Phytopathologia Mediterranea (2018), 57, 1, 115–121
DOI: 10.14601/Phytopathol_Mediterr-20896

NEW OR UNUSUAL DISEASE REPORTS

Vascular wilt of teak (*Tectona grandis*) caused by *Fusarium oxysporum* in Brazil

Rafaela C. F. BORGES[1], Mônica A. MACEDO[1], Cléia S. CABRAL[1], Maurício ROSSATO[1], Maria G. FONTES[1], Maria D. M. SANTOS[1], Maria A. FERREIRA[2], Maria E. N. FONSECA[3], Ailton REIS[3] and Leonardo S. BOITEUX[1,3]

[1] Plant Pathology Department, University of Brasília, Brasília–DF, Brazil
[2] Plant Pathology Department, University of Lavras–MG, Brazil
[3] National Center for Vegetable Crops Research (CNPH), Embrapa Hortaliças, Brasília–DF, Brazil

Summary. Commercial plantations of teak (*Tectona grandis* L.f.) are affected by many economically important fungal diseases under Brazilian conditions. Teak plants exhibiting distinctive vascular wilt symptoms were observed in Mirassol do Oeste (MT), Brazil. Trunk samples of the affected trees were collected, disinfected, and plated onto potato dextrose agar. Fungal cultures obtained displayed morphological characteristics typical of the *Fusarium oxysporum* species complex. A representative *F. oxysporum* isolate was used in pathogenicity assays. Teak plants displayed symptoms similar to those observed under field conditions approx. 60 d after root-dipping inoculation. Amplicons corresponding to segments of the translation elongation factor 1-α (TEF-1α) and RNA polymerase II second largest subunit (RPB2) genes were obtained using as template the genomic DNA extracted from two *Fusarium* isolates obtained from teak. Phylogenetic analyses of the amplicon sequences placed the isolates into the same cluster of isolates belonging to the *F. oxysporum* species complex. To our knowledge, this is the first report of vascular wilt of teak caused by *F. oxysporum* in the Neotropical region.

Key words: molecular diagnosis vascular disease, t.

Introduction

Teak (*Tectona grandis* Lf; Lamiaceae family) is a tropical forest species, which is native from the monsoon areas of India, Myanmar (Burma), Laos, and Thailand (Narayanan *et al*., 2007). Teak wood has high commercial value due to its aesthetic qualities, durability, and resistance to environmental factors (Mesquita *et al*., 2017).

Teak introduction and commercial cultivation in Brazil was initiated in the early 1970s, in the boundaries as well as within the Legal Amazon region. Since then, several economically important diseases of fungal etiology, associated with extensive yield and quality losses, have been reported in commercial plantations in this warm tropical region. These include rust caused by *Olivea tectonae* (Cabral *et al*., 2010); vascular wilt caused by *Ceratocystis fimbriata* (Firmino *et al*., 2012) and, more recently, trunk canker disease caused by *Lasiodiplodia theobromae* isolates (Borges *et al*., 2015).

In field surveys carried out in 2014, vascular disease of teak, with a peculiar set of symptoms, was found in commercial forests in Mirassol do Oeste, Mato Grosso State-MT, Brazil. *Fusarium oxysporum* isolates were consistently isolated from these affected trees. In the present study, we carried out morphometrical analyses and pathogenicity assays of these isolates, aiming to characterize the causal agents associated with this vascular disease, which was previously unreported in Brazil. To confirm the identity of isolated fungi at the species level, molecular analyses were carried out using genomic information from the partial translation elongation factor 1-α (TEF1-α) and RNA polymerase second largest subunit (RPB2)

Corresponding author: R.C.F. Borges
E-mail: rafaelafal@hotmail.com

www.fupress.com/pm
Firenze University Press
ISSN (print): 0031-9465
ISSN (online): 1593-2095

genes. These are the most phylogenetically informative genomic regions and the best represented sequence databases of *Fusarium* (O'Donnel *et al*., 2010).

Materials and methods

In 2014, teak trees with typical vascular wilt symptoms were observed in field inspections in Mirassol do Oeste (MT), Brazil. Trunks disks with dark heartwood and sapwood dark lesions (Figure 1) were collected and subjected to a series of disinfection steps with 70% alcohol for 1 min, followed by submersion of the plant tissues in 2% of sodium hypochlorite for 1 min and a final washing step in double distilled water for 1 min. The disinfected trunk fragments were then transferred to Petri plates containing 2% water agar, and maintained at 25 ± 1°C with a photoperiod 12 h during 3 d. Agar plugs (0.5 mm diam.) containing fungal structures were then transferred to Petri plates containing Saltwater Nutrient Agar (SNA), and these plates were then incubated under the same conditions. Pure fungal cultures were examined under a light microscope (Leica DM 2500) and photographed using a Leica DFC 490 camera. Morphometrical analyses of the fungi were performed using the Leica QWin-Plus program. The type of phialides as well as the lengths and widths of macroconidia, microconidia, and chlamydospores (n = 30) were annotated using fungal samples from 25 d-old cultures.

To confirm the identity of the vascular wilt causal agent at the species level, a set of analyses were carried out for two representative fungal isolates ('FusTeca2' and 'FusTeca3'), using the genomic information from (TEF1-α) and (RPB2) genes (O'Donnel *et al*., 2010). Genomic DNA from fungal samples was extracted using the 2× CTAB buffer and organic solvents with minor modifications (Boiteux *et al*., 1999). PCR assays were carried out using total genomic DNA with the primers EF1 and EF2 targeting the TEF-1α region, and fRPB2-5f and fRPB2 targeting the RPB2 region (O'Donnel *et al*., 2010).

The TEF-1α and RPB2 amplicons were gel-purified and directly sequenced in both directions. The identities of the sequences were confirmed to correspond to the TEF-1α and RPB2 regions via BLAST, and their alignments were conducted using MAFFT software (Katoh *et al*., 2013) as a plugin of Geneious R8 (Kearse *et al*., 2012). A phylogenetic tree was constructed using the Bayesian inference method (two million chains; 25% burn-in), using concatenated sequences

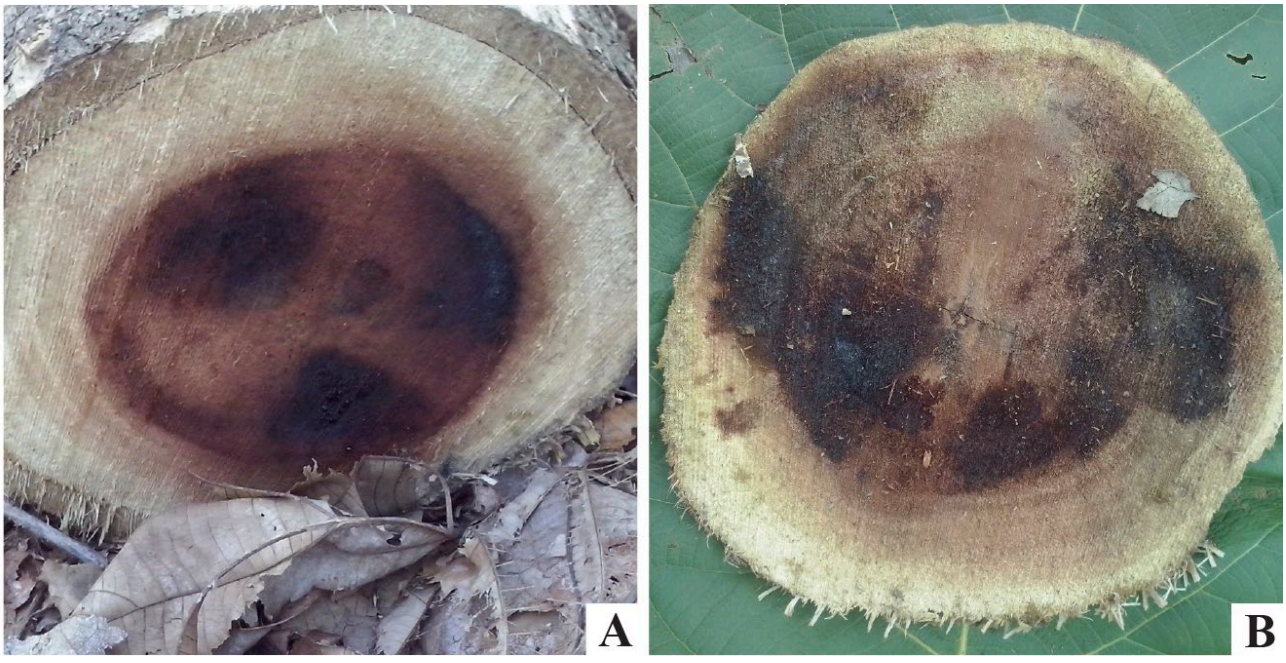

Figure 1. Cross sections of teak (*Tectona grandis*) tree trunks displaying heartwood and sapwood (xylem) darkening as well as black vascular areas (a and b).

Table 1.

Species	Strain	RPB2	TEF
Fusarium anthophilum	NRRL 25214	KU171696	KU171716
Fusarium babinda	NRRL 25539	KU171698	KU171718
Fusarium bulbicola	NRRL 22947	KU171699	KU171719
Fusarium dlaminii	NRRL 13164	KU171701	KU171721
Fusarium euwallaceae	NRRL 62626	KU171702	KU171722
Fusarium foetens	NRRL 38302	KU171703	KU171723
Fusarium gaditjirri	NRRL 45417	KU171704	KU171724
Fusarium miscanthi	NRRL 26231	KU171705	KU171725
Fusarium oxysporum	FusTeca2	MF170550	MF170551
Fusarium oxysporum	FusTeca3	MF170552	MF170553
Fusarium oxysporum	CIB_11	LN828100	LN828039
Fusarium oxysporum	CIB_13	LN828102	LN828041
Fusarium oxysporum	CIB_23	LN828106	LN828045
Fusarium oxysporum	LEMM_110248	LN828073	LN828011
Fusarium oxysporum	LEMM_110414	LN828079	LN828017
Fusarium oxysporum	LEMM_122066	LN828096	LN828034
Fusarium proliferatum	NRRL 62905	KU171707	KU171727
Fusarium redolens	NRRL 22901	KU171708	KU171728
Fusarium solani	LEMM_110029	LN828048	LN827959
Fusarium solani	LEMM_111347	LN828058	LN827970
Fusarium succisae	NRRL 13298	KU171712	KU171732
Fusarium thapsinum	NRRL 22049	KU171713	KU171733

of partial TEF-1α (420 bp alignments) and RPB2 (298 bp alignments) of two *Fusarium* isolates from teak ('FusTeca2' and 'FusTeca3') as well as 20 reference sequences of *Fusarium* isolates available at the GenBank (Table 1). *Fusarium solani* was employed as outgroup in the analyses. The analyses employed GTR model (Tavaré, 1986) with gamma-distributed variation rate across sites chosen by Mega 7.0 (Kumar *et al*., 2016).

For pathogenicity assays, three elite teak clones (named clones A, B, and C) were inoculated with one representative fungal isolate ('FusTeca2'). Inoculation of teak plants (80 d after transplanting) was carried out via root-dipping, using a suspension of *F. oxysporum* microconidia (adjusted to 10^6 mL^{-1}). Sterile water suspension was used in the inoculation of the control plants. The inoculated plants were maintained under greenhouse conditions and visually evaluated for disease up to 90 d after inoculation.

Results

The symptoms of previously unreported disease were initially found in tree trunks, which displayed black darkening of heartwood and sapwood vascular areas (Figure 1). External symptoms of leaf yellowing, leaf abscission and wilting were also observed in the analyzed trees.

On culture medium, the microconidia of the fungal isolates had lengths of 5–10 µm and widths of 2–4 µm (Figure 2a, b, c, d and h). The microconidia

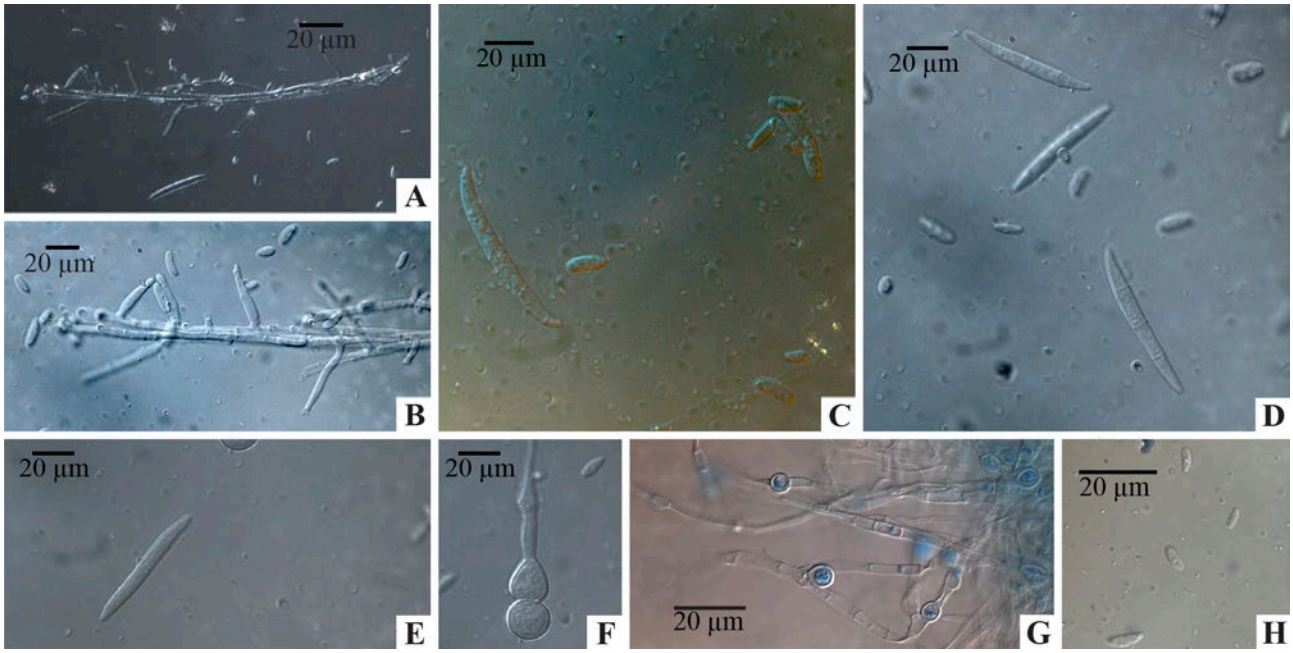

Figure 2. Morphological characteristics of a teak-infecting *Fusarium oxysporum* isolate. Conidiophores and phialides (a and b). Microconidia (oval to ellipsoid and slightly curved) with no septae (a, b, c, d, and h). Falcate and slightly curved macroconidia with three to five septae (c, d, and e). Globose to oval chlamydospores formed at the ends of hyphae or interspersed in the mycelium, which were found single, in pairs, or in chains (f and g).

were predominantly oval to ellipsoid, slightly curved (without septae) (Figure 2a, b, c and d), and organized in false heads produced in short monophialides (Figure 2a and b). Macroconidia (lengths 19–45 µm and widths 3–6 µm) were falcate, moderately curved, and each with three to five septae (Figure 2c, d and e). Chlamydospores were globular and formed at the ends or interspersed within the hyphae occurring singly, in pairs, or in chains (Figure 2f and g).

The three teak clones used in the pathogenicity assays exhibited wilting, and associated symptoms of premature leaf dropping and vascular browning, followed by plant collapse and death about 60 d after inoculation (Figure 3). Fungi isolated from affected plants onto PDA displayed the same morphological and molecular characteristics of the inoculated isolate (see above).

The TEF-1α sequences obtained from the *Fusarium* isolates 'FusTeca2' and 'FusTeca3' were deposited in the GenBank database as 'FusTeca2' (MF170551) and 'FusTeca3' (MF170553). RPB2 sequences of these two isolates were also deposited in the GenBank database as 'FusTeca2' (MF170550) and 'FusTeca3' (MF170552).

Both of these *Fusarium* isolates clustered with other *F. oxysporum* isolates, with maximum values of posterior probability (Figure 4). These phylogenetic analyses confirmed that the causal agent of this vascular wilt of teak belonged to the *F. oxysporum* species complex.

Discussion

The *Fusarium oxysporum* species complex (FOSC) is a cosmopolitan group of soil-borne pathogens that has been reported in association with vascular wilt diseases in a wide range of plant species (O'Donnell *et al*., 2010). Approximately 80 host-specific *formae speciales* are described within the FOSC. The three teak clones used in the pathogenicity assays exhibited wilting symptoms after inoculation with one isolate morphologically and molecularly identical to the *Fusarium* isolates re-isolated on PDA, thus fulfilling the Koch's postulates for this pathogen. Molecular markers have been developed to identify various *formae speciales* (Lievens *et al*., 2008). Given the high level of phylogenetic diversity and large number of

Figure 3. Pathogenicity assay of one *Fusarium oxysporum* isolate ('FusTeca2') from teak (*Tectona grandis*): seedlings of teak (80 d after transplanting) were inoculated via root-dipping using microconidium suspension (a). Typical damage induced by the *F. oxysporum* isolate in teak seedlings (b). Overview of teak seedlings in the pathogenicity assay with the *F. oxysporum* isolate (c). Distinct levels of vascular browning symptoms after inoculation of teak seedlings with the *F. oxysporum* isolate (c, d, e, and f).

formae speciales, multilocus DNA sequence typing currently represents the most robust approach for characterizing these fungal variants (Lievens *et al*., 2008; O'Donnell *et al*., 2009; 2010). However, pathogenicity assays are still the standard technique for identifying host-specific pathogens within members of the FOSC.

In the present study, the infectivity of this *F. oxysporum* isolate was only confirmed in teak. A host range assessment needs to be performed to confirm if this isolate represents a new *forma specialis* of *F. oxysporum*.

The TEF-1α and RPB2 genomic regions are usually employed in *formae speciales* discrimination assays, mainly because of their adequate levels of phylogenetic signal (O'Donnell *et al*., 2010). Due to their importance and utility, these regions were used in the present study in concatenated analyses (with

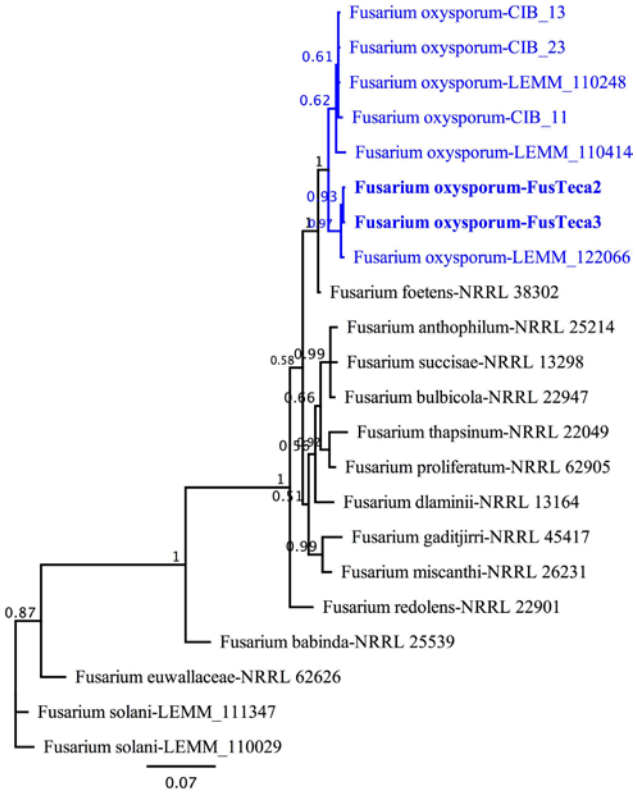

Figure 4. Phylogenetic tree obtained by Bayesian inference (GTR+G model) of the concatenated partial translation elongation factor1-α (TEF1-α) and RNA polymerase second largest subunit (RPB2) genes sequences along with other 20 reference *Fusarium* isolates available at the GenBank.

three phylogenetic methods). The results strongly indicated a clustering of *Fusarium* isolates from teak with a set of reference *F. oxysporum* isolates. No base differences were found in alignments of the isolates 'FusTeca2' and 'FusTeca3' with other reference *F. oxysporum* isolates, corroborating these high levels of genetic similarity.

There are no formal reports of FOSC members infecting teak in other continents, including Asia. To our knowledge, this is the first report of *F. oxysporum* in teak in the Neotropics, and probably the first worldwide report of vascular wilt caused by *F. oxysporum* in this host species. The geographic origins of the *F. oxysporum* isolates for which we found the greatest sequence similarities from the BLASTn search were mainly from countries in Asia (data not presented). This suggests that *F. oxysporum* from teak in Brazil is more likely to be exotic than endemic. Teak is a tropical forest species found dispersed across India, Myanmar, Laos, and Thailand, and several members of FOSC are well-known to be transmitted via contaminated seeds and/or seedlings (Reis *et al*., 2008; Cabral *et al*., 2014) or as endophytes. Therefore, this potentially new *forma specialis* of *F. oxysporum* from teak was likely to be introduced into Brazil via contaminated plant material.

The commercial teak production areas in Brazil are expanding, but some soil-borne diseases have already caused serious yield and quality losses. Description of this new vascular disease caused by *F. oxysporum* provides important new knowledge to stimulate the teak production industry and researchers to search for development of effective vascular wilt management strategies.

Literature cited

Boiteux L.S., M.E.N. Fonseca, and P.W. Simon, 1999. Effects of plant tissue and DNA purification method on randomly amplified polymorphic DNA-based genetic fingerprinting analysis in carrot. *Journal of the American Society for Horticultural Science* 124, 32–36.

Borges R.C.F., M. D. M. Santos, M.A. Macedo, I. Martins, A.G. Nascimento, A.C. Cafe-Filho, L.S. Boiteux, M.E.N. Fonseca, C.A. Inácio and S.C. M. Mello, 2015. A trunk canker disease of *Tectona grandis* induced by *Lasiodiplodia theobromae* in Brazil. *New Disease Report* 31, 26.

Cabral P. G. C., A. S. Capucho, O.L. Pereira, E. Maciel-Zambolim, R.L. Freitas and L. Zambolim, 2010. First report of teak leaf rust disease caused by *Olivea tectonae* in Brazil. *Australasian Plant Disease Notes* 5, 113–114.

Cabral C.S., K.R. Brunelli, H. Costa, M.E.N. Fonseca, L.S. Boiteux, and A. Reis, 2014. Identification of *Fusarium oxysporum* f. sp. *lactucae* race 1 as the causal agent of lettuce wilt in Brazil. *Tropical Plant Pathology* 39, 197–202.

Firmino A.C., H.J. Tozze Jr, and E.L. Furtado, 2012. First report of *Ceratocystis fimbriata* causing wilt in *Tectona grandis* in Brazil. *New Disease Reports* 25, 24.

Katoh K and D.M. Standley, 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30, 772–780.

Kearse M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes and A. Drummond, 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28, 1647–1649.

Kumar S., G. Stecher and K. Tamura, (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33, 1870–1874.

Lievens B., M. Rep and B.P.H.J Thomma, 2008. Recent developments in the molecular discrimination of formae speciales of *Fusarium oxysporum*. *Pest Management Science* 64, 781–788.

Mesquita R.R.S., J.C. Gonçalez and M.H. de Paula, 2017. Comportamento da madeira de *Tectona grandis* frente ao intemperismo. *Revista Floresta* 47, 29–36.

Narayanan C., S.A. Wali, N. Shukla, R. Kumar, A.K. Mandal and S.A. Ansari, 2007. RAPD and ISSR markers for molecular characterization of teak (*Tectona grandis*) plus trees. *Journal of Tropical Forest Science* 19, 218–225.

O'Donnell K., C. Gueidan, S. Sink, P.R. Johnston, P.W. Crous, A. Glenn, R. Riley, N.C. Zitomer, P. Colyer, C. Waalwijk, T. Lee, A. Moretti, S. Kang, H. S. Kim, D. M. Geiser, J. H. Juba, R. P. Baayen, M. G. Cromey, S. Bithell, D. A. Sutton, K. Skovgaard, R. Ploetz, H. Corby Kistler, M. Elliott, M. Davis and B.A.J. Sarver, 2009. A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. *Fungal Genetics and Biology* 46, 936–948.

O'Donnell K., D.A. Sutton, M.G. Rinaldi, B.A. Sarver, S.A. Balajee, H.J. Schroers, R.C. Summerbel, V.A.R.G. Robert, P. W. Crous, N. Zhang, T. Aoki, K. Jung, J. Park, Y. H. Lee, S. Kang, B. Park and D. M. Geiser, 2010. Internet-accessible DNA sequence database for identifying fusaria from human and animal infections. *Journal of Clinical Microbiology* 48, 3708–3718.

Reis A., L.S. Boiteux and R.F. Vieira, 2008. Search for sources of wide-spectrum resistance to *Fusarium oxysporum* f. sp. *basilici* isolates in accessions of *Ocimum* species. *Journal of General Plant Pathology* 74, 375–381.

Tavaré S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* 17, 57–86.

Accepted for publication: March 15, 2018
Published online: May 15, 2018