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A NEW SPECIES OF ACAULOSPORA ASSOCIATED WITH COCOA IN JAVA AND BALI (INDONESIA)

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ABSTRACT

During an investigation of Vesicular Arbuscular Mycorrhizal (VAM) fungi in the soils from plantations of *Theobroma cacao* L. (cocoa) in Java and Bali, a yellowish brown spore of a VAM fungus was regularly recovered by wet sieving. It appeared to be an undescribed species in the genus *Acaulospora*, and is described here for the first time as *A. walkeri*. The fungus is abundant and widely distributed under cocoa in Java and Bali.

INTRODUCTION

Samples of the top 10–20 cm of soils were collected from under cocoa at plantation sites distributed throughout Java and Bali. Following wet sieving and decanting, spores were removed under a dissecting microscope from suspensions in water or 5% Formaldehyde using swan-necked forceps. At all of the eleven sites investigated in Java and Bali an unknown species of *Acaulospora* was the most abundant spore type recovered, the average density being 440 spores per kg soil, with figures as high as 1278 spores per kg being recorded for one site (Kramadibrata 1989). In view of the importance of this taxon within the community of VAM fungi in soil under cocoa in Indonesia it is here described as a new species *Acaulospora walkeri*.

***Acaulospora walkeri* Kramadibrata & Hedger, sp. nov.**

Sporae in solo singulatim veniuntur, hyalinae et hyphae lateraliter exorientes, quae ipsa sporifero e sacculo producitur, sacculo ipso (sit spora statu maturo) collapsa. Sacculus sporiferus hyalinus vel albus, globosus vel subglobosus, 80–120 x 100–150 µm diam., muro indutus hyalino vel viridi, 0.5–1.0 µm lato et hyphae laterali quae sacculi in regione circa 200 x 10–20 µm et e spora fastigata est. Sacculus ipse nonnumquam minute asperatus minutos ob soli particulos est, modo granulorum in superficie visu.

Sporae globosae vel subglobosae vel reniformes, pallidae-brunnae vel flavo-brunnae ad brunnae, 140–200 x 140–200 µm diam., in superficie laeves licet etiam ab eo investigentur quod anglice redditur "scanning electron microscope", saepius circa 40–80 µm e sacculo sporifero productae, et hyphae hyalinae plusminusve 5 mm latae, et quaeque separatae cicatrice globoso vel irregulari, 10–20 µm diam.

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Spores are found singly in soil, arising laterally from a hyaline hypha, which is itself produced from a sporiferous saccule which collapses as the spore matures. The sporiferous saccule is hyaline to white, globose to subglobose, 80–120 x 100–150 μm diameter, with a hyaline to greenish wall, 0.5–1.0 μm thick. It gives rise to a hypha \approx 200 μm long, which is 10–20 μm broad near the sporiferous saccule, but which tapers to 5 μm at its tip near the spore. The sporiferous saccule itself is sometimes minutely roughened on account of minute particles of soil, which appear like granules on the surface.

Spores are globose or subglobose or reniform, pale brown or yellow-brown to brown, 140–200 x 140–200 μm diameter. The surface is smooth; no surface markings were found under the scanning electron microscope. Spores are formed at about 40–80 μm distant from the sporiferous saccule, from a hyaline hypha \approx 5 μm broad, and each is separated by a globose to irregular scar 10–20 μm wide left on the spore.

The wall is structured in two wall groups, A and B. Wall group A consists of walls 1 and 2. Wall 1 is a smooth unit wall, sometimes difficult to rupture, hyaline to pale yellow, or yellow-brown in colour, 0.5–1.0 μm thick. Wall 2 is also a smooth unit wall but is hyaline and 1.0–2.0 μm thick. It is tightly bound to wall 1. Wall group B consists of two walls, 3 and 4. Wall 3 is hyaline, unit, sometimes finely ornamented, 0.5–1 μm thick. Wall 4 is amorphous, 1.0–2.0 μm thick and sometimes difficult to see.

The muronym (Walker 1983, Morton 1986) is A(UU) B(U o A). The murograph is shown in figure 1. The sporiferous saccule, spores and wall structures are illustrated in figures 2–6.

CHEMICAL CHARACTERISTICS

Reactions to Melzer's reagent are: (a) fresh spores from pot culture, walls 1 and 2 do not react, walls 3 and 4 become purple; (b) after prolonged storage in 5% formaldehyde, walls 1 and 2 remain unreactive, but walls 3 and 4 colour brown in Melzer's, and ornamentation in wall 3 becomes obvious.

ETYMOLOGY

The specific epithet is given in honour of Dr. C. Walker.

COLLECTIONS EXAMINED

HOLOTYPE: West Java Province - Cianjur County: XII State Plantation Company (Rajamandala Plantation), Rama 2, 28 April 1987, KK 277. Deposited at Herbarium Bogoriense, Bogor, Indonesia (BO).

ISOTYPES: West Java Province - Cianjur County: XII State Plantation Company (Rajamandala Plantation), Rama 1, 28 April 1987, KK 275; Rama 3, 28 April 1987, KK 202 and KK 202a. Banjar County: XIII State Plantation Company (Batulawang Plantation), Batugajah Plantation, BG4, 6 May 1987, KK 322 and KK 323; Pangandaran Plantation, PND 1, 8 May 1987, KK 144; PND 5, 8 May 1987, KK 340; PND6, 8 May 1987, KK 345.

Central Java Province - Jepara County: XVII State Plantation Company (Beji Plantation), Beji Tengah 11, 3 April 1987, KK 228.

East Java Province - Malang County: Pancursari Subdistrict: Pancursari village, MLGS2, 30 March 1987, KK 212.

Bali Province - Jembrana County: Pekutatan Subdistrict: Puluhan village, Bali 2, 25 March 1987, KK s.n.

All isotypes are deposited in Herbarium Bogoriense except for KK 212 which is deposited in Mycological Herbarium, Department of Biological Sciences, University of Wales at Aberystwyth, Dyfed, U.K.

POT CULTURE: Pot cultures with *Lolium perenne* have been made, mixed with a *Glomus* species. Attempts are being made to produce single species pot cultures.

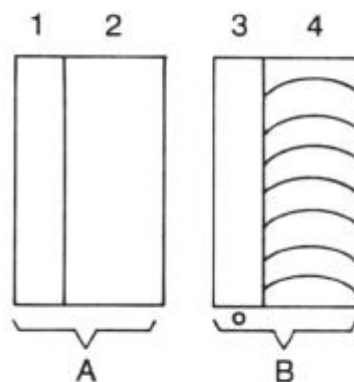


Fig. 1. Murograph of spores of *Acaulospora walkeri*. Arabic numerals indicate each wall in order from the outside of the spore; letters indicate wall groups. The conventions used are described by Walker (1983) and Morton (1986).

DISCUSSION

All collections of spores of *A. walkeri* made from cocoa soils throughout Java and Bali appeared to be very similar. Most field collections from Bali lacked sporiferous saccules, but were clearly *A. walkeri*. Wall group A in some intact spores appeared to consist of three walls, but careful examination of crushed spores never confirmed the presence of a third wall. Some spores also had outer walls (wall 1) which were somewhat reddish brown. Wall group B (walls 3 and 4) was always distinct and easily separable from wall group A when spores were crushed. By crushing it was usually possible to see the two tightly bound walls 3 and 4, but sometimes, in intact spores, the two walls were more difficult to distinguish. The ornamentation on wall 3 was most obvious in formaldehyde preserved spores, especially in Melzer's reagent. The amorphous nature of wall 4 was obvious in spores mounted in lactophenol. Spores of *A. walkeri* could easily be picked out under the dissecting microscope from sample preserved in 5% Formaldehyde, because of their smooth surface, and glittering or sparkling appearance.

A. walkeri resembles the description of *A. delicata* Walker, Pfeiffer & Bloss (1986) but the spores of the latter are smaller ($80-125 (150) \times 80-110 (140) \mu\text{m}$ in *A. delicata*, compared to $140-200 \times 140-200 \mu\text{m}$ in *A. walkeri*) but both have a sparkling appearance. Fresh spores of *A. walkeri* are also much darker in colour than those of *A. delicata*. Although *A. walkeri*, like *A. delicata*, has two thin unit walls in wall group A, they are rather rigid compared with those in *A. delicata*.

Wall group B in *A. walkeri* resembles group B in *A. delicata*, since wall 3 can be finely ornamented in both, but in spores of *A. delicata* wall 4 is membranous, not amorphous as in *A. walkeri*. *A. walkeri* is also similar to *A. appendicula* Spain, Sieverding & Shenck (Shenck, Spain, Sieverding & Howeler 1984) the spores of which are slightly larger (170–250 (–390) μm). However, the spores of *A. appendicula* are a dull yellowish cream to orange tan colour, and the wall structure is different. Although *A. appendicula* has four walls in two groups they are all unit walls. Wall group A is thicker than in *A. walkeri*, i.e. 8–16 (–20) μm for wall 1 and 4–6 μm for wall 2, which also differs in having a reticulum. In wall group B, wall 3 is much thicker than in *A. walkeri*, 4–8 μm , and also has an alveolate reticulum; in addition, wall 4 (2–10 μm thick) is a unit wall.

With regard to its mycorrhizal associations in the field, this spore type was found in soil samples collected from plantations, but a number of other higher plants were commonly present, and thus could also have acted as phytobionts. They included grasses, clove, banana, coconut, and cardamom. *A. walkeri* was also collected from soils in Nongkojajar, Purwodadi, East Java, not planted with cocoa. Here the hosts could have included apple, clove and coffee.

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Acaulospora walkeri. — Fig. 2. Crushed spore (S) attached to collapsed sporiferous saccule (SS); wall groups A & B are arrowed. — Fig. 3. Crushed spore with intact wall group A (arrowed) and ruptured wall group B (arrowed). — Fig. 4. Crushed spore showing details of wall structure; walls 1 & 2 (wall group A) are arrowed and can be seen separated from walls 3 & 4 (wall group B) also arrowed. — Fig. 5. Scanning electron micrograph of groups of spores showing range of shape and size. — Fig. 6. Scanning electron micrograph showing smooth surface of spore and circular attachment scar (as) left by the subtending hypha.

