

RESEARCH PAPERS

## Genetic diversity in *Macrophomina phaseolina*, the causal agent of charcoal rot

MAME P. SARR<sup>1</sup>, M'BAYE NDIAYE<sup>2</sup>, JOHANNES Z. GROENEWALD<sup>3</sup> and PEDRO W. CROUS<sup>3,4,5</sup>

<sup>1</sup> Centre National de Recherches Agronomiques, Laboratoire de phytopathologie ISRA/CNRA.BP: 53 Bambey, Senegal

<sup>2</sup> Centre Régional AGRHYMET, Département Formation et Recherche, Niamey, Niger

<sup>3</sup> CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

<sup>4</sup> Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

<sup>5</sup> Wageningen University and Research Centre (WUR), Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

**Summary.** *Macrophomina phaseolina* (*Botryosphaeriaceae*) is an important soil- and seed-borne pathogen. This pathogen has a broad geographic distribution, and a large host range. The aim of the present study was to determine the genetic variation among a global set of 189 isolates of *M. phaseolina*, isolated from 23 hosts and 30 soil samples in 15 countries. To achieve this goal a multi-gene DNA analysis was conducted for the following five loci, ITS, TEF, ACT, CAL and TUB. Based on these results two well-defined clusters could be delineated, one corresponding to *M. phaseolina* s. str., for which a suitable epitype is designated. The second clade corresponds to *M. pseudophaseolina*, a novel species occurring on *Abelmoschus esculentus*, *Arachis hypogaea*, *Hibiscus sabdarifa* and *Vigna unguiculata* in Senegal. No consistent correlation was found among genotype, host and geographic location, and both species could even occur on the same host at the same location. Although *M. pseudophaseolina* is presently only known from Senegal, further research is required to determine its virulence compared to *M. phaseolina*, and its geographic distribution.

**Key words:** genetic diversity, Senegal, soilborne pathogen, systematics, *Tiarospora phaseolina*.

### Introduction

*Macrophomina phaseolina*, the causal agent of charcoal rot, is a soil- and seed-borne polyphagous pathogen. It causes diseases of more than 500 crop and non-crop species, including economically important hosts such as soybean, common bean, corn, sorghum, cowpea, peanut and cotton (Dhingra and Sinclair, 1977; Ndiaye *et al.*, 2010) (Figure 1). The fungus has a worldwide distribution, but is regarded as economically more important in subtropical and tropical countries with semi-arid climates (Wrather *et al.*, 1997, 2001).

*Macrophomina phaseolina* induces diseases on a range of crops, ranging from seedling blight, root and stem rot, wilt, and pre- to post-emergent damping off, which result in decreased stem height, girth, root and head weight, or death, of affected plants (Raut, 1983). The abundant production of minute black sclerotia of the fungus cause the rotted tissues to become blackened, and for this reason the various diseases are known as charcoal rot. Wrather and Koenning (2010) stated that average yield losses due to charcoal rot in the USA were estimated at about 27 million bushels of soybeans per year from 1996 to 2009. In the Sahelian zone of West Africa (including Burkina Faso, Niger and Senegal), charcoal rot causes an average yield loss of 10%, which is equivalent to 30,000 t of cowpea with an estimated value of \$US 146 million for Niger and Senegal (Ndiaye,

Corresponding author: M.P. Sarr  
Fax: +221 973 63 48  
E-mail: sarrapenda@hotmail.com



**Figure 1.** Disease symptoms of *Macrophomina* (charcoal rot) in infested fields. A. Advanced level of infestation on cowpea (*Vigna unguiculata*) in an experimental field with a drip irrigation system (Agrhymet, Niger). B. Charcoal rot symptoms in a sorrel (*Hibiscus sabdarifa*) field during the rainy season (Agrhymet, Niger). C. Early infection in a young cowpea (*Vigna unguiculata*) plant, in an experimental field during the rainy season (Bambey, Senegal).

2007). However, when conditions are favourable for the growth and development of *M. phaseolina*, infections can result in total crop failures (Orellana, 1971; Tikhonov *et al.*, 1976; Jimenez *et al.*, 1983). Although it is an important plant pathogen, several case reports are known of *M. phaseolina* also acting as an opportunistic human pathogen (Tan *et al.*, 2008; Srinivasan *et al.*, 2009), especially in immunosuppressed patients, including those receiving prophylactic antifungal therapy (Arora *et al.*, 2012).

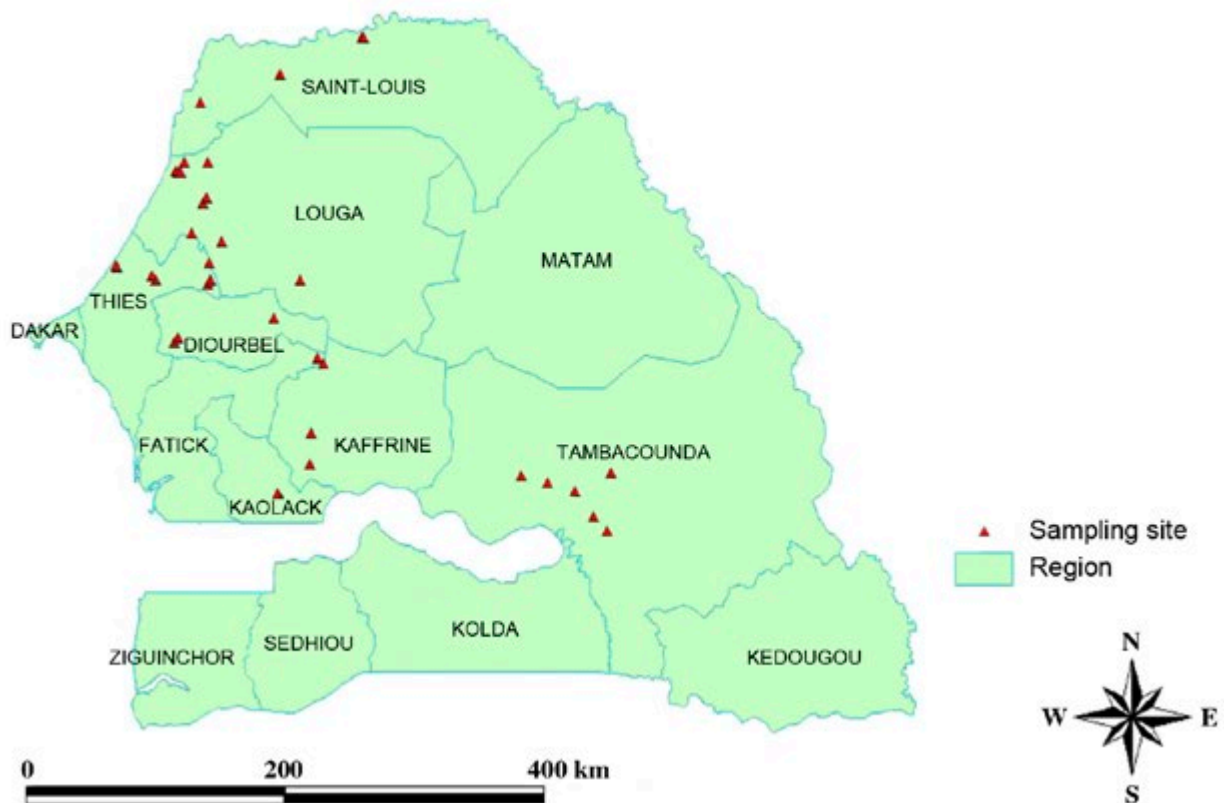
*Macrophomina phaseolina* produces asexual structures, microsclerotia and pycnidia, which can be detected in soil and host tissue using quantitative real-time PCR assays (Babu *et al.*, 2011). Microsclerotia can survive in soil for 2–15 y, or in root debris for longer periods (Cook *et al.*, 1973; Papavizas, 1977; Short *et al.*, 1980; Baird *et al.*, 2003). Once the host tissues start to decompose, microsclerotia are released into the soil (Hartman *et al.*, 1999). Microsclerotia enable the fungus to survive adverse environmental conditions in the field (Short *et al.*, 1980). Pycnidia develop readily on plant tissues, and range from being immersed to erumpent, each opening via a central ostiole, through which hyaline, aseptate, ellipsoid to ovoid conidia are discharged (Dhingra and Sinclair, 1977).

*Macrophomina phaseolina* (= *Tiarospora phaseolina*) is the type species of *Macrophomina*, which is a distinct genus in the *Botryosphaeriaceae* (Crous *et al.*, 2006; Slippers *et al.*, 2013). Although five species

have previously been described in *Macrophomina* (Mycobank, accessed November 2013), recent phylogenetic studies suggest that the genus could be monotypic (Phillips *et al.*, 2013). Morphologically, *Macrophomina* resembles species of *Tiarospora*, as members of both genera have conidia with hyaline apical appendages. However, *Tiarospora* lacks microsclerotia, the conidia do not turn brown with age, and its conidiogenous cells do not have conspicuous percurrent proliferations (Crous *et al.*, 2006).

Several recent studies have been devoted to characterising the genetic and pathogenic variability of *M. phaseolina*. Advances in molecular techniques and refined PCR-based technology, such as Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), and Amplified Fragment Length Polymorphism (AFLP), have contributed to a better understanding of the genetic and pathogenic variability within populations of this pathogen (Fuhlbohms, 1997; Su *et al.*, 2001; Jimenez, 2011). In studying Brazilian populations, Almeida *et al.* (2003) reported that although a single root could be infected by more than one genotype, the overall diversity in populations was low, and that the pathogen probably lacked a sexual cycle. Several studies have focused on developing sets of microsatellite markers to study population variation in *M. phaseolina* (Baird *et al.*, 2009, 2010; Arias *et al.*, 2011). Although these studies distinguished phylogenetic clades in their analyses, the clades generally did not

### Macrophomina sampling sites in Senegal March to May 2011



**Figure 2.** Senegalese cities where samples of different hosts of *Macrophomina* strains were collected.

correlate with geography or host, nor addressed speciation. The aim of the present study was, therefore, to employ multi-gene sequence analyses to examine a large sample of isolates representing different hosts and continents, to determine if all isolates represented a single species, or if more than one taxon were involved as the causes of charcoal rot.

## Materials and methods

### Fungal isolates

All strains of *M. phaseolina* available at the CBS-KNAW Fungal Biodiversity Centre (CBS) in the Netherlands, and from the working collection of Pedro Crous (CPC), maintained at CBS, were included in this study. Furthermore, from September to November 2011, charcoal rot-affected stems or

roots of cowpea (*Vigna unguiculata*), peanut (*Arachis hypogaea*), sorrel (*Hibiscus sabdarifa*), sorghum (*Shorghum bicolor*) and okra (*Abelmoschus esculentus*) were collected from different cropping regions of Senegal. Depending on the number of diseased foci, three to five plants were sampled per field, from the major crop production areas of Senegal. Details regarding the geographical and host origin of all the isolates are provided in Figures 2 and 3, and Table 1.

*Soil isolations.* Isolates were obtained from soil using a soil assay technique based on the method of Alabouvette (1976). Soil samples were dried in an oven at 37°C, crushed through a 1 mm mesh sieve, mixed thoroughly, and subsamples of 5 g were taken. Each subsample was submerged in 0.52% NaOCl for 10 min. The soil-NaOCl mixture was washed with distilled water through two sieves of, respectively,

180 and 45  $\mu\text{m}$  mesh sizes. The residue retained on the 45  $\mu\text{m}$  mesh sieve was incorporated into 100 mL of a semi-selective medium (SS medium) for *M. phaseolina*. This consisted of potato dextrose agar (PDA; 39 g L<sup>-1</sup>) maintained at 55°C in a water bath, with the following ingredients added: 1.5 mL 0.52% NaOCl, 1 mL 0.5% chloramphenicol dissolved in 95% alcohol, and 10 mL 2.25% quinterozone (PCNB). The medium was poured into 9 cm Petri dishes to solidify, and then incubated at 25°C.

*Host tissue isolations.* Uprooted plants were rinsed under running tap water, and blotted dry with a sterile paper towel. Separate subsamples of roots and stems of each plant were treated by immersion in 0.5% NaOCl for 10 min to eliminate secondary invaders, followed by rinsing for 30 min in sterile distilled water. Tissues excised from dying rootlets, necrotic taproots and stems were cut into 2–3 mm long fragments, and 15 of these were plated on three Petri dishes containing the above-described SS-medium and incubated at 30°C for 5–8 d.

Colonies of *M. phaseolina* each appeared as a ring of fluffy white mycelium surrounding a central area with black microsclerotia. Each colony was observed under the microscope for the presence of microsclerotia. Typical colonies from soil and host tissue isolations were propagated further onto PDA, and stored at 5°C (Table 1).

#### DNA isolation, amplification and analyses

Fungal colonies of 104 isolates obtained from host tissues and another 85 strains of *Macrophomina* from the CBS and CPC culture collections were established on 2% malt extract agar (MEA) plates for DNA isolation. For each isolate, total genomic DNA was extracted using UltraClean™ Microbial DNA isolation kits (Mo Bio Laboratories, Inc.) according to the manufacturer's protocol. The pure quantified DNA samples were stored in 2 mL tubes at 4°C for further use.

Five loci were amplified and sequenced, namely: the Internal Transcribed Spacer regions of the nuclear rDNA operon with the primers ITS 5 and ITS 4 (White *et al.*, 1990), part of the TEF-1 $\alpha$  gene region using primers EF1-728F (Carbone and Kohn, 1999) and EF2 (O'Donnell *et al.*, 1998); part of the TUB gene region using primers T1 (O'Donnell & Cigelnik 1997) and CYLTUB1R (Crous *et al.*, 2004b); part of the ac-

tin (ACT) gene region using primers ACT-512F and ACT-783R (Carbone and Kohn, 1999); and part of CAL gene region using primers CAL-228F and CAL-737R (Carbone and Kohn, 1999). However, some of these primer pairs failed to amplify with some of the isolates, so additional combinations were used. The primers EF1-728F and EF 986R were used for amplification of part of the TEF-1 $\alpha$  gene region, ACT-512F and ACT-2RD for part of the actin (ACT) gene region and T1-T22 for the partial beta-tubulin gene (O'Donnell and Cigelnik 1997).

The basic PCR protocol described by Lombard *et al.* (2010) was used and consisted of 2.5 units Fast-Start Taq polymerase (Roche Applied Science), 10  $\times$  PCR buffer, 0.25 mM of each dNTP, 0.5  $\mu\text{M}$  of each primer and  $\pm 30$  ng of fungal genomic DNA, made up to a total reaction volume of 12.5  $\mu\text{L}$ .

Amplification cycles were carried out for 2 min at 94°C as initial denaturation, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 48–55°C for 30 s, extension at 72°C for 1 min with a final extension step at 72°C for 10 min and a hold step at 10°C. Amplification conditions were the same for all loci, except for optimised MgCl<sub>2</sub> concentrations (1.26 mM for ITS and CAL, 1 mM for TEF-1 $\alpha$  and ACT, 0.75 mM for TUB) and for optimised annealing temperatures (48°C for ITS, 52°C for TEF-1 $\alpha$ , 55°C for CAL, ACT and TUB). Following PCR amplification, amplicons were visualised on 1% agarose gels stained with Gel-Red™ (Biotium Inc.) and viewed under ultra-violet light, and sizes of amplicons were determined against a HyperLadder™ I molecular marker (BIOLINE).

To ensure high quality sequences, amplified fragments were sequenced in both directions using the same primer pairs used for amplification. For this purpose, the BigDye terminator sequencing kit v. 3.1 (Applied Biosystems) and a 3730xl DNA Analyzer (Applied Biosystems) were used. All PCRs and sequencing reactions were performed on an Eppendorf Mastercycler Personal PCR (Eppendorf AG). The resulting sequences were analysed for homologies to sequences deposited in the NCBI's GenBank nucleotide databases using megablast searches. The genotypic differences were confirmed by manual inspection of the raw sequence data and the subsequent alignments were performed using the online interface of MAFFT v. 6 (Katoh and Toh, 2010), and manually corrected where necessary.

Congruency of the sequence datasets for the separate loci were determined using tree topolo-

**Table 1.** Details of *Macrophomina* strains included in the molecular and morphological analyses.

| Strain accession number <sup>1</sup> | Substrate of isolation                | Origin        | Structure and colour on PDA | GenBank accession numbers <sup>2</sup> |          |     |                |     |
|--------------------------------------|---------------------------------------|---------------|-----------------------------|--|----------|-----|----------------|-----|
|                                      |                                       |               |                             | ITS                                    | ACT      | CAL | TEF-1 $\alpha$ | TUB |
| <i>M. phaseolina</i>                 |                                       |               |                             |  |          |     |                |     |
| CBS 121.82                           | <i>Sesamum indicum</i> , seed         | —             |                             | KF951621                               | —        | —   | KF951995       | —   |
| CBS 162.25                           | <i>Eucalyptus</i> sp.                 | Uganda        |                             | —                                      | KF951803 | —   | KF951996       | —   |
| CBS 205.47                           | <i>Phaseolus vulgaris</i>             | Italy         |                             | KF951622                               | KF951804 | —   | KF951997       | —   |
| CBS 215.35                           | <i>Derris elliptica</i> , root        | Malaysia      |                             | KF951623                               | —        | —   | —              | —   |
| CBS 224.33                           | <i>Sesamum indicum</i>                | Uganda        |                             | KF951624                               | KF951805 | —   | KF951998       | —   |
| CBS 225.33                           | <i>Brassica rapa</i> , leaf           | Sierra Leone  |                             | KF951625                               | KF951806 | —   | KF951999       | —   |
| CBS 226.33                           | <i>Nicotiana tabacum</i>              | Palestine     |                             | KF951626                               | —        | —   | —              | —   |
| CBS 227.33                           | <i>Zea mays</i>                       | Palestine     |                             | KF951627                               | KF951807 | —   | KF952000       | —   |
| CBS 228.33                           | <i>Cajanus indicus</i>                | Ceylon        |                             | KF951628                               | KF951808 | —   | KF952001       | —   |
| CBS 229.33                           | <i>Cajanus indicus</i>                | Ceylon        |                             | KF951629                               | KF951809 | —   | KF952002       | —   |
| CBS 230.33                           | <i>Gossypium herbaceum</i> , seedling | Sudan         |                             | KF951630                               | KF951810 | —   | KF952003       | —   |
| CBS 231.33                           | <i>Saccharum officinarum</i>          | India         |                             | KF951631                               | KF951811 | —   | KF952004       | —   |
| CBS 270.34                           | <i>Vigna sinensis</i> , root          | USA: Missouri |                             | KF951632                               | KF951812 | —   | KF952005       | —   |
| CBS 271.34                           | <i>Chrysanthemum</i> sp., stem        | USA: Missouri |                             | KF951633                               | KF951813 | —   | KF952006       | —   |
| CBS 313.51                           | <i>Sorghum</i> sp.                    | Venezuela     |                             | KF951634                               | —        | —   | KF952007       | —   |
| CBS 416.62                           | <i>Arachis hypogaea</i>               | Portugal      |                             | KF951635                               | KF951814 | —   | KF952008       | —   |
| CBS 457.70 = IMI 147229              | <i>Phaseolus aureus</i> , seed        | Denmark       |                             | KF951636                               | KF951815 | —   | KF952009       | —   |
| CBS 458.70 = IMI 147230              | <i>Phaseolus mungo</i> , seed         | Denmark       |                             | KF951637                               | —        | —   | KF952010       | —   |
| CBS 459.70                           | <i>Abelmoschus esculentus</i> , seed  | —             |                             | KF951638                               | —        | —   | KF952011       | —   |
| CBS 460.70 = IMI 147232              | <i>Glycine max</i> , seed             | Denmark       |                             | KF951639                               | —        | —   | KF952012       | —   |
| CBS 461.70                           | <i>Phaseolus vulgaris</i> , seed      | Denmark       |                             | KF951640                               | KF951816 | —   | KF952013       | —   |
| CPC 11052 = BaraTN2                  | <i>Vigna unguiculata</i>              | Niger         |                             | KF951641                               | KF951817 | —   | KF952014       | —   |
| CPC 11053 = GGRMN6                   | <i>Vigna unguiculata</i>              | Niger         |                             | KF951642                               | —        | —   | KF952015       | —   |
| CPC 11054                            | <i>Vigna unguiculata</i>              | Niger         |                             | KF951643                               | KF951818 | —   | KF952016       | —   |

(Continued)

Table 1. (Continued)

| Strain accession number <sup>1</sup> | Substrate of isolation   | Origin  | Structure and colour on PDA | GenBank accession numbers <sup>2</sup> |          |     |                |     |  |
|--------------------------------------|--------------------------|---------|-----------------------------|--|----------|-----|----------------|-----|--|
|                                      |                          |         |                             | ITS                                    | ACT      | CAL | TEF-1 $\alpha$ | TUB |  |
| CPC 11055 = GGTMN4                   | <i>Vigna unguiculata</i> | Niger   |                             | KF951644                               | KF951819 | —   | KF952017       | —   |  |
| CPC 11056 = Bara TN7                 | <i>Vigna unguiculata</i> | Niger   |                             | KF951645                               | KF951820 | —   | KF952018       | —   |  |
| CPC 11057 = Bara RMIN2               | <i>Vigna unguiculata</i> | Niger   |                             | KF951646                               | KF951821 | —   | KF952019       | —   |  |
| CPC 11058 = Bara RMIN3               | <i>Vigna unguiculata</i> | Niger   |                             | KF951647                               | —        | —   | KF952020       | —   |  |
| CPC 11059 = P1TN2                    | <i>Vigna unguiculata</i> | Niger   |                             | KF951648                               | —        | —   | KF952021       | —   |  |
| CPC 11060 = BeRMIN 1                 | <i>Vigna unguiculata</i> | Niger   |                             | KF951649                               | —        | —   | KF952022       | —   |  |
| CPC 11061 = BeRMIN 2                 | <i>Vigna unguiculata</i> | Niger   |                             | KF951650                               | KF951822 | —   | KF952023       | —   |  |
| CPC 11062 = BeRMIN 3                 | <i>Vigna unguiculata</i> | Niger   |                             | KF951651                               | KF951823 | —   | —              | —   |  |
| CPC 11063 = CATMIN1                  | <i>Vigna unguiculata</i> | Niger   |                             | KF951652                               | —        | —   | KF952024       | —   |  |
| CPC 11064 = CATMIN2                  | <i>Vigna unguiculata</i> | Niger   |                             | KF951653                               | —        | —   | —              | —   |  |
| CPC 11065 = CATMIN3                  | <i>Vigna unguiculata</i> | Niger   |                             | KF951654                               | —        | —   | KF952025       | —   |  |
| CPC 11066 = CATN5                    | <i>Vigna unguiculata</i> | Niger   |                             | KF951655                               | KF951824 | —   | KF952026       | —   |  |
| CPC 11067 = CATMIN5                  | <i>Vigna unguiculata</i> | Niger   |                             | KF951656                               | —        | —   | KF952027       | —   |  |
| CPC 11068 = CATMIN6                  | <i>Vigna unguiculata</i> | Niger   |                             | KF951657                               | —        | —   | KF952028       | —   |  |
| CPC 11069 = Bara RN1                 | <i>Vigna unguiculata</i> | Niger   |                             | KF951658                               | —        | —   | KF952029       | —   |  |
| CPC 11070 = Bara TN4                 | <i>Vigna unguiculata</i> | Niger   |                             | KF951659                               | —        | —   | KF952030       | —   |  |
| CPC 11071 = Bara TN9                 | <i>Vigna unguiculata</i> | Niger   |                             | KF951660                               | —        | —   | KF952031       | —   |  |
| CPC 11072 = GGRMIN2                  | <i>Vigna unguiculata</i> | Niger   |                             | KF951661                               | —        | —   | KF952032       | —   |  |
| CPC 11073 = Bam1                     | Soil                     | Senegal |                             | KF951662                               | KF951825 | —   | KF952033       | —   |  |
| CPC 11074 = Bam2                     | Soil                     | Senegal |                             | KF951663                               | —        | —   | KF952034       | —   |  |
| CPC 11075 = Bam3                     | Soil                     | Senegal |                             | KF951664                               | —        | —   | —              | —   |  |
| CPC 11076 = Bam4                     | Soil                     | Senegal |                             | KF951665                               | —        | —   | KF952035       | —   |  |
| CPC 11077 = Bam6                     | Soil                     | Senegal |                             | KF951666                               | —        | —   | KF952036       | —   |  |
| CPC 11079 = Bam8                     | Soil                     | Senegal |                             | KF951667                               | —        | —   | KF952037       | —   |  |
| CPC 11080 = Bam9                     | Soil                     | Senegal |                             | KF951668                               | KF951826 | —   | KF952038       | —   |  |
| CPC 11082 = Bam11                    | Soil                     | Senegal |                             | KF951669                               | —        | —   | KF952039       | —   |  |

(Continued)

Table 1. (Continued)

| Strain accession number <sup>1</sup> | Substrate of isolation | Origin  | Structure and colour on PDA | GenBank accession numbers <sup>2</sup> |          |     |                |     |  |
|--------------------------------------|------------------------|---------|-----------------------------|--|----------|-----|----------------|-----|--|
|                                      |                        |         |                             | ITS                                    | ACT      | CAL | TEF-1 $\alpha$ | TUB |  |
| CPC 11083 = Bam12                    | Soil                   | Senegal |                             | KF951670                               | —        | —   | KF952040       | —   |  |
| CPC 11085 = Bam 14                   | Soil                   | Senegal |                             | —                                      | KF951827 | —   | KF952041       | —   |  |
| CPC 11086 = Bam16                    | Soil                   | Senegal |                             | KF951671                               | —        | —   | KF952042       | —   |  |
| CPC 11087 = Bam 17                   | Soil                   | Senegal |                             | —                                      | KF951828 | —   | —              | —   |  |
| CPC 11088 = Bam18                    | Soil                   | Senegal |                             | KF951672                               | KF951829 | —   | KF952043       | —   |  |
| CPC 11089 = Bam19                    | Soil                   | Senegal |                             | KF951673                               | KF951830 | —   | KF952044       | —   |  |
| CPC 11090 = Bam21                    | Soil                   | Senegal |                             | KF951674                               | —        | —   | KF952045       | —   |  |
| CPC 11091 = Bam22                    | Soil                   | Senegal |                             | KF951675                               | —        | —   | —              | —   |  |
| CPC 11093 = Bam24                    | Soil                   | Senegal |                             | KF951676                               | KF951831 | —   | KF952046       | —   |  |
| CPC 11094 = Kebe4                    | Soil                   | Senegal |                             | KF951677                               | KF951832 | —   | KF952047       | —   |  |
| CPC 11095 = Kebe5                    | Soil                   | Senegal |                             | KF951678                               | —        | —   | KF952048       | —   |  |
| CPC 11096 = Kebe6                    | Soil                   | Senegal |                             | KF951679                               | KF951833 | —   | KF952049       | —   |  |
| CPC 11098 = Kebe9                    | Soil                   | Senegal |                             | KF951680                               | KF951834 | —   | KF952050       | —   |  |
| CPC 11099 = Kebe10                   | Soil                   | Senegal |                             | KF951681                               | KF951835 | —   | KF952051       | —   |  |
| CPC 11100 = Kebe12                   | Soil                   | Senegal |                             | KF951682                               | —        | —   | KF952052       | —   |  |
| CPC 11101 = Kebe13                   | Soil                   | Senegal |                             | KF951683                               | —        | —   | KF952053       | —   |  |
| CPC 11102 = Kebe14                   | Soil                   | Senegal |                             | KF951684                               | —        | —   | KF952054       | —   |  |
| CPC 11103 = Kebe15                   | Soil                   | Senegal |                             | KF951685                               | —        | —   | KF952055       | —   |  |
| CPC 11104 = Kebe1                    | Millet                 | Senegal |                             | KF951686                               | KF951836 | —   | KF952056       | —   |  |
| CPC 11105 = Kebe16                   | Soil                   | Senegal |                             | KF951687                               | —        | —   | KF952057       | —   |  |
| CPC 11106 = Kebe 17                  | Soil                   | Senegal |                             | —                                      | —        | —   | KF952058       | —   |  |
| CPC 11107 = Kebe18                   | Soil                   | Senegal |                             | KF951688                               | KF951837 | —   | KF952059       | —   |  |
| CPC 11109 = Kebe25                   | Soil                   | Senegal |                             | KF951689                               | KF951838 | —   | KF952060       | —   |  |
| CPC 11110 = GG RM1                   | Millet                 | Niger   |                             | —                                      | KF951839 | —   | KF952061       | —   |  |
| CPC 11111 = GG RM2                   | Millet                 | Niger   |                             | KF951690                               | KF951840 | —   | KF952062       | —   |  |
| CPC 11112 = GG RM3                   | Millet                 | Niger   |                             | KF951691                               | —        | —   | KF952063       | —   |  |

(Continued)

Table 1. (Continued)

| Strain accession number <sup>1</sup> | Substrate of isolation    | Origin         | Structure and colour on PDA | GenBank accession numbers <sup>2</sup> |          |          |                |          |  |
|--------------------------------------|---------------------------|----------------|-----------------------------|--|----------|----------|----------------|----------|--|
|                                      |                           |                |                             | ITS                                    | ACT      | CAL      | TEF-1 $\alpha$ | TUB      |  |
| CPC 11113 = GGRM5                    | Millet                    | Niger          |                             | KF951692                               | —        | —        | KF952064       | —        |  |
| CPC 11114 = GGRM6                    | Millet                    | Niger          |                             | KF951693                               | —        | —        | KF952065       | —        |  |
| CPC 11115 = GGRM7                    | Millet                    | Niger          |                             | KF951694                               | KF951841 | —        | KF952066       | —        |  |
| CPC 13080 = HMP 18 - 3               | <i>Sesamum indicum</i>    | Mexico         |                             | KF951695                               | —        | —        | KF952067       | —        |  |
| CPC 13081 = HMP 48 - 1               | <i>Glycine max</i>        | Mexico         |                             | KF951696                               | —        | —        | KF952068       | —        |  |
| CPC 13082 = HMP 46 - 1               | <i>Sorghum bicolor</i>    | Mexico         |                             | KF951697                               | —        | —        | KF952069       | —        |  |
| CPC 13084 = HMP 47 - 1               | <i>Phaseolus vulgaris</i> | Mexico         |                             | KF951698                               | —        | —        | KF952070       | —        |  |
| CPC 13085 = HMP 53                   | <i>Phaseolus vulgaris</i> | Mexico         |                             | KF951699                               | —        | —        | KF952071       | —        |  |
| CPC 13086 = HMP 21-1                 | <i>Solanum melongena</i>  | Mexico         |                             | KF951700                               | —        | —        | —              | —        |  |
| CPC 21386                            | <i>Hibiscus sabdarifa</i> | Senegal: Louga | Feathery, grey              | KF951701                               | —        | —        | KF952072       | —        |  |
| CPC 21387                            | <i>Hibiscus sabdarifa</i> | Senegal: Louga | Feathery, grey              | KF951702                               | KF951842 | —        | KF952073       | —        |  |
| CPC 21388                            | <i>Hibiscus sabdarifa</i> | Senegal: Louga | Feathery, grey              | KF951703                               | KF951843 | KF951929 | KF952074       | KF952165 |  |
| CPC 21391                            | <i>Vigna unguiculata</i>  | Senegal        |                             | KF951704                               | —        | KF951930 | KF952075       | KF952166 |  |
| CPC 21392                            | <i>Vigna unguiculata</i>  | Senegal: Thiès | Dense, black                | KF951705                               | KF951844 | KF951931 | KF952076       | KF952167 |  |
| CPC 21393                            | <i>Vigna unguiculata</i>  | Senegal        |                             | —                                      | KF951845 | —        | —              | —        |  |
| CPC 21395                            | <i>Arachis hypogaea</i>   | Senegal: Louga | Feathery, grey              | KF951706                               | KF951846 | KF951932 | KF952077       | KF952168 |  |
| CPC 21399                            | <i>Arachis hypogaea</i>   | Senegal: Louga | Dense, black                | KF951707                               | KF951847 | KF951933 | KF952078       | KF952169 |  |
| CPC 21401                            | <i>Arachis hypogaea</i>   | Senegal: Louga | Feathery, grey              | KF951708                               | KF951848 | —        | KF952079       | —        |  |
| CPC 21405                            | <i>Vigna unguiculata</i>  | Senegal: Louga | Restricted, white           | KF951709                               | KF951849 | KF951934 | KF952080       | KF952170 |  |
| CPC 21406                            | <i>Vigna unguiculata</i>  | Senegal: Louga | Restricted, grey            | KF951710                               | KF951850 | KF951935 | KF952081       | KF952171 |  |
| CPC 21407                            | <i>Vigna unguiculata</i>  | Senegal: Louga | Feathery, grey              | KF951711                               | KF951851 | —        | KF952082       | KF952172 |  |
| CPC 21409                            | <i>Vigna unguiculata</i>  | Senegal: Louga | Feathery, grey              | KF951712                               | KF951852 | KF951936 | KF952083       | KF952173 |  |
| CPC 21410                            | <i>Vigna unguiculata</i>  | Senegal: Louga | Feathery, grey              | KF951713                               | KF951853 | KF951937 | KF952084       | KF952174 |  |
| CPC 21411                            | <i>Vigna unguiculata</i>  | Senegal: Louga | Feathery, grey              | KF951714                               | KF951854 | —        | KF952085       | KF952175 |  |
| CPC 21416                            | <i>Arachis hypogaea</i>   | Senegal: Louga | Feathery, grey              | KF951715                               | KF951855 | KF951938 | KF952086       | KF952176 |  |
| CPC 21419                            | <i>Arachis hypogaea</i>   | Senegal: Thiès | Dense, black                | KF951716                               | KF951856 | KF951939 | KF952087       | KF952177 |  |

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Table 1. (Continued)

| Strain accession number <sup>1</sup> | Substrate of isolation    | Origin               | Structure and colour on PDA | GenBank accession numbers <sup>2</sup> |          |          |          |          |
|--------------------------------------|---------------------------|----------------------|-----------------------------|--|----------|----------|----------|----------|
|                                      |                           |                      |                             | ITS                                    | ACT      | CAL      | TEF-1α   | TUB      |
| CPC 21420                            | <i>Vigna unguiculata</i>  | Senegal: Saint-Louis | Dense, black                | KF951717                               | KF951857 | KF951940 | KF952088 | KF952178 |
| CPC 21421                            | <i>Vigna unguiculata</i>  | Senegal: Saint-Louis | Feathery, grey              | KF951718                               | KF951858 | KF951941 | KF952089 | KF952179 |
| CPC 21423                            | <i>Vigna unguiculata</i>  | Senegal: Saint-Louis | Feathery, grey              | KF951719                               | KF951859 | KF951942 | KF952090 | KF952180 |
| CPC 21424                            | <i>Arachis hypogaea</i>   | Senegal: Diourbel    | Dense, black                | KF951720                               | —        | —        | KF952091 | KF952181 |
| CPC 21425                            | <i>Vigna unguiculata</i>  | Senegal: Louga       | Feathery, grey              | KF951721                               | KF951860 | KF951943 | KF952092 | KF952182 |
| CPC 21426                            | <i>Vigna unguiculata</i>  | Senegal: Louga       | Dense, black                | KF951722                               | KF951861 | KF951944 | KF952093 | KF952183 |
| CPC 21427                            | <i>Vigna unguiculata</i>  | Senegal: Louga       | Dense, black                | KF951723                               | —        | KF951945 | KF952094 | KF952184 |
| CPC 21428                            | <i>Vigna unguiculata</i>  | Senegal: Louga       | Feathery, grey              | KF951724                               | KF951862 | KF951946 | KF952095 | KF952185 |
| CPC 21429                            | <i>Vigna unguiculata</i>  | Senegal: Louga       | Dense, black                | KF951725                               | KF951863 | KF951947 | —        | KF952186 |
| CPC 21430                            | <i>Arachis hypogaea</i>   | Senegal: Thiès       | Feathery, grey              | KF951726                               | KF951864 | KF951948 | KF952096 | KF952187 |
| CPC 21431                            | <i>Hibiscus sabdarifa</i> | Senegal: Thiès       | Feathery, grey              | KF951727                               | KF951865 | KF951949 | KF952097 | KF952188 |
| CPC 21433                            | <i>Arachis hypogaea</i>   | Senegal: Diourbel    | Feathery, grey              | KF951728                               | KF951866 | KF951950 | KF952098 | KF952189 |
| CPC 21436                            | <i>Arachis hypogaea</i>   | Senegal: Thiès       | Dense, black                | KF951729                               | KF951867 | KF951951 | KF952099 | KF952190 |
| CPC 21437                            | <i>Arachis hypogaea</i>   | Senegal: Thiès       | Dense, black                | KF951730                               | KF951868 | —        | KF952100 | —        |
| CPC 21438                            | <i>Arachis hypogaea</i>   | Senegal: Thiès       | Dense, black                | KF951731                               | KF951869 | KF951952 | KF952101 | KF952191 |
| CPC 21441                            | <i>Vigna unguiculata</i>  | Senegal: Diourbel    | Feathery, grey              | KF951732                               | KF951870 | —        | KF952102 | KF952192 |
| CPC 21442                            | <i>Vigna unguiculata</i>  | Senegal: Diourbel    | Feathery, grey              | KF951733                               | KF951871 | KF951953 | KF952103 | KF952193 |
| CPC 21443                            | <i>Vigna unguiculata</i>  | Senegal: Diourbel    | Feathery, grey              | KF951734                               | KF951872 | KF951954 | KF952104 | KF952194 |
| CPC 21444                            | <i>Vigna unguiculata</i>  | Senegal: Diourbel    | Feathery, grey              | KF951735                               | KF951873 | KF951955 | KF952105 | KF952195 |
| CPC 21445                            | <i>Vigna unguiculata</i>  | Senegal: Diourbel    | Feathery, grey              | KF951736                               | KF951874 | KF951956 | KF952106 | KF952196 |
| CPC 21447                            | <i>Arachis hypogaea</i>   | Senegal: Kaolack     | Restricted, grey            | KF951737                               | KF951875 | KF951957 | KF952107 | KF952197 |
| CPC 21448                            | <i>Arachis hypogaea</i>   | Senegal: Kaolack     | Feathery, grey              | KF951738                               | KF951876 | KF951958 | KF952108 | KF952198 |
| CPC 21450                            | <i>Vigna unguiculata</i>  | Senegal: Diourbel    | Dense, black                | KF951739                               | KF951877 | —        | KF952109 | —        |
| CPC 21451                            | <i>Vigna unguiculata</i>  | Senegal: Diourbel    | Feathery, grey              | KF951740                               | KF951878 | KF951959 | KF952110 | KF952199 |
| CPC 21452                            | <i>Vigna unguiculata</i>  | Senegal: Diourbel    | Feathery, grey              | KF951741                               | KF951879 | KF951960 | KF952111 | —        |
| CPC 21453                            | <i>Vigna unguiculata</i>  | Senegal: Louga       | Feathery, grey              | KF951742                               | KF951880 | KF951961 | KF952112 | KF952200 |

(Continued)

Table 1. (Continued)

| Strain accession number <sup>1</sup> | Substrate of isolation        | Origin               | Structure and colour on PDA | GenBank accession numbers <sup>2</sup> |          |          |                |          |  |
|--------------------------------------|-------------------------------|----------------------|-----------------------------|--|----------|----------|----------------|----------|--|
|                                      |                               |                      |                             | ITS                                    | ACT      | CAL      | TEF-1 $\alpha$ | TUB      |  |
| CPC 21454                            | <i>Vigna unguiculata</i>      | Senegal: Diourbel    | Dense, black                | KF951743                               | KF951881 | —        | KF952113       | KF952201 |  |
| CPC 21455                            | <i>Vigna unguiculata</i>      | Senegal: Diourbel    | Dense, black                | KF951744                               | KF951882 | —        | KF952114       | KF952202 |  |
| CPC 21456                            | <i>Vigna unguiculata</i>      | Senegal: Louga       | Dense, black                | KF951745                               | KF951883 | KF951962 | KF952115       | KF952203 |  |
| CPC 21460                            | <i>Arachis hypogaea</i>       | Senegal: Louga       | Feathery, grey              | KF951746                               | KF951884 | KF951963 | KF952116       | KF952204 |  |
| CPC 21461                            | <i>Arachis hypogaea</i>       | Senegal: Louga       | Feathery, grey              | KF951747                               | KF951885 | —        | KF952117       | —        |  |
| CPC 21462                            | <i>Vigna unguiculata</i>      | Senegal: Louga       | Feathery, grey              | KF951748                               | KF951886 | —        | KF952118       | KF952205 |  |
| CPC 21463                            | <i>Vigna unguiculata</i>      | Senegal: Thiès       | Dense, black                | KF951749                               | KF951887 | KF951964 | KF952119       | KF952206 |  |
| CPC 21464                            | <i>Arachis hypogaea</i>       | Senegal: Louga       | Dense, black                | KF951750                               | —        | —        | —              | —        |  |
| CPC 21468                            | <i>Abelmoschus esculentus</i> | Senegal              | —                           | KF951751                               | —        | —        | —              | —        |  |
| CPC 21470                            | <i>Abelmoschus esculentus</i> | Senegal: Saint-Louis | Dense, black                | KF951752                               | —        | —        | KF952120       | —        |  |
| CPC 21474                            | <i>Abelmoschus esculentus</i> | Senegal: Saint-Louis | Restricted, grey            | KF951753                               | —        | —        | KF952121       | —        |  |
| CPC 21475                            | <i>Abelmoschus esculentus</i> | Senegal              | —                           | KF951754                               | —        | —        | —              | —        |  |
| CPC 21476                            | <i>Abelmoschus esculentus</i> | Senegal: Saint-Louis | Feathery, grey              | KF951755                               | KF951888 | KF951965 | KF952122       | KF952207 |  |
| CPC 21477                            | <i>Arachis hypogaea</i>       | Senegal: Tambacounda | Dense, black                | KF951756                               | KF951889 | KF951966 | KF952123       | KF952208 |  |
| CPC 21478                            | <i>Arachis hypogaea</i>       | Senegal: Tambacounda | Feathery, grey              | KF951757                               | KF951890 | KF951967 | KF952124       | KF952209 |  |
| CPC 21481                            | <i>Hibiscus sabdarifa</i>     | Senegal: Tambacounda | Feathery, grey              | KF951758                               | KF951891 | KF951968 | KF952125       | KF952210 |  |
| CPC 21483                            | <i>Arachis hypogaea</i>       | Senegal: Tambacounda | Restricted, black           | KF951759                               | —        | —        | —              | —        |  |
| CPC 21485                            | <i>Sorghum</i> sp.            | Senegal: Saint-Louis | Dense, black                | KF951760                               | KF951892 | —        | KF952126       | —        |  |
| CPC 21486                            | <i>Abelmoschus esculentus</i> | Senegal: Saint-Louis | Restricted, grey            | KF951761                               | KF951893 | KF951969 | KF952127       | KF952211 |  |
| CPC 21490                            | <i>Arachis hypogaea</i>       | Senegal: Tambacounda | Dense, black                | KF951762                               | KF951894 | KF951970 | KF952128       | KF952212 |  |
| CPC 21491                            | <i>Vigna unguiculata</i>      | Senegal: Tambacounda | Feathery, grey              | KF951763                               | —        | —        | —              | —        |  |
| CPC 21492                            | <i>Vigna unguiculata</i>      | Senegal: Tambacounda | Restricted, grey            | KF951764                               | —        | KF951971 | KF952129       | KF952213 |  |
| CPC 21495                            | <i>Vigna unguiculata</i>      | Senegal: Tambacounda | Feathery, grey              | KF951765                               | KF951895 | —        | KF952130       | —        |  |
| CPC 21496                            | <i>Vigna unguiculata</i>      | Senegal: Tambacounda | Feathery, grey              | KF951766                               | KF951896 | —        | KF952131       | —        |  |
| CPC 21497                            | <i>Vigna unguiculata</i>      | Senegal: Tambacounda | Feathery, grey              | KF951767                               | KF951897 | KF951972 | KF952132       | KF952214 |  |
| CPC 21498                            | <i>Arachis hypogaea</i>       | Senegal: Tambacounda | Feathery, grey              | KF951768                               | KF951898 | —        | KF952133       | KF952215 |  |

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Table 1. (Continued)

| Strain accession number <sup>1</sup> | Substrate of isolation        | Origin               | Structure and colour on PDA | GenBank accession numbers <sup>2</sup> |          |          |                |          |  |
|--------------------------------------|-------------------------------|----------------------|-----------------------------|--|----------|----------|----------------|----------|--|
|                                      |                               |                      |                             | ITS                                    | ACT      | CAL      | TEF-1 $\alpha$ | TUB      |  |
| CPC 21504                            | <i>Hibiscus sabdariffa</i>    | Senegal: Saint-Louis | Feathery, grey              | KF951769                               | KF951899 | KF951973 | KF952134       | KF952216 |  |
| CPC 21505                            | <i>Hibiscus sabdariffa</i>    | Senegal: Saint-Louis | Feathery, grey              | KF951770                               | KF951900 | —        | KF952135       | KF952217 |  |
| CPC 21506                            | <i>Vigna unguiculata</i>      | Senegal: Diourbel    | Feathery, grey              | KF951771                               | KF951901 | KF951974 | KF952136       | KF952218 |  |
| CPC 21508                            | <i>Abelmoschus esculentus</i> | Senegal              | —                           | KF951772                               | —        | —        | —              | —        |  |
| CPC 21512                            | <i>Abelmoschus esculentus</i> | Senegal: Saint-Louis | Dense, black                | KF951773                               | KF951902 | KF951975 | KF952137       | KF952219 |  |
| CPC 21513                            | <i>Vigna unguiculata</i>      | Senegal: Diourbel    | Dense, black                | KF951774                               | KF951903 | KF951976 | KF952138       | KF952220 |  |
| CPC 21514                            | <i>Vigna unguiculata</i>      | Senegal              | —                           | KF951775                               | —        | —        | —              | —        |  |
| CPC 21515                            | <i>Vigna unguiculata</i>      | Senegal              | —                           | KF951776                               | KF951904 | —        | —              | —        |  |
| CPC 21516                            | <i>Vigna unguiculata</i>      | Senegal: Diourbel    | Feathery, grey              | KF951777                               | KF951905 | —        | KF952139       | KF952221 |  |
| CPC 21517                            | <i>Vigna unguiculata</i>      | Senegal              | —                           | KF951778                               | —        | —        | KF952140       | —        |  |
| CPC 21518                            | <i>Vigna unguiculata</i>      | Senegal: Diourbel    | Feathery, black             | KF951779                               | KF951906 | KF951977 | KF952141       | —        |  |
| CPC 21519                            | <i>Vigna unguiculata</i>      | Senegal: Diourbel    | Feathery, black             | KF951780                               | KF951907 | KF951978 | KF952142       | KF952222 |  |
| CPC 21520                            | <i>Vigna unguiculata</i>      | Senegal: Diourbel    | Feathery, black             | KF951781                               | KF951908 | KF951979 | KF952143       | KF952223 |  |
| CPC 21521                            | <i>Vigna unguiculata</i>      | Senegal: Diourbel    | Feathery, black             | KF951782                               | KF951909 | KF951980 | KF952144       | KF952224 |  |
| CPC 21532                            | <i>Vigna unguiculata</i>      | Senegal: Thiès       | Dense, black                | KF951783                               | KF951910 | KF951981 | KF952145       | KF952225 |  |
| CPC 21534                            | <i>Vigna unguiculata</i>      | Senegal: Thiès       | Restricted, black           | KF951784                               | KF951911 | KF951982 | KF952146       | KF952226 |  |
| CPC 21535                            | <i>Vigna unguiculata</i>      | Senegal: Thiès       | Feathery, grey              | KF951785                               | KF951912 | —        | KF952147       | KF952227 |  |
| <i>M. pseudophaeoscolina</i>         |                               |                      |                             |  |          |          |                |          |  |
| CPC 21394 = CBS 137166               | <i>Vigna unguiculata</i>      | Senegal: Thiès       | Dense, black                | KF951786                               | KF951913 | KF951983 | KF952148       | KF952228 |  |
| CPC 21398                            | <i>Arachis hypogaea</i>       | Senegal: Louga       | Feathery, grey              | KF951787                               | KF951914 | —        | KF952149       | KF952229 |  |
| CPC 21400                            | <i>Arachis hypogaea</i>       | Senegal: Louga       | Feathery, grey              | KF951788                               | KF951915 | —        | KF952150       | KF952230 |  |
| CPC 21402                            | <i>Arachis hypogaea</i>       | Senegal: Louga       | Dense, black                | KF951789                               | KF951916 | KF951984 | KF952151       | KF952231 |  |
| CPC 21403                            | <i>Arachis hypogaea</i>       | Senegal: Louga       | Dense, black                | KF951790                               | KF951917 | KF951985 | KF952152       | KF952232 |  |
| CPC 21417 = CBS 137165               | <i>Arachis hypogaea</i>       | Senegal: Louga       | Dense, black                | KF951791                               | KF951918 | KF951986 | KF952153       | KF952233 |  |
| CPC 21422                            | <i>Vigna unguiculata</i>      | Senegal: Saint-Louis | Feathery, grey              | KF951792                               | KF951919 | —        | KF952154       | KF952234 |  |

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Table 1. (Continued)

| Strain accession number <sup>1</sup> | Substrate of isolation        | Origin               | Structure and colour on PDA | GenBank accession numbers <sup>2</sup> |          |          |                |          |  |
|--------------------------------------|-------------------------------|----------------------|-----------------------------|--|----------|----------|----------------|----------|--|
|                                      |                               |                      |                             | ITS                                    | ACT      | CAL      | TEF-1 $\alpha$ | TUB      |  |
| CPC 21458                            | <i>Arachis hypogaea</i>       | Senegal: Louga       | Dense, black                | KF951793                               | KF951920 | KF951987 | KF952155       | KF952235 |  |
| CPC 21459                            | <i>Arachis hypogaea</i>       | Senegal: Louga       | Dense, black                | KF951794                               | KF951921 | KF951988 | KF952156       | KF952236 |  |
| CPC 21500 = CBS 137167               | <i>Hibiscus sabdariffa</i>    | Senegal: Saint-Louis | Dense, black                | KF951795                               | KF951922 | KF951989 | KF952157       | KF952237 |  |
| CPC 21501                            | <i>Hibiscus sabdariffa</i>    | Senegal: Saint-Louis | Feathery, black             | KF951796                               | KF951923 | KF951990 | KF952158       | KF952238 |  |
| CPC 21502                            | <i>Hibiscus sabdariffa</i>    | Senegal: Saint-Louis | Feathery, grey              | KF951797                               | KF951924 | KF951991 | KF952159       | KF952239 |  |
| CPC 21503                            | <i>Hibiscus sabdariffa</i>    | Senegal: Saint-Louis | Dense, black                | —                                      | —        | —        | KF952160       | —        |  |
| CPC 21511 = CBS 137168               | <i>Abelmoschus esculentus</i> | Senegal              | —                           | KF951798                               | —        | —        | —              | —        |  |
| CPC 21524                            | <i>Hibiscus sabdariffa</i>    | Senegal: Saint-Louis | Dense, black                | KF951799                               | KF951925 | KF951992 | KF952161       | KF952240 |  |
| CPC 21525                            | <i>Hibiscus sabdariffa</i>    | Senegal: Saint-Louis | Dense, black                | KF951800                               | KF951926 | —        | KF952162       | KF952241 |  |
| CPC 21527                            | <i>Hibiscus sabdariffa</i>    | Senegal: Saint-Louis | Dense, black                | KF951801                               | KF951927 | KF951993 | KF952163       | KF952242 |  |
| CPC 21528                            | <i>Hibiscus sabdariffa</i>    | Senegal: Saint-Louis | Dense, black                | KF951802                               | KF951928 | KF951994 | KF952164       | KF952243 |  |

<sup>1</sup> CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht; The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, Basingstoke, U.K.

<sup>2</sup> ITS: internal transcribed spacers and intervening 5.8S nrDNA; ACT: partial actin gene; CAL: partial calmodulin gene; TEF-1 $\alpha$ : partial translation elongation factor 1-alpha gene; TUB: partial beta-tubulin gene.

gies of 70% reciprocal Neighbour-Joining bootstrap trees with Maximum Likelihood distances that were compared visually to identify conflicts between partitions (Mason-Gamer and Kellogg, 1996; Guedan *et al.*, 2007). A maximum parsimony analysis was performed on the combined alignments with Phylogenetic Analysis Using Parsimony (PAUP) v. 4.0b10 (Swofford, 2003), using the heuristic search option with 100 random sequence additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. Branches of zero length were collapsed and the first 1,000 multiple, equally most parsimonious trees were saved. The robustness of the trees obtained was evaluated by 1,000 bootstrap replications (Hillis and Bull, 1993). All characters were weighted equally and alignment gaps were treated as new state data. Tree length, consistency indices (CI), retention indices (RI), rescaled consistency indices (RC) and homoplasy indices (HI) were calculated for the resulting trees. The resulting phylogenetic tree (Figure 3) was printed with Geneious v. 5.5.4 (Drummond *et al.*, 2011), and the layout of the tree for publication was carried out using in Adobe Illustrator v. CS5.1. Sequences derived in this study were lodged at GenBank, the alignment in TreeBASE ([www.treebase.org/treebase/index.html](http://www.treebase.org/treebase/index.html)), and taxonomic novelties in MycoBank ([www.Mycobank.org](http://www.Mycobank.org); Crous *et al.*, 2004a).

## Morphology

Observations were made with a Zeiss V20 Discovery stereo-microscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and an AxioCam MRc5 camera and software. Colony characters and pigment production were noted after 3 d of growth on PDA at 25°C. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970). Morphological descriptions were based on colonies sporulating on sterile pine needles on water agar (PNA; Smith *et al.*, 1996).

## Results

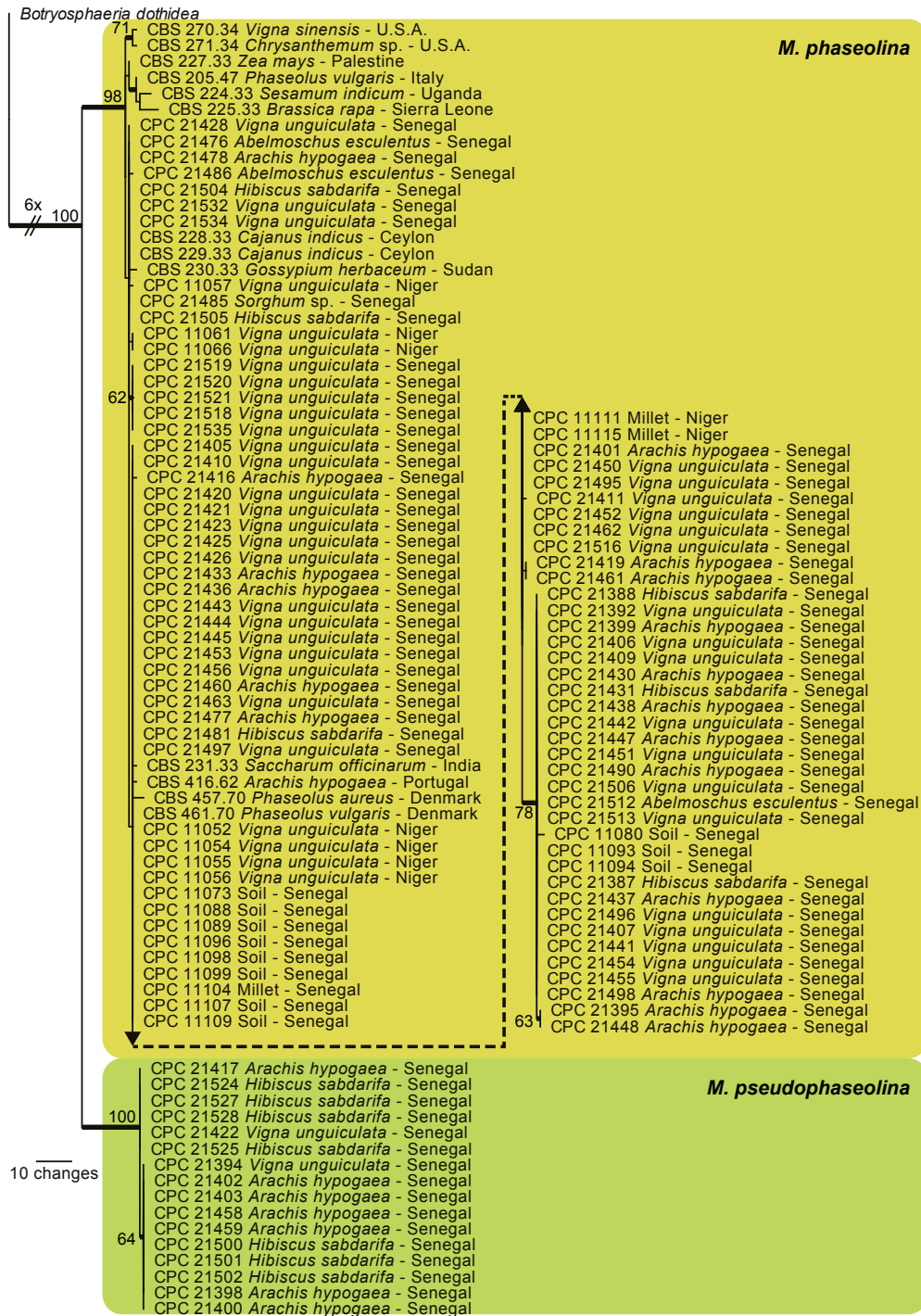
### Phylogeny

One hundred and four *Macrophomina* isolates were obtained from five plant species and six regions in Senegal (Figure 2, Table 1). The majority of these

isolates were sequenced for all five loci, and these data were combined with the sequence data from additional strains from different hosts and continents available from the CBS and CPC collections. Two phylogenies were generated. The first was based on 118 isolates (60 isolates from six regions in Senegal and 58 CBS strains) and the *Botryosphaeria dothidea* outgroup sequence (ITS, TEF-1 $\alpha$  and ACT; Figure 3). The second phylogeny was based on 60 isolates (60 isolates from six regions in Senegal) and the *Botryosphaeria dothidea* outgroup sequence (all five loci; data not shown, alignment and tree available in TreeBASE).

The first analysis (combined ITS, TEF-1 $\alpha$  and ACT alignment) is based on 119 isolates (including the outgroup sequence) and the resulting dataset of 1047 characters, including alignment gaps which are treated as fifth base, consisted of 879 constant characters, 123 variable parsimony-uninformative characters and 45 parsimony-informative characters. The maximum of 1,000 equally most parsimonious trees were obtained (TL = 189; CI = 0.931; RI = 0.979; RC = 0.911; HI = 0.069, of which the first tree is presented in Figure 3). The second analysis (combined 5-gene alignment) is based on 61 isolates (including the outgroup sequence) and the resulting dataset of 2,171 characters, including alignment gaps which are treated as fifth base, consisted of 1,842 constant characters, 262 variable parsimony-uninformative characters and 67 parsimony-informative characters. Ninety equally most parsimonious trees (TL = 341; CI = 0.988; RI = 0.995; RC = 0.983; HI = 0.012) were obtained and the resulting phylogenetic tree confirms the topology obtained from the 3-gene phylogeny (data not shown, available from TreeBASE).

In the phylogenetic tree (Figure 3), *Macrophomina* isolates are divided into two main clades that are treated below as two distinct species. The first main clade has a bootstrap support value (BS) of 98% and contains the majority of *Macrophomina* strains, including all the CBS and older CPC strains used in this study. Although some clustering of strains occurs in this main clade, e.g. the two strains from USA (CBS 270.34 and CBS 271.34), bootstrap support values are generally low and not significant at species level. The strain from Italy (CBS 205.47), designated below as ex-epitype culture of *M. phaseolina*, clusters in this clade and therefore all the strains in this main clade constitute the real *M. phaseolina*. The second main clade (BS = 100%) contains 16 isolates, all of



**Figure 3.** The first of 1,000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined three-gene (ITS, TEF-1 $\alpha$ , ACT) sequence alignment. The scale bar shows ten changes, and bootstrap support values from 1,000 replicates are shown at the nodes. Culture collection numbers, substrate and country of origin are shown for each sequence, and coloured blocks indicate the two *Macrophomina* species. Branches present in the strict consensus tree are thickened and the tree was rooted to sequences of *Botryosphaeria dothidea* (obtained from the genome data available at: <http://genome.jgi.doe.gov/Botdo1/Botdo1.home.html>).

which were recently collected from diverse hosts from Senegal. This clade is phylogenetically distinct from the *M. phaseolina* clade in all analyses. A novel species is therefore introduced below for these distinct isolates.

### Taxonomy

**Macrophomina phaseolina** (Tassi) Goid., *Annali Sper. agr. N.S.* **1**: 457 (1947)

*Basionym*: *Macrophoma phaseolina* Tassi, *Bull. Lab. Ort bot. Siena* **4**: 9 (1901)

= *Tiarosporella phaseoli* (Maubl.) Aa, *Verh. Kon. Ned. Akad. Wetensch., Sectie 2*, **68**: 4 (1977)

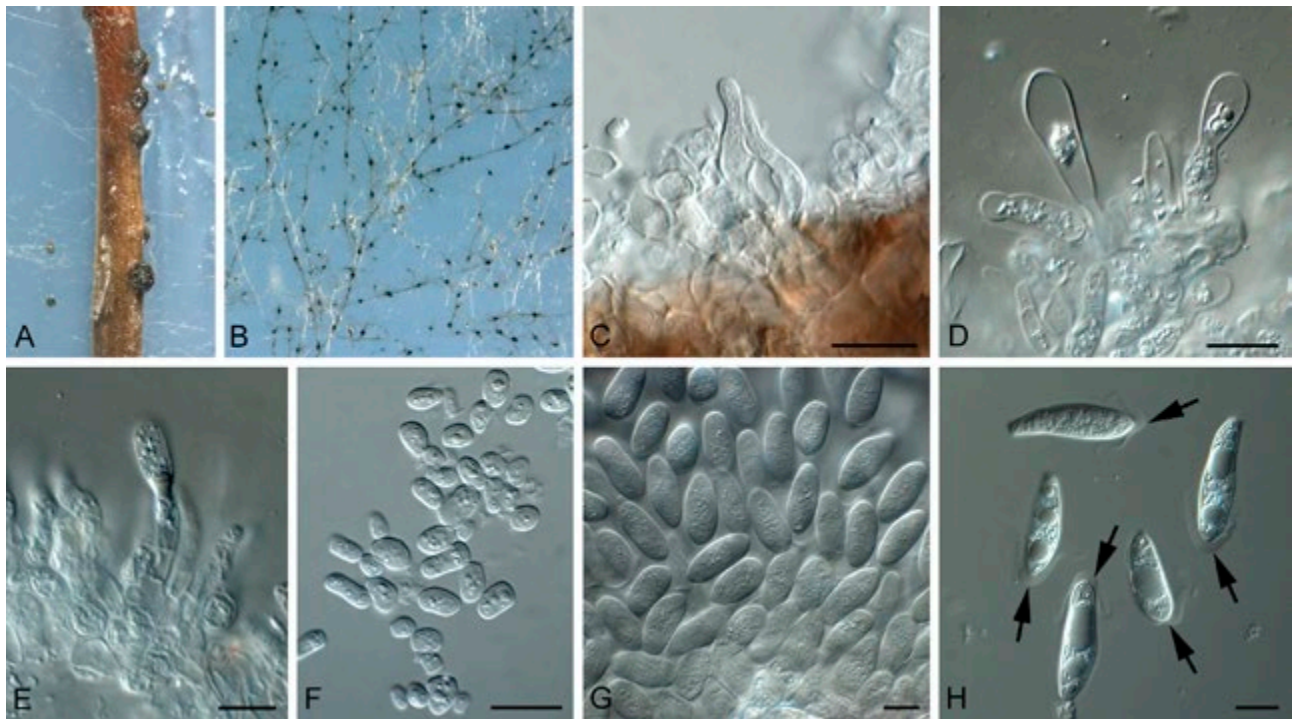
(Figure 4)

Additional synonyms listed by Holliday and Punithalingam (1988).

*Sclerotia* developing on PNA or in the water agar, black, smooth, hard, 100–400  $\mu\text{m}$  diam. *Conidiomata* pycnidial, dark brown to black, solitary or gregarious, up to 300  $\mu\text{m}$  diam., each opening by a central ostiole; wall multilayered, cells dark brown, thick-

walled. *Conidiogenous cells* lining the inner surface of the conidioma, hyaline, short obpyriform to sub-cylindrical, proliferating several times percurrently near the apex, 6–12  $\times$  4–6  $\mu\text{m}$ ; young conidiogenous cells covered by a mucous layer that extends over the apex of the developing conidium. *Conidia* ellipsoid to obovoid, (19–)22–26(–30)  $\times$  (6–)8(–9)  $\mu\text{m}$  (av. 24  $\times$  8  $\mu\text{m}$ ); immature conidia hyaline, enclosed in a mucous sheath, that upon dehiscence encloses the top half of the conidium, transformed into two lateral tentaculiform, apical mucoid appendages (type C; Nag Raj, 1993); mature conidia becoming medium to dark brown, each with a granular outer layer that in some cases appears pitted, without any mucoid appendages; conidial hilum frequently with marginal frills. *Microconidia* aseptate, hyaline, smooth, guttulate to granular, straight to curved, ellipsoid to sub-cylindrical to irregular, 5–8(–10)  $\times$  3–5  $\mu\text{m}$ .

*Culture characteristics*. Colonies with even margins, and abundant, fluffy aerial mycelium. On PDA buff, turning vinaceous buff to pale olivaceous grey with dense, black sclerotial masses. Colonies after 2 d



**Figure 4.** *Macrophomina phaseolina* (CBS 205.47). Pycnidia forming on pine needle agar. B. Sclerotia on tap water agar. C–E. Conidiogenous cells. F. Microconidia. G, H. Macroconidia with apical appendages (arrows). Scale bars = 10  $\mu\text{m}$ .

not growing at 6 or 9°C, growing 9–10 mm diam. at 12°C, optimal growth at 30–36°C (80 mm diam.), and still growing at 40°C (25–40 mm diam.). On average growing faster than isolates of *M. pseudophaseolina*.

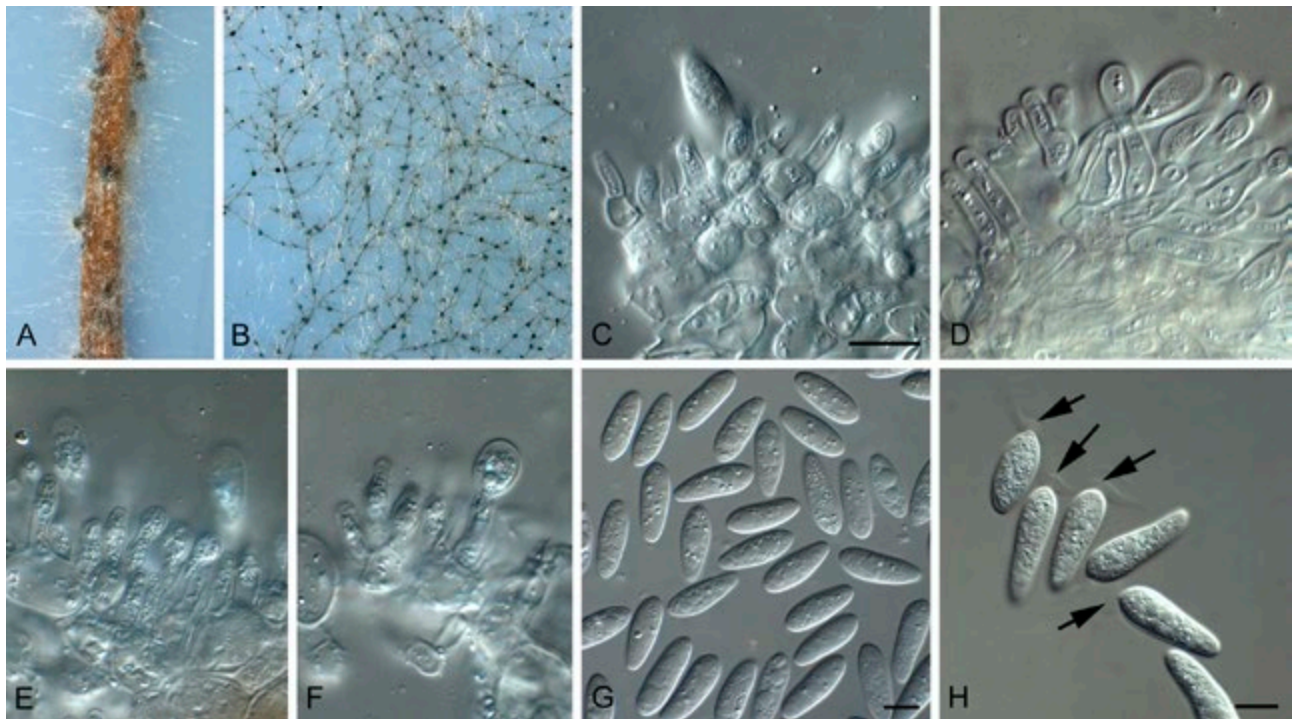
*Specimen examined: Italy:* Siena Botanical Garden, on leaves of *Phaseolus* sp., Sept. 1901, holotype Siena. Italy, on *Phaseolus vulgaris*, Mar. 1947, G. Goidánich, epitype designated here CBS H-21519, “MBT176946”, culture ex-epitype CBS 205.47.

***Macrophomina pseudophaseolina* Crous, Sarr & Ndiaye, sp. nov.**  
Mycobank MB807468  
(Figure 5)

*Etymology:* Named for morphological similarity to *M. phaseolina*.

*Diagnosis.* *Conidia* hyaline, smooth, granular to guttulate, ellipsoid to obovoid, (19–)20–24(–27) × (7.5–)8(–9) μm (av. 22 × 8 μm).

*Sclerotia* developing on PNA or in the water agar, black, smooth, hard, 100–400 μm diam. *Conidiomata* pycnidial, dark brown to black, glabrous, solitary or gregarious, globose to slightly elongated, up to 300 μm diam., each opening by a central ostiole; wall of five to six layers of dark brown, thick-walled *textura angularis*. *Conidiophores* reduced to conidiogenous cells or each with a supporting cell, hyaline, ampulliform, branched at base or not, 15–20 × 5–8 μm. *Conidiogenous cells* lining the inner surface of the conidioma, hyaline, subcylindrical, each proliferating several times percurrently near the apex, 8–15 × 3–4 μm; young conidiogenous cells each covered by a mucous layer that extends over the apex of the developing conidium. *Conidia* hyaline, smooth, granular to guttulate, ellipsoid to obovoid, widest in upper third, apices subobtuse, bases truncate, 2.5–3 μm diam., with minute marginal frills, (19–)20–24(–27) × (7.5–)8(–9) μm (av. 22 × 8 μm); immature conidia hyaline, each enclosed in a mucous sheath, that upon dehiscence encloses the top half of the conidium, transformed into two lateral tentaculiform, apical mucoid appendages (type C; Nag Raj, 1993); mature



**Figure 5.** *Macrophomina pseudophaseolina* (CPC 21417). Pycnidia forming on pine needle agar. B. Sclerotia on tap water agar. C–F. Conidiogenous cells. G, H. Macroconidia with apical appendages (arrows). Scale bars = 10 μm.



conidia becoming medium to dark brown, without any mucoid appendages.

**Culture characteristics.** Colonies with even margins, and abundant, fluffy aerial mycelium. On PDA buff, turning vinaceous buff to pale olivaceous grey, with dense, black sclerotial masses. Colonies after 2 d not growing at 6 or 9°C, growing 4–5 mm diam. at 12°C, optimal growth at 30–36°C (80 mm diam.), and still growing at 40°C (10–15 mm diam.).

**Specimen examined. Senegal:** Louga, on *Arachis hypogaea*, 2011, M.P. Sarr, holotype CBS H-21518, culture ex-type CPC 21417 = CBS 137165.

**Notes.** Morphologically *M. phaseolina* is very similar to *M. pseudophaseolina*, except that conidia of the latter are somewhat shorter. These two species occur in the same regions and on the same hosts in Senegal. *Macrophomina pseudophaseolina*, however, is only known from Senegal, occurring on *Abelmoschus*, *Arachis*, *Hibiscus* and *Vigna*. ITS analysis indicated that the two species can be distinguished on three nucleotide duplications in the first internal transcribed spacers. In ACT there are two fixed nucleotide differences, and on CAL 15, TEF-1 $\alpha$  22, and TUB 15 fixed nucleotide differences (see 5-gene alignment in TreeBASE). However, the TEF-1 $\alpha$  was problematic for obtaining a good consensus sequence, possibly due to the presence of an extra copy or pseudogene copy of the gene in the genome. All TEF-1 $\alpha$  trace files used in this study were therefore carefully evaluated and judged against the data from the other loci. A frequent problem would be that the sequence in the one orientation would not match the one from the other direction, i.e. the one direction would be from *M. phaseolina* and the other from *M. pseudophaseolina*. Whether this is a remnant of the speciation event requires further investigation, preferably using whole genome sequence data from both species.

## Discussion

The genus *Macrophomina* is based on the type species, *M. phaseolina*, which was originally described from *Phaseolus* collected in Italy. During the course of this study, *M. phaseolina* was resolved as a widely distributed pathogen occurring on a broad host range. Furthermore, one isolate in the collection, originating from *Phaseolus* in Italy (CBS 205.47),

proved suitable as the epitype, thus helping to fix the genetic application of the name (Cannon *et al.*, 2012). The concept of *Macrophomina* as employed by Phillips *et al.* (2013) in their recent treatment of the *Botryosphaeriaceae*, was correct, representing a genus distinct from *Tiarospora*, a morphologically similar genus in the family (Crous *et al.*, 2006).

*Macrophomina phaseolina* has previously been considered as heterogeneous with regard to morphology, physiology, ecology and generic characteristics (Indera *et al.*, 1986; Babu *et al.*, 2010; Ndiaye *et al.*, 2010). In the present study, a multigene analysis (ITS, TEF, ACT, CAL and TUB) representing a large sample of *Macrophomina* isolates from many hosts and geographical locations provided robust support for the identification of a new species from Senegal, named *M. pseudophaseolina*. The phylogenetic analysis clusters 16 strains from three hosts (*Arachis hypogaea*, *Hibiscus sabdarifa* and *Vigna unguiculata*) and four locations in Senegal (Podor, Saint-louis, Louga and Thiès) in the *M. pseudophaseolina* clade, and 102 strains from 12 countries and 17 hosts in the *M. phaseolina* clade. Very little is known about the distribution of *M. pseudophaseolina*, and although all isolates available to us originate from Senegal, it is possible that this fungus also has a wider distribution.

Based on the resulting three-gene tree (ITS, TEF, ACT) (Figure 3), isolates could not be allocated to specific groups according to host or geographic origins. Some isolates from the same host or location tended to group together (e.g. CPC 21527 and CPC 21528; CPC 21500, CPC 21501 and CPC 21502; CPC 21425 and CPC 21426). However, some strains from the same host or location separated into the two respective species, e.g. *M. phaseolina* (CPC 21399) and *M. pseudophaseolina* (CPC 21400). Therefore, no correlation was observed between hosts and geographic locations. Our results supported those of Vandemark *et al.* (2000), who concluded that based on AFLP analysis, it was not possible to correlate DNA polymorphisms with geographic location or host. Most other studies have also failed to find any associations in populations between DNA genotypes and host origins (Almeida *et al.*, 2003; Reyes-Franco *et al.*, 2006). Jana *et al.* (2003), however, used one specific RAPD primer (OPA-13), with which it was possible to distinguish *M. phaseolina* isolates from soybean, sesame, groundnut, chickpea, cotton, common bean, and other hosts. These differences were at population level, however, and not indicative of species.

## Acknowledgements

We are grateful to Dr Ndiaga Cissé, Director of the Regional Centre for Drought Improvement and Adaptation and Pr Ibrahim Ndoye at Cheikh Anta Diop University for their invaluable assistance, and the Generation Challenge program (GCP) and the Collaborative Research Support Program (CRSP) for financial support.

We also thank the Senegalese Agricultural Research Institute, particularly the staff of the phytopathology lab at Bambey.

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Accepted for publication: January 7, 2014