueberries and the medicinal plant *Glycyrrhiza glabra* (Liu et al. 2016; Pandey et al. 2013; Soylu et al. 2011; Verma and Gupta 2007; Wright et al. 2008).

Fig. 4 Symptoms on fungal-inoculated jatropha leaves. Symptoms as a result of *Nigrospora sphaerica* (isolate 4IL) inoculation on plant genotype 19 observed from the adaxial surface of the leaf (**a**), and the abaxial surface of the leaf (**b**). Symptoms caused by *Diaporthe phaseolorum* (isolate 4CB) on plant genotype 10 seen from the adaxial surface of the leaf (**c**), and the abaxial surface of the leaf (**d**)

Given the variability of locations and conditions in which jatropha is produced, it is recommended to perform additional inoculation tests with the novel pathogens in field trials, to verify their behavior in non-controlled environmental conditions, determine the influence of climatic characteristics in the incidence

Table 3 List of fungi fulfilling Koch's postulates pathogenic to jatropha identified through molecular methods [a], among fungi isolated from leaf samples from different locations

Origin of Samples	Isolate Code	Identification after Re-Isolation	Query Cover	E value	Ident.	Accession
Argentina	1bAR2	*Phoma herbarum*	100%	0.0	100%	AB369456
India	12IC	*Alternaria alternata*	99%	0.0	100%	FJ809940
	4IL	*Nigrospora sphaerica*	99%	0.0	100%	HQ608063
	10IL	*Alternaria alternata*	99%	0.0	100%	KP278204
	13IL	*Phoma herbarum*	98%	0.0	100%	JX867221
	19IP	*Alternaria alternata*	99%	0.0	100%	FJ809940
	24IP	*Gibberella moniliformis*	99%	0.0	100%	GQ168842
Cameroon	4CB	*Diaporthe phaseolorum*	98%	0.0	99%	AY577815
	6CB	*Phoma herbarum*	98%	0.0	100%	JX867222
	18CB	*Fusarium* sp.	100%	0.0	100%	JF740930

[a] DNA extraction of fungal isolates was done using the PeqGold Fungal DNA Mini Kit and DNA amplification through PCR. PCR products were analyzed by gel electrophoresis, purified using the PeqGold Cycle-Pure Protocol Kit and sequenced by Microsynth AG. Sequences were manually trimmed and nucleotide queries were performed at the NCBI BLAST GenBank database. Species were assigned based on an E-value of 0.0 and a percentage of identity ≥ 99%

Table 4 Summary of resistance to pathogens of 20 jatropha genotypes to six fungal isolates with confirmed pathogenicity

Genotype	Pathogenicity of identified fungi						Total of pathogenic results per genotype
	Phoma herbarum (1bAR2)	*Nigrospora sphaerica* (4IL)	*Alternaria alternata* (10IL)	*Gibberella moniliformis* (24IP)	*Diaporthe phaseolorum* (4CB)	*Phoma herbarum* (6CB)	
G1	x	−	x	x	x	−	4
G2	x	x	x	x	x	x	6
G3	x	−	x	x	x	x	5
G4	x	−	x	x	x	x	5
G5	x	x	x	x	x	x	6
G6	x	x	x	x	x	x	6
G7	x	x	x	x	x	x	6
G8	x	−	x	x	x	x	5
G9	x	x	x	x	−	x	5
G10	x	−	x	x	−	x	4
G11	x	−	x	x	x	x	5
G12	x	−	x	x	−	x	4
G13	x	x	x	x	x	x	6
G14	x	x	x	x	−	x	5
G15	x	x	x	x	−	x	5
G16	x	x	x	−	x	x	5
G17	x	x	x	x	x	x	6
G18	x	x	x	x	x	x	6
G19	x	x	x	x	x	x	6
G20	x	x	x	x	x	x	6

(x) = symptomatic, (−) = non-symptomatic

and severity of symptoms, and evaluate extent of damage according to seasonal differences.

Screening of resistance to pathogens on 20 different jatropha genotypes

Most previous studies related to jatropha genotype evaluations focused on variables like plant height, seed weight, seed yield, oil content and phorbol ester content (Ahoton and Quenum 2012; Gohil and Pandya 2009). The current research is the first study of jatropha genotypes dedicated to resistance to pathogens. As documented in Table 4, according to the tested isolates, jatropha genotypes reacted differently in reference to resistance to pathogens, even when different isolates were identified as the same pathogen, such as the case of *P. herbarum*. In this study, *P. herbarum* was found to have pathogenic effects on leaves of all genotypes, except in genotype 1 when inoculations were done with the isolate obtained from Cameroon (6CB). This could indicate potential resistance of genotype 1 to *P. herbarum*. Nonetheless, *P. herbarum* was the only pathogen (besides *Alternaria alternata*) that showed pathogenicity in the majority of genotypes, therefore additional experiments under different environmental conditions and field tests registering the severity of the symptoms, would be useful to determine the optimal conditions that influence pathogenicity of *P. herbarum*. Furthermore, in order to verify the existence of different strains of this pathogen and its behavior to different environmental conditions, it is suggested to evaluate other genotypes for resistance of *P. herbarum*, including the use of isolates obtained from other locations, such as the isolate 13IL (Table 3) from India.

As seen in Table 4, genotypes 1, 10 and 12 were resistant to two of the six fungal isolates evaluated in this study; *N. sphaerica* was non-pathogenic to all three of these genotypes, all of which are lines (Table 4).

Genotype 1 showed resistance to *P. herbarum* isolate from Cameroon (6CB), while genotypes 10 and 12 were resistant to *D. phaseolorum*. These two genotypes (10 and 12) are free of phorbol esters (Montes et al. 2014) (Table 2), and showed resistance to the same two pathogens (*N. sphaerica and D. phaseolorum*). This may indicate that these genotypes (10 and 12) are more resistant to other diseases as compared to the other tested genotypes. However, further experiments are required to confirm this and to further consider such genotypes in breeding for disease resistant lines.

The presence of phorbol ester might be associated with resistance to some pathogens. Genotypes 3, 4, 8, 9, 11, 14, 15 and 16 showed resistance to one of the six tested fungal isolates. Four of the genotypes (3, 4, 8 and 11) were resistant to *N. sphaerica*. Three genotypes, (9, 14 and 15), all of them without presence of phorbol esters (Table 2), did not show any symptoms after being inoculated with *D. phaseolorum. Gibberella moniliformis* was non-pathogenic to genotype 16. Consequently, breeding for resistance to the jatropha pathogens *N. sphaerica* (non-pathogenic on seven genotypes) and *D. phaseolorum* (non-pathogenic on five genotypes) is of interest for subsequent research. Relationships between genotype characteristics and response to pathogens need to be further investigated in order to (i) determine the influence of phorbol ester presence in resistance to pathogens, and (ii) determine if lines or hybrids (Table 2) influence resistance to pathogens. However, in this study it seems there were no clear differences in response to pathogens when considering the characteristics of the different genotypes evaluated (Table 2); supplementary data is give in Online Resource 2. Additional experiments with susceptible genotypes as well as other genotypes not tested in this study, would be useful to determine the best productive varieties/genotypes with potential resistance to pathogens.

Genotypes 2, 5, 6, 7, 13, 17, 18, 19 and 20 showed susceptibility to all six fungal isolates (Table 4) evaluated in this research. Additional experiments with other pathogens would be valuable to determine their possible use in breeding selection processes. The observed effects of the six fungal isolates on different jatropha genotypes (Table 4) confirmed the pathogenicity of these fungi to jatropha as observed in Table 3. Further experiments are recommended to determine the different conditions influencing the infectiousness of the pathogens identified in this study, the potential resistance of different genotypes to other pathogens and the interactions in the triad genotype, environment and pathogen.

This research is the first to develop a detailed laboratory identification of fungal pathogens associated with jatropha samples obtained in different continents. Three fungi have been identified for the first time as pathogens of jatropha (*D. phaseolorum, G. moniliformis and N. sphaerica*). This research is also the first study on sources of resistance to pathogens of jatropha genotypes. Further experiments should be conducted to confirm pathogenicity of these mentioned novel pathogens in field trials, as well as to determine sources influencing resistance to pathogens in jatropha genotypes for consequent breeding purposes.

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