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MYCORRHIZAL HABIT AND SCLEROGENESIS OF *PHLEBOPUS SUDANICUS* (GYRODONTACEAE) IN SENEGAL

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ABSTRACT

INTRODUCTION

Among the Boletales only two boletes are known to produce sclerotia. *Boletinellus* (*Gyrodon*) *merulioides* (Schw.)Murr. formed sclerotia in pure culture (Pantidou, 1961) and in nature (Cotter & Miller, 1985) whereas *Boletus porosporus*(Imler)Watling produced sclerotia in mycorrhizal synthesis trials (Giltrap, 1979 *in* Cotter & Miller, 1985).

In this study we present the first tropical bolete, *Phlebopus sudanicus* Har.& Pat., able to produc sclerotia in nature, in pure culture and in mycorrhizal synthesis trials. The mycorrhizal status of *P.sudanicus* is substantiated. The *in vitro* production and the viability of the sclerotia is investigated.

MATERIALS AND METHODS

Field collections - Sclerotia and sporocarps of *P.sudanicus* (Fig1a,b) were collected under *Carapa procera* in a subguinean forest. Sporocarps were also collected at nine sites ranging from the sudano-guinean to the sahelian domain. The closest trees were *Acacia macrostachya*, *A.nilotica*, *A.raddiana*, *A.senegal*, *Cassia sieberiana*, *Dichrostachys glomerata*, *Parkia biglobosa*, and the introduced *Acacia holosericea*, *Casuarina equisetifolia*, *Prosopis chilensis*. Although mycelium and rhizomorphs of *P.sudanicus* were intimately tangled to the fine roots of the trees, ectomycorrhizas were never observed in the field. Sclerotia were preserved in FAA.

Laboratory cultures - Ten cultures of *P.sudanicus* were isolated from fresh sporocarps by transferring pieces of pileus trama into Petri dishes filled with MNM agar. Strains were subcultured on different media, at different pH, temperature and light levels to determine the best conditions inducing sclerogenesis.

Inoculum for mycorrhizal synthesis trials was prepared by inoculating 250ml Erlenmeyer flasks filled with vermiculite, peat and liquid MNM (145/5/150 by vol.) and incubating them at 30°C for 4 to 6 weeks. Mycorrhizal synthesis trials were performed in polyethylene containers filled with a sterilized mixture of a low fertility sand, vermiculite and peat (50/45/5 by vol.).

Seeds of trees used for mycorrhizal tests were surface sterilized with concentrated sulfuric acid, germinated on water agar and transferred into the containers. Forty ml inoculum of *P.sudanicus* was placed close to the lateral roots two weeks later. Seedlings were placed in a greenhouse, watered daily at field capacity and allowed to grow for 6-7 months.

Anatomy and histochemistry of sclerotia and mycorrhizas - Native sclerotia fixed in FAA and fresh, mature sclerotia produced in vitro were hand sectionned and stained with 0.5% Congo red for anatomical observation under the microscope. Sections of in vitro sclerotia were stained with toluidine blue O acidified to pH 1 with

HCl, with sudan black B in 70% ethanol, with Lugol's solution, with 1% amido black 10B in 7% acetic acid, with Nile blue to determine the main histochemical characteristics.

Fresh washed ectomycorrhizal roots were hand sectionned, cleared with 15% sodium hypochloride, rinsed in water and stained with 0.5% Congo red. Sections were mounted in 50% glycerol.

Viability of sclerotia - Mature sclerotia produced in vitro were stored in dry or wet sterile sand at 4°C, in sterile water at 4°C and in 50% glycerol at -80°C for 4 months to determine their viability. Sclerotia were also air dried and stored at 25°C for different periods of time.

RESULTS

Production of sclerotia in vitro - On MNM agar at 25°C and 30°C all the isolates of *P.sudanicus* produced sclerotia within 15 to 30 days growth (Fig.1c,d). Growth rate and sclerogenesis was higher at 30°C. At 40°C *P.sudanicus* was completely inhibited.

On the "starvation medium" (thiamine 100µg, agar 18g) growth was very weak and no sclerogenesis occured. On "Pachlewski" medium (Pachlewski & Pachlewska,1974) and malt extract agar (malt extr.16g, agar 18g) the sclerogenesis was comparable to that obtained on MNM. On PDA (dextrose 20g, potato extr.15g, agar 20g) sclerotia occured earlier and after 6 weeks the number of mature sclerotia was three times higher than on MNM (ca.30 v.10 per Petri dish). Sclerotia appeared also in the Erlenmeyer flasks when preparing inoculum for mycorrhizal tests. Influence of light on sclerogenesis seems to be negligeable as we obtained the same results in the dark and in full light. On PDA *P.sudanicus* was able to grow on a wide range of pH but the daily growth rate at 30°C decreased from 3.1mm at pH 4 to 1.3mm at pH 8. At pH 4-6 sclerogenesis occured in all replicates (5), at pH 7 in 80% and at pH 8 in 50% of the replicates. The fungus is thus acidocline.

Storage conditions and viability of sclerotia - Sclerotia stored for 4 months in sterile water or in sterile humid sand were able to germinate on MNM agar after 2-4 days incubation at 30°C. The cultures produced neosclerotia after ca. 2 weeks growth and their characteristics (e.g. clamp connections, colour) were identical to those of the mother cultures. Sclerotia stored for 4 months in dry sand or in glycerol at -80°C failled to germinate. Sclerotia air dried 1-2 days at 25°C germinated on MNM only after soaking them 1 night in sterile water. Sclerotia dried for 1-2 weeks were unable to germinate even after soaking.

Morphology and anatomy of sclerotia - Native sclerotia were tough, light brown, globulous, ellipsoidal or irregularly clon gated (as a result of coalescing sclerotia), with attached rhizomorphs (Fig.1b). *In vitro* sclerotia were ellipsoidal, 8-14mm thick, or irregularly lobed. Four main steps were observed during sclerogenesis : 1-small felty cream tuft (1-2mm diam.) appeared on a rhizomorph (Fig.1e); 2-young hemispherical (ca. 4mm diam.) sclerotium occured, felty, yellow to russet, with hyaline droplets (Fig.1f); 3-sclerotium became tough,slightly felty, light brown with brown exudating droplets; 4-evaporating droplets were covered by a wrinkled membranous sac and were finally replaced by dark brown spots on a light dull or glossy brown background (Fig.1g). Sclerotia were connected to basal rhizomorphs.

In cross sections four layers were distinguishable (Fig.1h) : a 25 to 50 µm thick trichoid of brown horn-shaped hairs, 2.5 µm diam. bearing clamp connections; a dark brown to black, 8-14 µm thick rind formed by hard to distinct compressed hyphae embedded in a matrix of melanised stuff; a 16-14 µm thick cortex formed by tangled hyphae of 2.5-4 µm diam. and bigger hyphae of 7-8 µm diam.; a medulla formed by intricated scleritiform, diverticulate and bone shaped hyphae, 10-18 µm diam., with thickened walls of 2.5 µm or more, accompanied by a few olive brown sap hyphae; cells of the medulla empty or with granular contents. Native sclerotia had a similar anatomy.

Histochemical characteristics of sclerotia - Toluidine blue O stained the membranes of the trichoid in blue and those of the cortical hyphae in green or blue green (presence of polyphenolics). The rind remained unstained. Cytoplasmic granules of the medulla stained violaceous red and are suspected to be polyphosphates. With Lugol's reagent the content of medullar cells stained mahogany allowing to suspect the presence of glycogen. Amido black 10B stained basic stuffs, including proteins, of the medulla in blue. Sudan black B stained acid and neutral lipidic inclusions of the medulla. Phospholipidic granules of the medulla stained blue in Nile blue A.

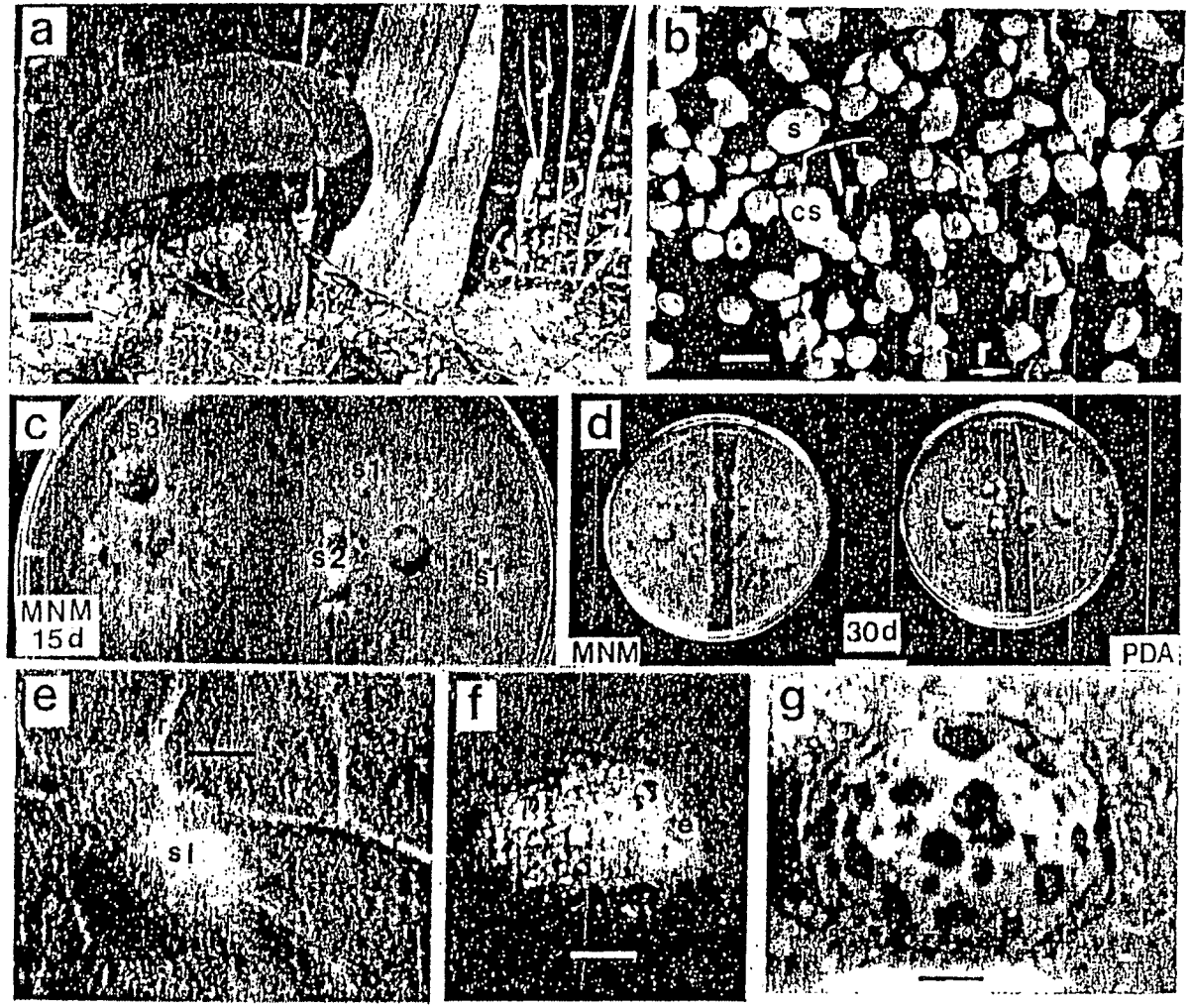
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Fig.1a, sporocarp of *P.sudanicus* (bar = 5cm); b, native sclerotia of *P.sudanicus* (s : sclerotia; cs : composite sclerotia; r : rhizomorphs; bar = 1cm); c, 15d old culture of *P.sudanicus* on MNM agar showing sclerotia at different stages of development (s1, s2, s3); d, 30d old cultures of *P.sudanicus* on MNM and on PDA media; e, sclerotium initial (si) connected to rhizomorphs (r; bar = 0.5mm); f, young sclerotium with hyaline droplets of exudate (e; bar = 2mm); g, mature sclerotium (r : rhizomorph; bar = 2mm); h, transverse section of a mature sclerotium (t : horn-shaped trichoid; r : rind; c : cortex; m : medulla; bar = 10μm); I, synthesized ectomycorrhizas of *Acacia chisholmii* + *P.sudanicus* (bar = 1mm); J, transverse section of *Acacia chisholmii* + *P.sudanicus* ectomycorrhiza (m : fungal mantle; h : Hartig net; c : cortical cell; s : stele; bar = 20μm).

Mycorrhizal synthesis trials - Four replicates of ten tree species were inoculated with *P.sudanicus*,viz. *Acacia ampliceps*, *A.chisholmii*, *A.holosericea*, *A.mangium*, *Casuarina equisetifolia* (australian species), *Pinus caribaea v. hondurensis* (meso-american species), *Acacia albida*, *A.raddiana*, *A.seyal*, *Afzelia africana* (local species in Sene gal). After 6-7 months growth, brownish yellow ectomycorrhizas were observed on *A.chisholmii* (Fig.1i), *A.holose ricea* and *A.mangium*. Transverse sections showed a mantle and a typical Haertig's net (Fig.1j). The size of inoculated and non inoculated seedlings were not significatively different.

On *P.caribaea v.hondurensis*, *P.sudanicus* induced the formation of dichotomized short roots covered by a yellowish, 25-60µm thick mantle. Transverse sections showed that Hartig's net was not developped. Sclerotia of *P.sudanicus* were found associated with the ectomycorrhizal roots of the containe rized pines.Inoculated pines had an increase of 150% in height, 61% in stem diam. and 173% in total dry weight in regard of non inoculated seedlings.

No ectomycorrhizas occured on *Acacia ampliceps*, *A.albida*, *A.raddiana*, *A.seyal*, *Afzelia africana* and *Casuarina equisetifolia* showing a probable incompatibility of the fungal strain.

DISCUSSION

Phlebopus sudanicus is the first tropical bolete known to produce large amounts of sclerotia in pure culture. During their development, the sclerotia produced numerous droplets of exudates surrounded by a membranous sac which became wrinkled, resulting from evaporation of the liquid exudate. This phenomenon was also observed on exudates of *Sclerotium rolfsii* Sacc. (Christias, 1980). Sclerotia of *P.sudanicus* had a typical layered structure with a tough protective rind and a medulla containing energy-storage material. Sclerotia were able to survive, in definite conditions, for at least 4 months. These features are good prerequisites for the production and the sto rage of sclerotia as mycorrhizal propagules.

The mycorrhizal status of *P.sudanicus* is established for the first time. Paradoxally the african *P.sudanicus* seems incompatible with the local *Acacia* species and compatible with some australian *Acacia* species. Following field observations of Fisch (*in* Trappe, 1962) *Phlebopus (Boletus) portentosus* (Berk.& Br.)Boedijn, a related species of *P.sudanicus* is mycorrhizal on *Eucalyptus* spp.

Further investigations are needed to establish the list of poten tial host trees of *P.sudanicus*.

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