Arbuscular mycorrhizal fungi associated with cassava (Manihot esculenta Crantz) in South Africa

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Abstract

The rhizospheres of cassava (Manihot esculenta) plants growing in Limpopo and Mpumulanga provinces in South Africa were sampled for the presence of arbuscular mycorrhizal fungi (AMF). The two provinces corresponded to high input commercial and low input subsistence agricultural soils, respectively. The Limpopo soils yielded Acaulospora scrobiculata, Glomus rubiforme and Gigaspora sp.1 whereas the Mpumulanga soils yielded Acaulospora scrobiculata, Acaulospora mellea, Acaulospora tuberculata, Glomus etunicatum, Glomus rubiforme, Gigaspora sp. 2 and Scutellospora sp., a total of eight species. The higher diversity in the Mpumulanga sites corresponded with lower soil nitrogen and total and available phosphorus levels. Descriptions of the species are given and the results are discussed in relation to AMF diversity found in other parts of Africa.

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Keywords: Acaulospora; Gigaspora; Glomeromycota; Glomus; Scutellospora

1. Introduction

Cassava (Manihot esculenta Crantz), a woody perennial shrub from the Neotropics is grown primarily for its edible storage roots and is Africa’s second most important crop in terms of calories consumed (Yaninek and Schulthess, 1993). Although cassava grows best in loamy, sandy soils of moderate fertility it can grow in soils too infertile to support other crops and is, therefore, often planted in marginal areas (Yaninek and Schulthess, 1993). In South Africa cassava is grown in KwaZulu-Natal, Mpumulanga and Limpopo provinces, largely by small-scale and subsistence farmers. It also has industrial uses and is grown by the Cassava Starch Manufacturing Company in Mpumulanga and Limpopo provinces who extract its starch for papermaking and other industrial products.

Studies on the growth of cassava in tropical South American ecosystems have shown the plant to be moderately to highly mycotrophic, depending on which species of arbuscular mycorrhizal fungus (AMF) is colonising the plant (Sieverding, 1989b) and the size of the cuttings from which it is propagated (Habte and Byappanahalli, 1994). Cassava is able to form associations with a wide diversity of AMF but in these tropical soils it is most commonly associated with Glomus manihotis (=Glomus clarum) (Sieverding, 1989a,b). In field studies which tested the response of cassava to different levels of fertilizer applications and combinations of single and mixed inocula, G. manihotis was found to be the most strongly invasive, effective and competitive AMF with the ability to tolerate higher levels of fertilizer application and considerably improve P inflow into the plant and dry mass (Sieverding and Toro, 1989). Inoculation with G. intraradices can also improve the salt tolerance of micropropagated cassava clones (Carretero et al., 2008) and the resistance of these clones to transplant stress and increase their shoot and root biomass (Carretero et al., 2009).

There have been a number of studies from Nigeria on AMF and the growth of cassava in alley inter-cropping systems with hedgerow woody legumes. While variables like topography and intercrop species became influential, overall the growth...
response of cassava to inoculation with various non-indigenous species of AMF (including *G. manihotis* and *G. deserticolum*) was a strongly positive one (Atayese et al., 1993; Osonubi et al., 1995; Fagbola et al., 1998a,b; Oyetunji et al., 2003; Liasu et al., 2006).

Although a number of studies documenting the species identity of the Glomeromycota in South Africa have been done many of the data have not been published but are still locked up in dissertations or theses (e.g. Dames, 1991; Mugerwa, 2007). However, Gaur et al. (1999) identified the AMF species associated with *Vangueria infausta*, an indigenous fruit tree, to be *Glomus etunicatum*, *G. intraradices*, *G. occultum* and *Gigaspora albida*. No studies on the Glomeromycota associated with cassava in South Africa have been undertaken and this paper describes AMF species associated with the rhizosphere of cassava plants growing in two different localities: one a high soil input, commercial farm and the other a low soil input, small scale farm. This study formed part of a larger project investigating the response of local cultivars of cassava to inoculation with indigenous isolates of AMF.

2. Materials and methods

2.1. Sources of AMF

Two separate geographical areas were sampled: (a) Limpopo Province (LP) site in Dendron region where cassava is grown commercially on a single farm (GPS position: 23° 22′ 49.25″ S and 29° 21′ 26.54″ E) and (b) Mpumulanga Province (MP) site in Thulamahashe region where cassava is grown as a subsistence crop in a rural farming area (GPS position: 24° 43′ 56.50″ S and 31° 08′ 67.60″ E). Four soil samples from around the stem in the tuber/root region of each of four separate cultivars were collected in the LP site, whereas four soil samples were collected from four adjacent farms all growing the same cultivar in the MP site. Due to the clumped, uneven distribution of AMF spores which requires very high replication for the distribution to near normality (Walker et al., 1982) samples were bulked: samples from each cultivar in the LP site were bulked and homogenized whereas samples from each farm in the MP site were bulked and homogenized.

2.2. AMF spore density

Spores were extracted from three 200 g sub-samples of each bulked sample by the wet-sieving and centrifugation method described by Brundrett et al. (1994), using sieve sizes of 45 μm, 125 μm, 212 μm and 425 μm. Spore density is expressed in terms of unit mass of dry soil. Soil dry mass, soil N, soil total P and soil available P was determined from sub-samples by standard methods (Murphy and Riley, 1962; Sibbensen, 1978; Anderson and Ingram, 1993).

2.3. Spore identification

Semi-permanent microscope slide mounts of representative spores were made using Polyvinyl alcohol-lacto-glycerol (PVLG) mounting medium with and without Melzer’s reagent (Koske and Tessier, 1983; Morton et al., 1993). Identification of AMF was based on morphological characteristics of spores as described by Schenck and Pérez (1990) and descriptions provided by the International Culture Collection of VA Mycorrhizal Fungi (INVAM) (http://invam.caf.wvu.edu/myc_info). Spores were examined by bright field illumination and Nomarski differential interference contrast (Zeiss Axioskop) and in some instances, laser confocal microscopy (LSM 410 Invert Zeiss Laser Scan Microscope).

2.4. Trap pot cultures

Since quality of field-collected spores can be variable and of poor quality due to predation (Morton et al., 1993), trap pot cultures of soil from both sites were established to confirm identifications made from field samples. Equal amounts of field soil, autoclaved (121 °C, 103.4 kPa, 3 h) potting soil and autoclaved acid-washed river sand were mixed into free-draining 2 l pots. Germinated seeds of *Sorghum bicolor* (cv. PAN) were sown and plants grown under greenhouse conditions with the addition of a commercial fertilizer (Multifeed — applied at a rate of 25 mg kg⁻¹ soil) when plants started showing signs of nutrient deficiency. After 12 w of growth, pots were dried for 12 d and reseeded. This procedure involved excising the shoots of the host plant at the soil line, removing the top 2 cm of pot contents, breaking up the root bole in the pots (without removing them) and covering the new seeds with the removed top layer. Plants were grown for another 12 w, dried for 12 d and spores extracted for identification.

3. Results

3.1. Soil phosphorus and nitrogen

The soils from the two provinces used for spore counts and trapping were chemically analyzed for nitrogen and phosphorus levels. Compared to the MP soil, the LP soil was higher in total P by a factor of 1.4, in available P by a factor of 3.0 and in total N by a factor of 2.8 (Table 1).

3.2. Spore density and distribution

Two species of AMF were identified from spores isolated from original soil samples from LP sites (*G. rubiforme* and *G. manihotis*) and from MP sites (*G. intraradices* and *G. rubiforme*).

<table>
<thead>
<tr>
<th>Site</th>
<th>Total P (μg P g⁻¹ soil)</th>
<th>Plant available P (μg P g⁻¹ soil)</th>
<th>Total N (μg N g⁻¹ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP</td>
<td>143.3 ± 0.1</td>
<td>9.3 ± 0.6</td>
<td>427.7 ± 52.5</td>
</tr>
<tr>
<td>MP</td>
<td>100.7 ± 0.1</td>
<td>3.1 ± 0.3</td>
<td>151.9 ± 56.2</td>
</tr>
</tbody>
</table>

Values are the means (±SD) of five pseudo-replicates.
**Table 2**
Spore density (no. spores 200 g⁻¹ soil) and AMF composition of rhizosphere samples associated with four cassava cultivars in the Limpopo Province (LP) site.

<table>
<thead>
<tr>
<th>Cassava cultivar</th>
<th>AMF species</th>
<th>Spore density</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-400</td>
<td>Glomus rubiforme</td>
<td>212±70</td>
</tr>
<tr>
<td></td>
<td>Gigaspora sp.1</td>
<td>261±138</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>472</td>
</tr>
<tr>
<td>T-200</td>
<td>Glomus rubiforme</td>
<td>240±57</td>
</tr>
<tr>
<td></td>
<td>Gigaspora sp.1</td>
<td>15±9</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>255</td>
</tr>
<tr>
<td>TN</td>
<td>Glomus rubiforme</td>
<td>256±72</td>
</tr>
<tr>
<td></td>
<td>Gigaspora sp.1</td>
<td>56±20</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>312</td>
</tr>
<tr>
<td>TM</td>
<td>Glomus rubiforme</td>
<td>214±55</td>
</tr>
<tr>
<td></td>
<td>Gigaspora sp.1</td>
<td>10±6</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>224</td>
</tr>
</tbody>
</table>

Values are the means (±SD) of five pseudo-replicates.

**Gigaspora** sp.1) (Table 2) and two different species were identified from spores isolated from original soil samples from MP sites (G. etunicatum and Scutellospora sp.) (Table 3). In the LP site the total spore number from the T-400 cultivar sample was between 1.5 and 2 times greater than the total spore numbers from the other cultivar samples (Table 2). In addition, whereas the ratio of G. rubiforme to Gigaspora sp. 1 was approximately 1:1 in the T-400 sample, the number of G. rubiforme spores was between 4 and 20 times greater than those of Gigaspora sp 1 in the other cultivar samples (Table 2). On average the total spores numbers from the MP site were double those of the LP site with Glomus spp. being the most common in both localities. Trap pot cultures produced higher diversity with the LP pots selecting for Acaulospora scrobiculata in addition to the original two species and the MP pots selecting for Glomus rubiforme, Acaulospora scrobiculata, A. mellea, A. tuberculata and a new species of Gigaspora in addition to the species found in the original soil samples. Thus, there were two species (G. rubiforme and A. scrobiculata) which were common to both localities.

**Table 3**
Spore density (no. spores 200 g⁻¹ soil) and composition of AMF of rhizosphere samples associated with cassava in four farm sites in Mpumulanga Province (MP).

<table>
<thead>
<tr>
<th>Farm site</th>
<th>AMF species</th>
<th>Spore density</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glomus etunicatum</td>
<td>690±80</td>
</tr>
<tr>
<td></td>
<td>Scutellospora sp.</td>
<td>126±15</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>816</td>
</tr>
<tr>
<td>2</td>
<td>Glomus etunicatum</td>
<td>586±71</td>
</tr>
<tr>
<td></td>
<td>Scutellospora sp.</td>
<td>45±6</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>631</td>
</tr>
<tr>
<td>3</td>
<td>Glomus etunicatum</td>
<td>665±74</td>
</tr>
<tr>
<td></td>
<td>Scutellospora sp.</td>
<td>17±3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>682</td>
</tr>
<tr>
<td>4</td>
<td>Glomus etunicatum</td>
<td>559±69</td>
</tr>
<tr>
<td></td>
<td>Scutellospora sp.</td>
<td>25±7</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>584</td>
</tr>
</tbody>
</table>

Values are the means (±SD) of five pseudo-replicates.

### 3.3. Species identification

**Glomus etunicatum** (Becker & Gerdemann) (INVAM reference accession NE108A; Becker and Gerdemann, 1977)

Spores orange to red brown to very dark in older spores, globose to subglobose, 60–150 μm, mean=129 μm (n=269) (Fig. 1a,b). Spore wall consists of two layers, L1 and L2 (Fig. 1b). L1 is the outer mucilaginous layer (showing some plasticity and an uneven outer surface); 2.5 μm thick which may degrade as the spore ages to develop a granular appearance (Fig. 1b,c,d). L2 consists of thin adherent sub-layers (laminae), light orange-brown to red-brown in colour (Fig. 1b,c,d); 5.0–6.1 μm thick (Fig. 1b,c,d) but thickening to 7.5 μm in the region of the subtending hypha (Fig. 1d). The subtending hypha is 8.5 μm thick with two layers continuous with the two layers of the spore wall (Fig. 1c). An occlusion between the innermost sub-layer of the laminate layer of the spore wall is present which resembles a septum (Fig. 1c,d).

**Glomus rubiforme** (Gerdemann & Trappe) Almeida & Schenck (Almeida and Schenck, 1990)

Sporocarps resembling a miniature blackberry, dark brown, subglobose to irregular, 300 μm–540 μm, mean=430 μm (n=16) (Fig. 1e). Sporocarp consists of a single layer of chlamydospores, not arranged side by side, surrounding a central plexus of hyphae, peridium absent (Fig. 1f,g). Chlamydospores are yellow brown to dark brown, ovoid to ellipsoidal, 57 μm–135 μm, mean=114.5 μm (n=41) (Fig. 1f,g). Spore wall is laminate, 7.0 μm thick (Fig. 1h), with a small pore opening into a thick-walled subtending hypha (Fig. 1h) and with perforated projections on inner surface (Fig. 1h). The outer wall is hyaline, but frequently absent in mature spores (Fig. 1g).

**Acaulospora mellea** Spain & Schenck (INVAM reference accession BR983A; Schenck et al., 1984)

Sporocarps orange brown, 87.5 μm–135 μm (mean=118 μm) (n=28). Spore wall consists of three layers, L1, L2 and L3 (Fig. 2a,b,c,d). L1 is thin and hyaline (Fig. 2b,d) and sloughs on many spores (Fig. 2a,c); L2 consists of laminae (Fig. 2d) with a smooth surface if outer layer has sloughed (Fig. 2a,c); L3 is yellow-brown (Fig. 2a) and also consists of laminae (Fig. 2a) which can separate from each other but generally merge to be part of the spore wall (Fig. 2a,b,c,d). There are two flexible hyaline germinal inner walls that that separate from each other and the spore wall (Fig. 2a,c). Germinal inner wall 1 (gw1) is a bilayered hyaline wall which separates clearly from the spore wall but the two layers do not often separate from each other (Fig. 2b,d). Germinal inner wall 2 (gw2) consists of two adherent hyaline layers (Fig. 2a,b,c,d) with gw2L1 showing granular excrescences or “beads” (Fig. 2d) and gw2L2 staining red-purple in Melzer’s reagent (Fig. 2d). A cicatrix (scar showing region of contact between spore and saccule neck during spore synthesis) is circular to oval-shaped, 7.5 μm (Fig. 2a).

**Acaulospora scrobiculata** Trappe (INVAM reference accession BR984; Spain, 1992)

Pale yellow to straw-coloured spores, 80 μm–165 μm, mean=118 μm (n=100), globose to sub-globose (Fig. 2e),
Spore wall consists of three layers, L1, L2 and L3. L1 hyaline, degrading early in spore wall differentiation, often absent in mature spores (Fig. 2e). L2 consists of laminae with ovoid concave depressions on surface (Fig. 2e,g,h). L3 is regarded as a discrete component of the spore wall as it sometimes separates slightly with defined boundaries (Fig. 2h). Two flexible hyaline inner walls (iw1 and iw2) can be seen as separate when spore is broken (Fig. 2f). Two tightly adherent layers form iw1 (Fig. 2g,h) and iw2, with iw2L1 forming granular excrescences or “beads”, whereas iw2L2 (Fig. 2h) stains reddish brown in Melzer’s reagent (Fig. 2g). A circular cicatrix, indicating region of contact between spore and saccule is present (Fig. 2e).

*Acaulospora tuberculata* Janos & Trappe (INVAM reference accession VZ103E; Janos and Trappe, 1982)
Fig. 2. a–d, *Acaulospora mellea*: (swL1) thin, hyaline outer layer of spore wall; (swL2 and swL3) laminated inner layers of spore wall; (gw1) first bilayered hyaline germinal inner wall; (gw1L1) outer layer of inner wall one; (gwL2) inner layer of inner wall one; (gw2) second hyaline inner wall; (gw2L1) outer layer of second inner wall with beads; (gw2L2) inner layer of second inner wall staining red-purple in Melzer’s reagent. e–h, *Acaulospora scrobiculata*: (sw) spore wall; (swL1) hyaline outer layer of spore wall; (swL2) second laminate layer of spore wall with concave depressions; (swL3) inner layer of spore wall; (iw1) first hyaline inner wall consisting of two tightly adherent layers; (iw2) second hyaline inner wall; (iw2L1) outer layer of inner wall two with granular beads; (iw2L2) inner layer of inner wall two stains red-brown in Melzer’s reagent.
Spores red-orange to dark red-brown in colour, globose to sub-globose, 126 μm–270 μm, mean = 209 μm (n = 32) (Fig. 3a). Spore wall consists of three layers (L1, L2, L3) (Fig. 3c,d). L1 is hyaline, 1.2 μm thick that remains after tubercles on L2 have formed. L2 thicken by formation of red-brown sub-layers (laminae) (Fig. 3c,d) and forms polygonal spines or tubercles (Fig. 3b). L3 is yellow-brown to red-brown in colour and mostly appears to be an inner sub-layer of L2 (Fig. 3c,d). Two flexible, hyaline inner walls (iw1 and iw2) (Fig. 3a) are present. Two layers of near equal thickness comprise iw1 (L1 and L2) (Fig. 3c,d). Similarly, iw2 comprises two adherent hyaline layers (L1 and L2) and L2 stains pinkish red to red-brown in Melzer’s reagent (Fig. 3c,d).

Gigaspora sp. 1

Spores pale yellow to yellow-green in colour, globose to sub-globose, 260–410 μm mean = 320 μm (n = 30) (Fig. 3e). Spores are borne terminally on bulbous, suspensor-like sporogenous cell, 40 μm at widest point (Fig. 3f). Spores turn pale orange-brown in Melzer’s reagent with no distinctive difference in colouration of spore wall (Fig. 3f). Spore wall is thin (8 μm thick) and one is unable to distinguish different layers from these micrographs but laminae are apparent (Fig. 3f).

Gigaspora sp. 2

Spores are orange-brown to dark-brown in colour, globose to sub-globose and even ellipsoid, 310 μm–500 μm, mean = 422 μm (n = 25) (Fig. 4a,b). Spores are borne terminally on a bulbous suspensor-like sporogenous cell, 60 μm at widest point (Fig. 4b). Spore wall (22.5 μm–27 μm thick) consists of outer layer (Fig. 4b), laminate layer (Fig. 4b,c,d), and what appears to be an inner papillate layer (Fig. 4c).

Scutellospora sp.

Spores cream to pale magenta or even light brown in colour, globose to sub-globose, sometimes ellipsoid, 153–218 μm,
mean = 185 µm (n=57) (Fig. 4e). Spores are borne terminally on a bulbous suspensor-like sporogenous cell, 22.0–24.0 µm at widest point (Fig. 4e,f,g,h). Spore wall (18.0–20.0 µm) distinctly bi-layered (Fig. 4f,g,h) with outer spore wall and laminate inner spore wall of equal thickness. One flexible inner wall with darker-coloured germination shield is present (Fig. 4e, f,g). The germination shield is ovoid, 88 × 66 µm in diameter with paired germination holes (Fig. 4g).
4. Discussion

The AMF diversity of the original field soil samples was very low with only two species from each locality being isolated. This low diversity is not unusual as spores isolated directly from a field soil sample may represent only those AMF with sufficient root-colonizing activity and biomass to trigger sporulation, and in arid sites it is found that little or no sporulation occurs but roots are clearly colonized (Morton et al., 1993). Trapping is therefore necessary to create conditions for root colonizing activity and sporulation of all indigenous species present. Trap pots were able to stimulate spore germination of an additional species from the LP (high input) soil (total three) and an additional five species from the MP (low input) soil (total seven). This diversity is on the low side in terms of the general scale proposed by Sieverding (1991) of 6–9 species in high-input, intensive agriculture, 10–15 species in low-input systems and 16–21 species in natural ecosystems. The lower species diversity in the LP soil may be indirectly related to higher fertilizer-derived nutrient levels in these soils. For example, high external availability of P for N-sufficient plants can lead to reduced root colonization by AMF (Smith and Read, 1997) and P uptake characteristics by external hyphae can vary greatly between fungi (Smith et al., 2000) so that these soils may be selecting only for high P-tolerant strains. Although, initial spore counts in the MP soil were on average double those of the LP soil it would be difficult to propose a causal relationship between sporulation and soil fertility as sporulation is dependent on a range of edaphic, seasonal and host determinants (Morton et al., 1993). For the original LP samples, it was observed that the total spore number associated with the T-400 cultivar was double those of the other cultivar samples and that there was a much lower ratio of G. rubiforme to Gigaspora sp. 1 spores associated with T-400 which suggests that it is (a) more mycotrophic and, (b) that some degree of host–fungus specificity is operating. However, to prove (a) would require supportive evidence from physiological and nutritional studies, and the nature of specificity in this symbiosis is still debatable and a complex one, probably operating at a number of levels (Sanders, 2002) and these data are too slight for anything but speculation. Nevertheless, the analysis did allow us to choose appropriate cultivars for subsequent pot experiments testing the response of cassava to AMF inoculation.

G. etunicatum is an extremely widespread species with many ecotypes (Becker and Gerdemann, 1977) and has been found associated with cassava in Brazil (De Souza et al., 1999). There are six reports of G. etunicatum from the African continent. Musoko et al. (1994) detected the species in undisturbed secondary semi-deciduous moist forest in Cameroon, and Mason et al. (1992) identified the species in a similar forest system in Cameroon but where Terminalia ivorensis had been planted after site clearance. The species has also been found in Terminalia plantations in Côte d’Ivoire (Wilson et al., 1992). The species has been associated with the wild fruit tree, Vangueria infausta in South Africa (Gaur et al., 1999) and Stutz et al. (2000) identified G. etunicatum and three unnamed Glomus species from the rhizosphere of plants growing in the Namib Desert in Namibia. More recently, it has been identified in different farming systems in Malawi (Jefwa et al., 2006). G. rubiforme also appears to be a cosmopolitan species found in many soil types (Khade, 2008), which may explain its commonality to both LP and MP sites: it is also known by the synonyms, G. pachycaulis and G. indica from Taiwanese and Indian isolates (Khade, 2008). There are two other reports of G. rubiforme being isolated in Africa, from Terminalia plantations and undisturbed semi-deciduous moist forest in Cameroon (Mason et al., 1992; Musoko et al., 1994).

In Africa, A. mellea and A. scrobiculata have been identified in low input farm, forest and grassland soils in Western Kenya (Shepherd et al., 1996) and in the Terminalia and undisturbed semi-deciduous moist forests of Cameroon (Mason et al., 1992; Musoko et al., 1994) and A. scrobiculata was found in Malawi (Jefwa et al., 2006). These two species are considered facultative symbionts and appear adapted to a wide range of soils and host species, appearing in soils of widely differing pH and nutrient availability (Sieverding, 1991; Shepherd et al., 1996) which may account for the presence of A. scrobiculata in both LP and MP sites. The present study represents the first report of A. tuberculata from Africa but the species is considered to be extremely difficult to sporulate in culture (Morton et al., 1993) which may account for its apparent low presence on the continent. All three species of Acaulospora have been found associated with cassava in Brazilian soils (De Souza et al., 1999).

The two Gigaspora spp. isolated could not be identified as being any of the species in the INVAM collection when their spore descriptions were compared (http://invam.caf.wvu.edu/fungi/taxonomy/Gigasporaceae/Gigaspora/Gigaspora.htm) and could not be identified by the dichotomous key of Bentivenga and Morton (1995). Both species were precluded from being G. rosea on the basis of colour, as G. rosea has a distinctive pale pink tint and is also smaller than these two species and usually has a much darker-coloured sporogenous cell than the spore wall. Gigaspora sp. 1 is similar to G. albida in the following respects: the spore wall has a similar colour, the laminae of the wall are not robust, and the sporogenous cell is similar in colour to the spore but Gigaspora sp. 1 is slightly larger in average diameter than G. albida, has a thinner wall and there is no evidence of dark purple staining of the L2 layer in Melzer’s reagent. Gigaspora sp. 2 is darker in colour and much wider in average diameter and with a wider sporogenous cell width than that of G. albida. Gigaspora sp. 1 has a similar diameter range as G. gigantea but is not as brightly coloured, the wall is much thinner and there is no distinctive dark red-purple staining of the L2 layer in Melzer’s reagent. Gigaspora sp. 2 is darker in colour and much wider in average diameter and with a wider sporogenous cell width than that of G. albida. Gigaspora sp. 1 has a similar diameter range as G. gigantea but is not as brightly coloured, the wall is much thinner and there is no obvious distinctive staining of the L2 layer in Melzer’s reagent. Gigaspora sp. 2 is precipitated from being G. gigantea on the basis of colour and a larger average spore diameter. Gigaspora sp. 1 is in the same size range as G. margarita but there is no distinctive dark red-purple staining of the spore wall in Melzer’s reagent. Gigaspora sp. 2 has a darker colour and larger spore diameter than G. margarita. In comparison to G. decipiens, Gigaspora sp. 1 is somewhat smaller in average diameter and more yellow-green in colour; the wall is much thinner and does not stain very dark purple in Melzer’s reagent. Gigaspora sp. 2 is slightly larger than G. decipiens in average diameter and much darker in colour, although composite wall diameter is similar. In addition, Gigaspora sp. 1 and sp. 2
showed no evidence of the roughened, warty outer walls described by Koske and Walker (1985) and characteristic of *G. dipapillosa*, *G. coralloidea*, *G. heterogama*, *G. gregaria*, *G. verrucosa* and *G. persica*.

Nine species of *Scutellospora* have been reported from Africa. Two species, *Scutellospora pellucida* and *Scutellospora coralloidea* were associated with the *Terminalia* plantations in Cameroon (Mason et al., 1992) while *Scutellospora pellucida* and *Scutellospora gregaria* were associated with similar plantations in Côte d’Ivoire (Wilson et al., 1992). *Scutellospora pellucida* was also isolated from nutrient-depleted farm soils in Kenya, in addition to four other *Scutellospora* spp. and two different species were reported from farms soils in Malawi (Jefwa et al., 2006). In terms of colour and size the *Scutellospora* sp. isolated in this study is closest to *S. pellucida* but lack of information on the inner wall structure makes a definitive identification untenable.

The diversity of AMF in South Africa, as indicated by this study, appears to be low compared with some other studies from Africa which have shown higher levels, especially in tropical ecosystems. Musuko et al. (1994) isolated 11 species or species aggregates from undisturbed moist forest in Cameroon whereas Mason et al. (1992) identified 17 species from Cameroonian *Terminalia* plantations with a strong overlap between those identifications and the ones from the present study. Wilson et al. (1992) identified 41 species in Côte d’Ivoire; Jefwa et al. (2006) isolated 12 species in Malawi; but Dalpé et al. (2000) found only five species associated with the legume, *Faidherbia albida*, in Senegal with no overlap between their identifications and the species from this study. However, more intensive sampling, especially seasonal, would be required to ascertain the full suite of AMF in these soils supporting cassava.

In conclusion, this study provides a valuable contribution to the database of the Glomeromycota found in both South African and African soils. The presence of *Glomus manihotis*, a common symbiont of cassava in South American tropical systems, was not detected.

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