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RESEARCH ARTICLE

Dieback and pod rot caused by *Lasiodiplodia theobromae* and *L. iraniensis* in native accessions of cacao (*Theobroma cacao*) from Amazonas, Peru

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Abstract
Lasiodiplodia spp. are known to cause canker, rot and dieback symptoms in several crops worldwide. In this study, two *Lasiodiplodia* species were identified as pathogens of native cacao accessions from the department of Amazonas, Peru, causing dieback and pod rot on young and old stems. We evaluated the macro and micro morphological characteristics, and conducted a molecular identification based on a phylogenetic analysis with a multilocus dataset with informative loci for the genus. Microscopic examination revealed the presence of immature conidia that were initially hyaline, subovoid, unicellular, and double-layered, which became reddish brown with a central septum and longitudinal grooves at maturity. In the phylogenetic analysis, we identified our isolates as *L. theobromae* and *L. iraniensis* with strong bootstrap support values. Koch's postulates were fulfilled after the re-isolation of the same species from diseased tissues of cacao fruits and stems after an artificial inoculation. Therefore, in this study, we report for the first time *L. theobromae* and *L. iraniensis* infecting native cacao plants in Amazonas, Peru.

Keywords Cacao dieback · Cacao pod rot · Chocolate · Tropical phytopathogens

Introduction

Theobroma cacao L. (Malvaceae) is a native plant from South America, widely cultivated in the world tropics (Motamayor et al. 2002). The Amazonian region shared between Peru, Ecuador, Colombia and Brazil harbors the highest diversity of the crop and the most ancient evidence of its domestication and consumption (Bustamante et al. 2022; Osorio-Guarín et al. 2017; Thomas et al. 2012). In the Amazonas department, Peru, *T. cacao* is produced mainly under organic and agroforestry systems, representing the main source of income for 11,623 families (Oliva et al. 2020). In 2016, the cacao produced in the provinces of Bagua and Utcubamba, in Amazonas, became protected by the Peruvian appellation rules under the name "Cacao Amazonas Peru", in recognition for its superior qualities of flavor and aroma (Oliva et al. 2020).

Fungal diseases are the main limiting factor of the crop worldwide (Huda-Shakirah et al. 2022). Currently, the main fungal diseases reported on Peruvian cacao farms are frosty pod rot (caused by *Moniliophthora roreri*), witches' broom (*M. perniciosa*), and the emerging thread blight disease (*Marasmius infestans*) (Díaz-Valderrama et al. 2020; Huaman-Pilco et al. 2023). Additionally, cacao is affected by other yield-reducing pathogens such as *Lasiodiplodia* spp. (Puig et al. 2021; Rahim et al. 2022). *Lasiodiplodia* is a cosmopolitan genus of fungal pathogens, with a predominance in tropical climates causing wood diseases (dieback, canker, leaf blight, and root and fruit rot) on a widespread variety of economically important crops (Mohali et al. 2005; Zheng et al. 2021). Its capacity to survive in the soil and vegetative residues makes its phytosanitary management challenging (Picos-Muñoz et al. 2014). In recent years, multiple

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species of this genus have been reported in different crops (Moreira-Morrillo et al. 2021). These include *L. iraniensis* described for the first time from diseased plants of *Salvadora persica*, *Juglans* spp., *Eucalyptus* spp., *Citrus* spp., and *Prunus* spp. in Iran (Abdollahzadeh et al. 2010). This pathogen was also associated with mandarin (Al-Sadi et al. 2013), mango (Marques et al. 2013; Rodríguez-Gálvez et al. 2017; Sakalidis et al. 2011), *Bougainvillea spectabilis* (Li et al. 2015), and *Anacardium occidentale* (Netto et al. 2017). *Lasiodiplodia brasiliensis* was first described causing papaya stem-end rot (Netto et al. 2014), and *L. jatrophicola* and *L. euphorbicola* on nuts in Brazil (Machado et al. 2014). In Italy, *L. citricola* was associated with trunk diseases in grapevines (Carlucci et al. 2015). The most common species, *L. theobromae*, is pathogenic on more than 500 hosts (perennial fruit and nut trees, vegetables and ornamentals) causing cankers, necrosis and rotting of stems and roots (Gnanesh et al. 2022).

The germplasm botanical garden of native cacao accessions from Northern Peru, managed by the *Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva* (INDES-CES) of the National University Toribio Rodríguez de Mendoza de Amazonas (UNTRM-A) (Oliva-Cruz et al. 2021, 2022) often harbors trees with a high incidence of symptoms of dieback on twigs, fruit rotting, and stem and leaf necrosis, typical of *Lasiodiplodia* spp. infection, which affects their performance. In this study we aimed to conduct a morphological and molecular identification of the causal agents of these *Lasiodiplodia*-like symptoms in the INDES-CES cacao germplasm collection.

Table 1 KUELAP herbarium voucher numbers of *Lasiodiplodia* spp. isolates and source

Species	Strain	Voucher numbers	Source
L. theobromae	INDES-JHP23	KUELAP-2941	Stem
L. theobromae	INDES-JHP40	KUELAP-2942	Stem
L. theobromae	INDES-JHP57	KUELAP-2943	Stem
L. theobromae	INDES-JHP60	KUELAP-2944	Leaf
L. iraniensis	INDES-JHP61	KUELAP-2945	Stem
L. theobromae	INDES-JHP62	KUELAP-2946	Leaf

Materials and methods

Sampling, incidence, and isolation

Between November 2021 and March 2022, we conducted phytosanitary inspections of native cacao accessions in the INDES-CES cacao germplasm collection, located in the Cajaruro district, Utcubamba province, Amazonas department, Peru (E: 792283; N: 9364101; 648 m a.s.l.). The samples were collected in February 2022. A total of 10 accessions (220 plants in total) were evaluated. We collected samples of diseased cacao tissues from plants showing dieback on young stems, and leaf spots (Fig. 1). The incidence of stem dieback was determined using the formula: $I\ (\%) = (ni/\ N) \times 100$ (ni: total number of plants affected, N: total number of plants evaluated) posed by Rodríguez-Gálvez et al. (2017).

Small sections ($\sim 1 \times 1\ cm^2$) of symptomatic tissues were sterilized in 2% sodium hypochlorite (NaClO) for 2 min. They were then washed in sterile distilled water for 1min, three times. The surface-sterilized sample was dried

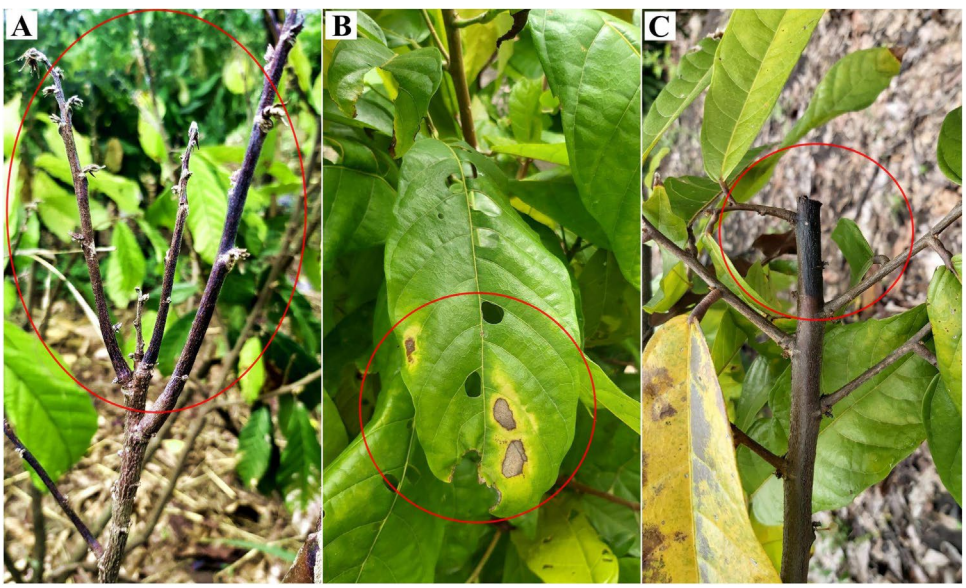

Fig. 1 Sample types of diseased cacao tissues associated with *Lasiodiplodia* spp. in the INDES-CES germplasm botanical garden. **A** Tree showing terminal branch dieback. **B** Leaf spot. **C** Branch showing rot symptoms after pruning

with a sterile paper towel, transferred to potato dextrose agar medium (PDA), and incubated at 28 °C for 3–5 days. Six fungal strains were obtained using the hyphal tip subculture technique (Table 1).

Morphological identification

The macro and micro morphological characteristics of isolates were evaluated and compared to characteristics reported in previous relevant literature (Phillips et al. 2013; Sakalidis et al. 2011). Three isolates for each strain were incubated at 28 °C for 12 h day/night, and the colony diameter was measured with a digital caliper every 24 h for three days. The isolates were checked every week for the formation of conidiomata, which were observed and photographed on a stereoscope Nikon SMZ18 (Tokyo, Japan). Conidia morphology (cell wall thickness, form, color and presence or absence of septa) was evaluated on an inverted microscope OLYMPUS DP74 (Tokyo, Japan). Conidia were mounted on slides using lactophenol. We measured at least fifty micro structures. Each isolate was preserved in a metabolically inactive state as dried culture in the KUELAP herbarium of the UNTRM-A (voucher numbers in Table 1).

Molecular identification and phylogenetic analysis

Total genomic DNA was extracted from mycelium growing on PDA using the Wizard® DNA kit (Promega, Madison, WI, USA.) as indicated by the manufacturer. PCR assays were performed to amplify the internal transcribed spacer region (ITS1, 5.8S and ITS2 rDNA regions; ITS), and the partial translation elongation factor 1-α (*tef1*), β tubulin (*tub2*) and RNA polymerase II subunit (*rpb2*) genes. We used the primer pairs ITS1F/ITS4, EF1-688F/EF1-1251R, Bt2a/Bt2b, and rpb2-LasF/rpb2-LasR, respectively, as in Huda-Shakirah et al. (2022). PCR products were sequenced at Macrogen (Seoul, South Korea). Raw sequences were edited and assembled with Sequencher 5.4.6. Cleaned sequences and other sequences from reference specimens (Table 2) were aligned with MUSCLE (Edgar 2004) in MEGA 11.0 (Kumar et al. 2018). We generated a concatenated dataset with the four loci obtained with Seaview (Gouy et al. 2010). A maximum likelihood phylogenetic analysis with the concatenated dataset was performed in the CIPRES gateway (Miller et al. 2010). The phylogenetic tree was mid-point rooted and edited with FigTree 1.4.4. (Rambaut 2018).

Pathogenicity tests

Pathogenicity tests were conducted to fulfill Koch's postulate on cacao stems and fruits. Tests on stems were conducted following the mycelial disc method (Puig et al. 2021). Stems of seedlings were superficially disinfected with 70% alcohol. Then, a small wound (5 mm long) was made on each disinfected stem with a sterile scalpel. A disk (5 mm in diameter) of five-day-old isolates grown on PDA at 28 °C were inoculated on two two-month-old cacao seedlings for each *Lasiodiplodia* strain; an additional seedling was inoculated with a disc without the fungus for each isolate as a control. Finally, the inoculated areas were covered with parafilm. All inoculated plants were maintained at 28 °C in a growth chamber.

Pathogenicity tests were also conducted on healthy and mature harvested cacao fruits. Three fruits were used for each of the six strains, two of them were inoculated and one was used as a control. Whole fruits were surface-disinfected in 2% NaClO for 2 min, then washed in sterile distilled water. They were then dried and placed on sterilized Petri dishes within clean and NaClO-disinfected plastic containers. Two UV-sterilized sheets of paper towel humidified with sterile water were placed next to Petri dishes to maintain the relative humidity high. Fruits were wounded with a sterile scalpel to a depth of approximately 5 mm, and mycelium discs (5 mm in diameter) were placed on the wounds. No-fungus PDA disks were used on control fruits. The containers were sealed with stretch film and incubated at 28 °C for 8 days. Pathogenicity tests on seedlings and fruits were replicated twice. When the disease symptoms appeared, the pathogen was re-isolated using the same isolation procedure described earlier. A morphological characterization of the new isolate was performed to confirm that it was the one originally inoculated.

Results

Incidence evaluation

A total of 220 plants in a plot of approximately 0.25 ha were evaluated within the INDES-CES germplasm collection (Table 3). Regressive stem dieback of the cacao crop was detected in all accessions. Accession INDES-50 had the highest incidence of dieback and leaf spot, with 63.6% of plants affected, followed by INDES-67 (59.1%), INDES-54 (57.6%) and INDES-53 (54.5%). The other accessions showed incidence percentages below 50% namely INDES-55 (22.7%), INDES-24 (24.2%), INDES-31 (24.2%), INDES-83 (27.3%), INDES-27 (36.4%), INDES-65 (36.4%).

Morphological characterization of the strains

All six strains had the same growth rate with a mean of 23.47 ± 0.186 (standard error) mm/day at 28 °C after 3 days in PDA medium. We observed dense light-gray mycelium on the first days of growing, turning dark-gray as the days

Table 2 GenBank accession numbers of DNA sequences of *Lasiodiplodia* spp. included in the phylogenetic study

Species	Isolate	Host	Location	GenBank accession number			
				ITS	tef1-α	tub2	rpb2
L. brasiliensis	CBS123095	*Theobroma cacao*	Cameroon	MT587423	MT592135	MT592615	MT592309
L. brasiliensis	CBS115447	*Psychotria tutcheri*	Hong Kong	MT587422	MT592134	MT592614	MT592308
L. brasiliensis	CMM4015[a]	*Mangifera indica*	Brazil	JX464063	JX464049	–	–
L. brasiliensis	CSM11	*Theobroma cacao*	Venezuela	MF436018	MF436006	MF435998	–
L. citricola	CBS124707[a]	*Citrus* sp.	Iran	GU945354	GU945340	KU887505	KU696351
L. citricola	CBS124706	*Citrus* sp.	Iran	GU945353	GU945339	KU887504	KU696350
L. crassispora	CBS118741[a]	*Santalum album*	Australia	DQ103550	EU673303	KU887506	KU696353
L. crassispora	CBS125626	*Vitis vinifera*	South Africa	MT587424	–	MT592617	MT592312
L. euphorbiicola	CMM3609[a]	*Jatropha curcas*	Brazil	KF234543	KF226689	KF254926	–
L. euphorbiicola	CMM3651	*Jatropha curcas*	Brazil	KF234553	KF226711	KF254937	–
L. euphorbiicola	CMW33268	*Adansonia* sp.	Unknown	KU887131	KU887008	KU887430	KU887367
L. hormozganensis	CBS124709[a]	*Olea* sp.	Iran	GU945355	GU945343	KU887515	KU696361
L. hormozganensis	CBS124708	*Mangifera indica*	Iran	GU945356	GU945344	KU887514	KU696360
L. iraniensis	CBS124710[a]	*Salvadora persica*	Iran	GU945348	GU945336	KU887516	KU696363
L. iraniensis	CBS124711	*Juglans* sp.	Iran	GU945347	GU945335	KU887517	KU696362
L. iraniensis	CMW35881	*Adansonia* sp.	Unknown	KU887092	KU886970	KU887464	KU887388
L. iraniensis	**INDES-JHP61**	***Theobroma cacao***	**Perú**	**OR428219**	**OR468302**	**OR468314**	**OR468308**
L. lignicola	CBS134112[a]	*Dead wood*	Thailand	JX646797	KU887003	JX646845	KU696364
L. lignicola	MFLUCC110656	*Dead wood*	Thailand	JX646798	JX646863	JX646846	–
L. mahajangana	CBS124925[a]	*Terminalia catappa*	Madagascar	FJ900595	FJ900641	KU887518	KU696365
L. mahajangana	CBS124926	*Terminalia catappa*	Madagascar	FJ900596	FJ900642	KU887519	KU696366
L. pseudotheobromae	CBS116459[a]	*Gmelina arborea*	Costa Rica	EF622077	EF622057	EU673111	KU696376
L. pseudotheobromae	CBS116460	*Acacia mangium*	Costa Rica	EF622078	EF622058	KU198428	MT592322
L. theobromae	CBS164.96[a]	Fruit on coral reef coast	Indonesia: New Guinea	AY640255	AY640258	EU673110	KU696383
L. theobromae	CBS214.50	*Cajanus cajan*	India	MT587440	MT592152	MT592637	MT592333
L. theobromae	CMW13490	*Eucalyptus urophylla*	Venezuela: Acarigua	KY473071	KY473019	KY472962	KY472888
L. theobromae	CMM4019	*Mangifera indica*	Brazil	JX464096	JX464026	–	–
L. theobromae	CSM57	*Theobroma cacao*	Venezuela	MF436029	MF436017	MF435999	–
L. theobromae	M400	*Theobroma cacao*	USA: Puerto Rico	MN446021	MN536705	MN536694	–
L. theobrome	**INDES-JHP23**	***Theobroma cacao***	**Perú**	**OR428215**	**OR468298**	**OR468310**	**OR468304**
L. theobrome	**INDES-JHP40**	***Theobroma cacao***	**Perú**	**OR428216**	**OR468299**	**OR468311**	**OR468305**
L. theobrome	**INDES-JHP57**	***Theobroma cacao***	**Perú**	**OR428217**	**OR468300**	**OR468312**	**OR468306**
L. theobrome	**INDES-JHP60**	***Theobroma cacao***	**Perú**	**OR428218**	**OR468301**	**OR468313**	**OR468307**
L. theobrome	**INDES-JHP61**	***Theobroma cacao***	**Perú**	**OR428220**	**OR468303**	**OR468315**	**OR468309**
L. viticola	CBS128313[a]	hybrid grape Vignoles	USA	HQ288227	HQ288269	HQ288306	KU696385
L. viticola	CBS128314	Chardonel	USA	HQ288228	HQ288270	HQ288307	KU696386

Sequences in bold were generated in this study

[a]Ex-type isolates

progressed (Fig. 2). After 40–60 days, conidiomata were observed in the deep black colony.

All strains showed morphological characteristics typical of *Lasiodiplodia* spp. (Phillips et al. 2008, 2013), such as, slowly maturing conidia, subovoid to ellipsoid ovoid in shape, with a broadly rounded apex and a truncated base tapering towards the base. Immature conidia were initially double-layered, hyaline and unicellular (Fig. 2). Mature conidia became dark-reddish brown with a central septum, a thick cell wall, and forming longitudinal striations (Fig. 2). Mean length and width of mature conidia did not differ among strains INDES-JHP23 (24.75 × 13.81 µm), INDES-JHP40 (24.98 × 14.08 µm), INDES-JHP57 (24.76 × 13.75 µm), INDES-JHP60 (25.17 × 14.29 µm), INDES-JHP61 (23.16 × 13.31 µm), INDES-JHP62 (25.11 × 13.98 µm) on average.

Table 3 Evaluation of the incidence of dieback in the INDES-CES cacao germplasm collection

Cacao accession	Number of evaluated plants	Number of Infected plants	Incidence (%)
INDES 50	22	14	63.6
INDES 67	22	13	59.1
INDES 54	33	19	57.6
INDES 53	22	12	54.5
INDES 27	11	4	36.4
INDES 65	11	4	36.4
INDES 83	11	3	27.3
INDES 24	33	8	24.2
INDES 31	33	8	24.2
INDES 55	22	5	22.7

Molecular identification and phylogenetic analysis of the strains

Phylogenetic analysis with the multi-locus concatenated data set (ITS, *tef1*, *tub2*, and *rpb2*) identified the species of the six strains obtained from *T. cacao* symptomatic tissues at the INDES-CES germplasm collection. Isolates INDES-JHP23, INDES-JHP40, INDES-JHP57, INDES-JHP60, INDES-JHP62 grouped with the type and reference specimens of *L. theobromae* with a strong bootstrap support of 76%, while isolate INDES-JHP61 clustered with *L. iraniensis* type and reference specimens with a support of 100% (Fig. 3).

Pathogenicity tests of the strains

Both *L. theobromae* and *L. iraniensis* isolates were pathogenic to cacao stems and fruits, inducing lesions of similar appearance (Fig. 4). Stems inoculated with *L. theobromae* and *L. iraniensis* showed visible lesions four days after inoculation. Symptoms appeared as a dark brown lesion, progressing longitudinally from the inoculation sites. The infected tissue turned brown and rotted, subsequently causing dieback after four weeks. Then, black conidiomata were observed on the diseased area (Supplementary Figure S1). These symptoms and signs were the same as those observed in the field. Control seedlings did not develop any symptoms. Additionally, symptoms such as black lesions were observed on fruits inoculated with *L. theobromae* and *L. iraniensis* after two days of inoculation, covering the entire surface as the days progressed after 30 days (Supplementary Figure S2). At this point, a large amount of gray mycelium was observed on the entire surface of the fruit. The fungus was then reisolated from infected stems and pods, showing macro and micro morphological characteristics identical to those observed in the field, fulfilling Koch's postulate.

Discussion

In the present study, *L. theobromae* and *L. iraniensis* were identified as the causal agents of cacao young stems dieback and fruit rot in the INDES-CES native cacao germplasm collection. In recent years, the severity and damage of *Lasiodiplodia* spp. have increased, causing various phytosanitary problems in different crops including cacao (Gnanesh et al. 2022; Pereira et al. 2006; Pisco-Ortiz et al. 2024). The symptoms observed at the INDES-CES germplasm collection had been commonly associated with *Phytophthora* damage due to their similar symptoms on fruits and shoots by local cacao producers. Previous investigations described that *Lasiodiplodia* species transitioned from endophytic to opportunistic pathogens, and they are now considered a threat to different crops of agricultural interest including cacao (Ali et al. 2020; Salvatore et al. 2020). Also, it has been shown that plants subjected to biotic and abiotic stresses are more susceptible to fungi of the genus *Lasiodiplodia* (Moreira-Morrillo et al. 2021; Pereira et al. 2006). In addition to this, mechanical pruning in cacao is a recommended management practice, but the wounds caused by this activity provide an access point for *Lasiodiplodia* infection.

Stem dieback and leaf spot were detected in all cacao accessions evaluated at the INDES-CES germplasm collection. However, different levels of incidence were observed. The clones with the highest incidence (50–65%) were INDES-53, INDES-54, INDES-50, INDES-67. On the other hand, those that showed lower incidence (20–40%) were INDES-24, INDES-27, INDES-31, INDES-55, INDES-65, INDES-83. In recent months, the increase in the incidence of *Lasiodiplodia* diseases is causing concern. Pruning wounds not only provide an entry point for pathogens, but are also a source of stress to the plant (Adu-Acheampong et al. 2012), which may be the cause of the increased incidence of the disease (Rodríguez-Gálvez et al. 2017). Rains and high humidity also play a role on *Lasiodiplodia* diseases as they favor the production of fungal spores which can be disseminated by raindrops and wind (Vásquez-López et al. 2008). The incidence of *Lasiodiplodia* spp. is also influenced by temperature above 30 °C, water stress and low levels of plant nutrition (Gunamalai et al. 2023).

Symptoms observed in the field, as well as those obtained from pathogenicity tests in this study, revealed substantial similarity to those of dieback diseases caused by *Lasiodiplodia* spp. These included a fast-growing, cream-white colony in the first few days, turning dark to black as the days progressed; conidia were dark brown, striated, ellipsoidal and uniseptate (Mbenoun et al. 2008). However, the fungus may show aseptate conidia when young, but these conidia may develop septa as they mature (Burgess et al. 2006; Phillips et al. 2013). In addition, the microscopic

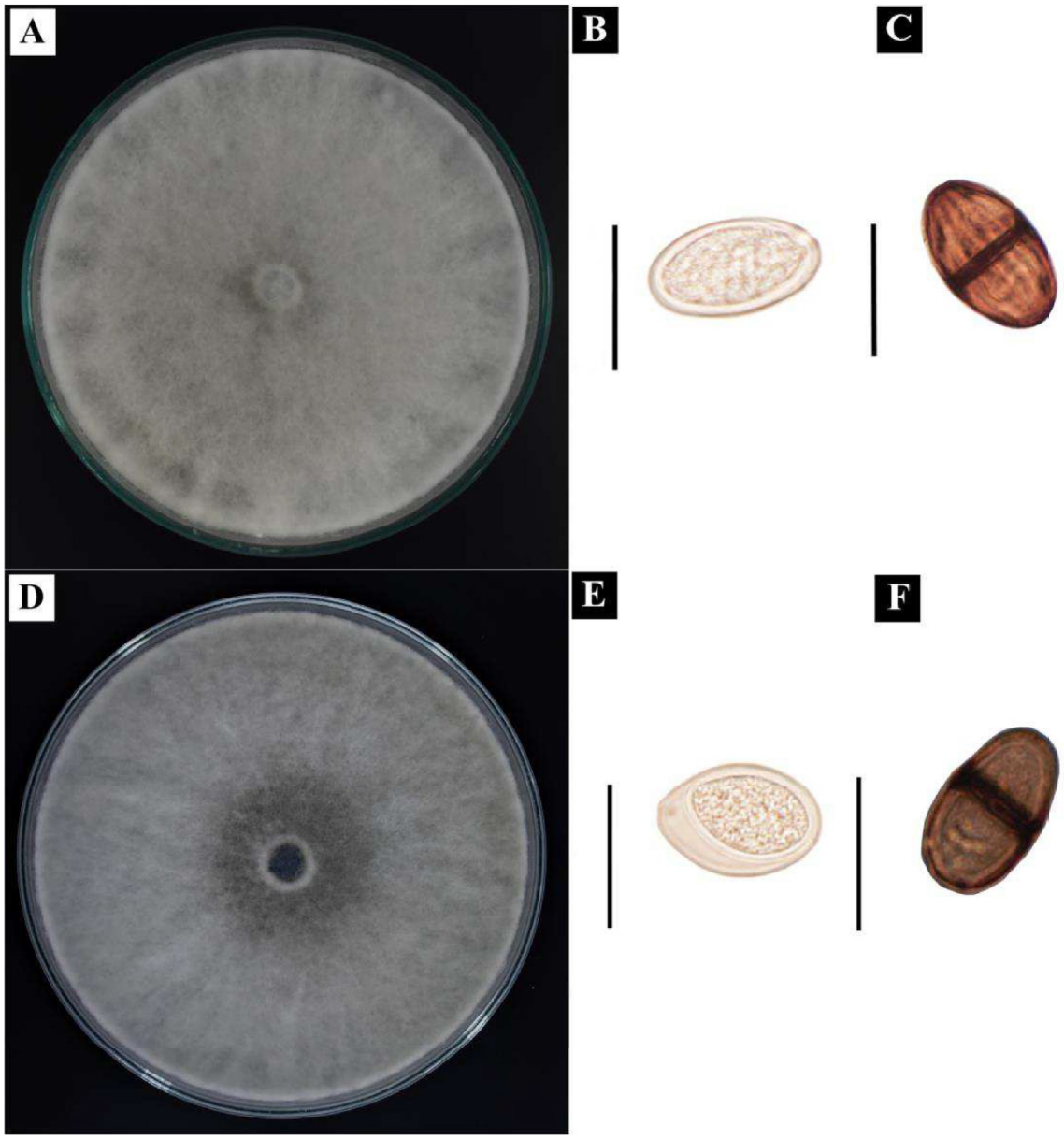

Fig. 2 Colony and conidia characteristics of *Lasiodiplodia* spp. **A–C** *Lasiodiplodia theobromae* INDES-JHP40 macro and micromorphological characteristics: a seven-day old colony in PDA culture medium; **B** hyaline and aseptate immature conidia; **C** mature conidia. **D–F** *Lasiodiplodia iraniensis* INDES-JHP61 macro and micromorphological characteristics: **D** seven-day old colony in PDA culture medium; **E** immature conidia; **F** mature conidia. Photographs of conidia were taken at × 100 (oil immersion). Scale bar = 20 µm

morphological characteristics of the conidia were consistent with those reported for this species in previous studies (Coutinho et al. 2017; Huda-Shakirah et al. 2022). The shape and color of mature conidia, as well as the presence of septa and longitudinal striae were important features for the identification of *Lasiodiplodia* spp. However, even though *L. theobromae* has slightly larger conidia than *L. iraniensis* (23.6–28.8 × 13–15.4 µm vs 22.51–26.09 × 12.75–14.97 µm, respectively), it is not sufficient to distinguish them (Abdollahzadeh et al. 2010; Marques et al. 2013). Here, we also found *L. theobromae* (23.4–26.8 × 12.8–15.2 µm; $n = 50$) has slightly larger conidia than *L. iraniensis* (22.2–25.4 × 11.9–14.2 µm; $n = 50$). However, given that morphological characters are insufficient to identify *Lasiodiplodia* species, molecular phylogeny has become an important tool for species identification (Marques et al. 2013; Pavlic et al. 2004). Other studies showed that phylogenetic analysis with a single locus, such as ITS, is unable to determine species in the genus *Lasiodiplodia*, so additional loci are required (Alves et al. 2005; Ismail et al. 2012). In

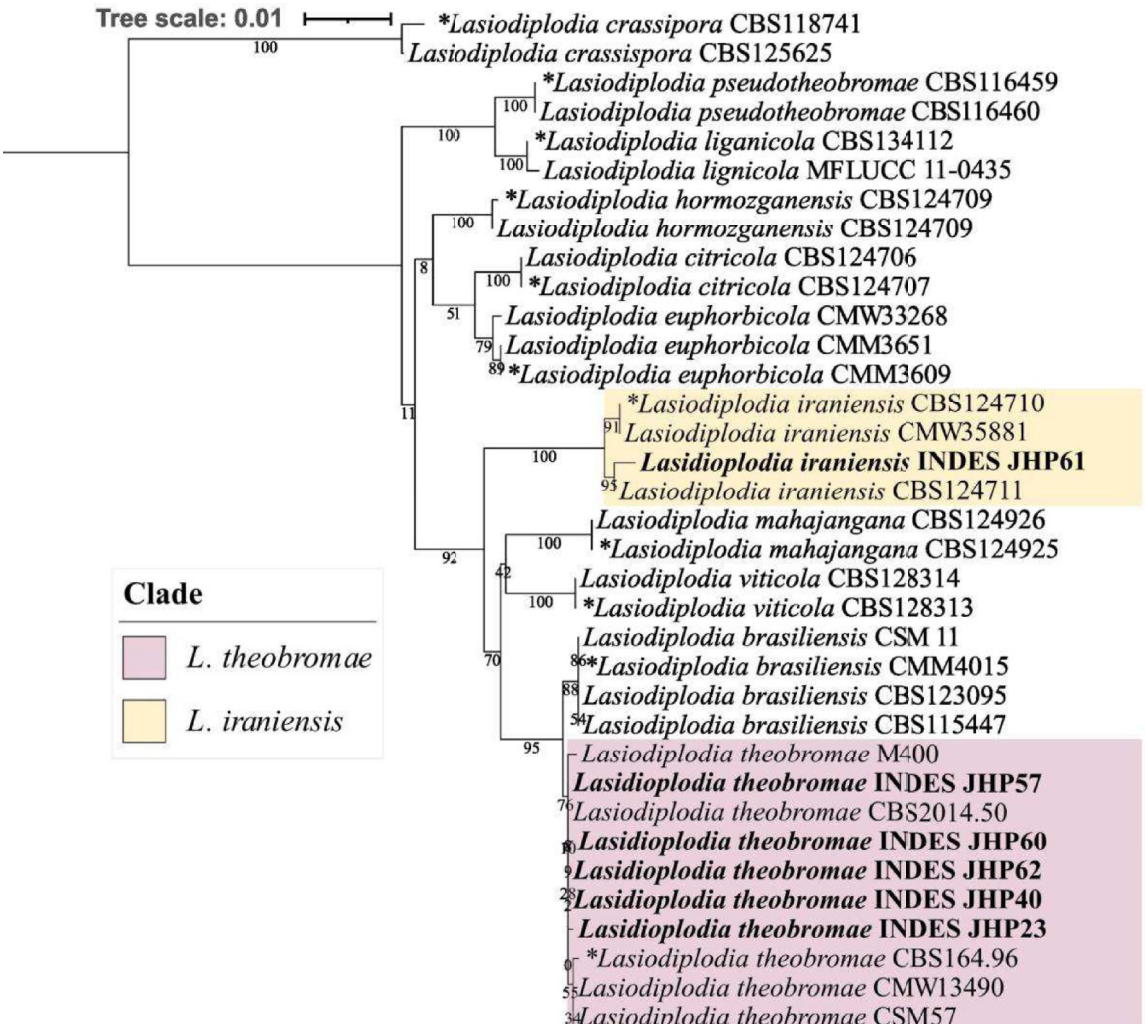

Fig. 3 Phylogenetic tree based on maximum likelihood analysis inferred from a multi-locus concatenated alignment of internal transcribed spacer (ITS) sequences, the partial translation elongation factor 1-α gene (*tef1*), β-tubulin genes (*tub2*) and RNA polymerase II subunit (*rpb2*). Numbers on nodes represent bootstrap values (only values greater than 50% are indicated). Isolates sequenced in this study are shown in bold. Type specimens are marked with an asterisk. *Lasiodiplodia crassispora* was included as outgroup

recent studies, taxonomists have frequently used highly conserved protein-coding genes such as *tef1*, *tub2*, and ITS to construct species-resolving phylogenies (Phillips et al. 2019; Slippers et al. 2014).

The phylogenetic analysis with the concatenated dataset of ITS, *tub2*, *rpb2* and *tef1* sequences clearly placed our isolates within the *L. theobromae* and *L. iraniensis* species clusters with reference specimens from previously published studies (Netto et al. 2014; Phillips et al. 2008, 2013). *Lasiodiplodia theobromae* was the most frequent species in this study, as also found in previous studies (Marques et al. 2013; Netto et al. 2014). This confirms the wide distribution of *L. theobromae* throughout the INDES-CES cacao germplasm bank in Amazonas, Peru. Only one strain of *L. iraniensis* was found. This species was first described in Iran and can infect different hosts, such as *Salvadora persica*, *Juglans* sp. *Citrus* sp. and *Mangifera indica* (Abdollahzadeh et al. 2010). It has also been reported in Brazil on *Mangifera indica* (Al-Sadi et al. 2013; Marques et al. 2013), *Bougainvillea spectabilis* (Li et al. 2015), *Anacardium occidentale* (Netto et al. 2017), and coffee (Ramos et al. 2023).

Finally, the pathogenicity of *L. theobromae* and *L. iraniensis* was confirmed after inoculation of cacao stems and fruits. Even though *L. iraniensis* had a lower prevalence, it was a species that showed the same aggressiveness as *L. theobromae* during pathogenicity tests. Therefore, both species are a threat to this crop. These findings are relevant for management strategies since the disease significantly reduces cacao production upon favorable conditions such as intense rainfall, prolonged drought and the presence of

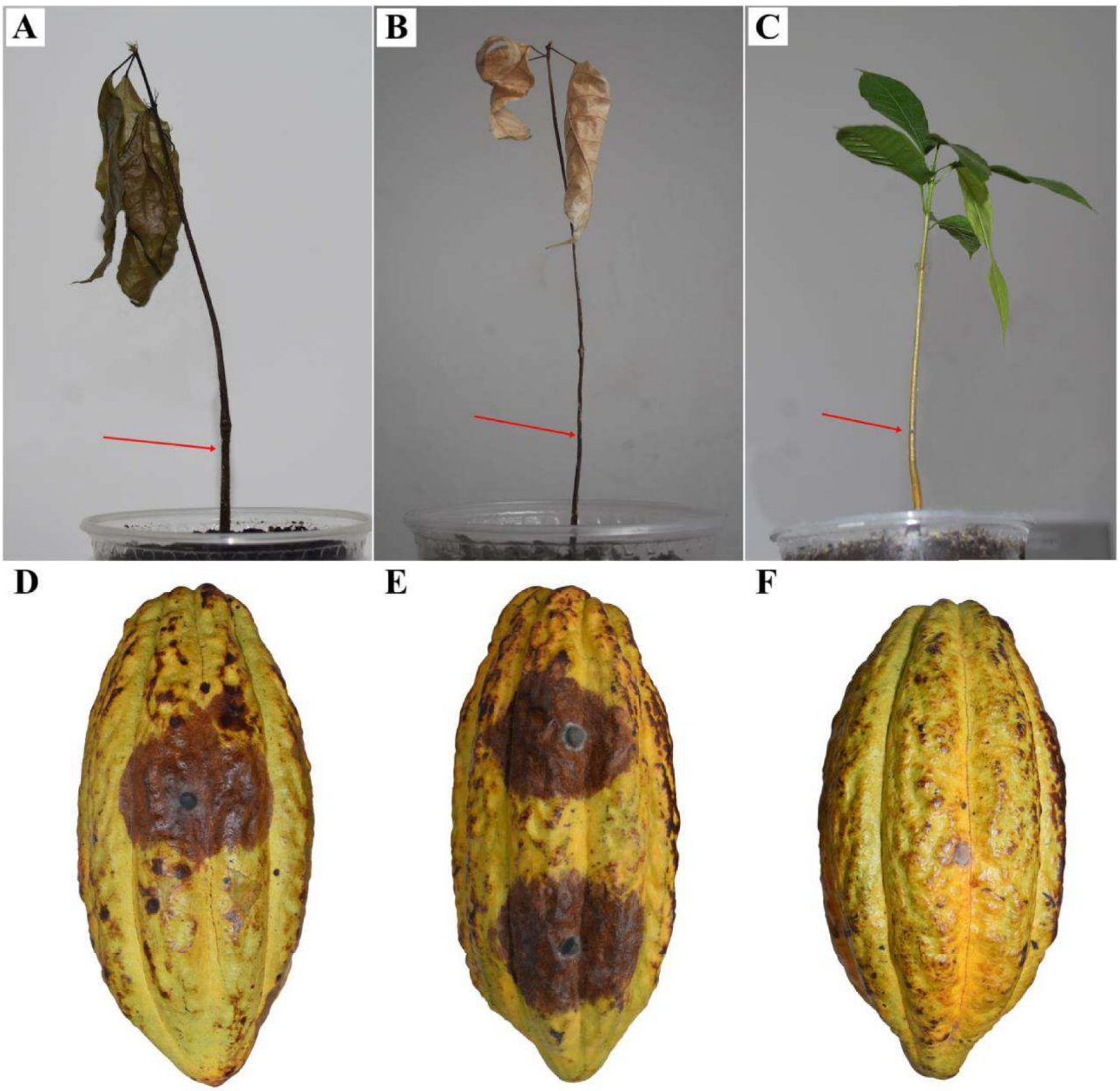

Fig. 4 Pathogenicity of *Lasiodiplodia* spp. on cacao seedlings and fruits. **A**, **B** Stem rot caused by *L. theobromae* INDES-JHP23 and *L. iraniensis* INDES-JHP61, respectively, four weeks after inoculation. **C** Control plant. Red arrows show the point of inoculation. **D**, **E** Fruit rot caused by *L. theobromae* INDES-JHP62 and *L. iraniensis* INDE-JHP61, respectively, three days after inoculation; the dark brown zone shows that the fungi are still growing and colonizing healthy fruits. **F** Control fruit inoculated with a disk of PDA without the pathogen showing no visible symptoms

wounds on the plant. We therefore report for the first time *L. theobromae* and *L. iraniensis* are the causal agents of dieback and leaf spots in cacao plantations from Northern Peru. This research may also be useful for future studies and could help to find effective management strategies of this disease that represents a threat to cacao cultivation in the Amazonas department, Peru.

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Author contributions JHP collected samples, led the conduction of all experiments, analyzed the data, and wrote the original draft of the manuscript, EHD and AFHP collected samples, conducted pathogenicity tests, and revised and edited versions of the manuscript; SMOC secured funds for the development of this study, and revised early versions of the manuscript; JRDV secured funds for the development of this study, supervised the study, analyzed the data, revised and proofread the manuscript.

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Data availability All sequences generated in this study are publicly available under the NCBI Accession Numbers: OR428215-20, OR468298-315 (For details, see Table 2).

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Consent to participate This study does not involve human subjects, so consent to participate was not necessary.

Human and animal rights None of the authors conducted experiments involving human participants or experimental animals.

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