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Investigation of *Ampelomyces* sp. Associated with Twig Yellowing in Southern and Eastern Namibia Occurring on Namibian *Acacia erioloba*

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Acacia **tree species are the most common tree in Namibia. This is probably because they are better adapted to the arid conditions that predominate in Namibia.** ***Acacia karoo*****,** ***Acacia mellifera*****,** ***Acacia hebeclada*****,** ***Acacia erioloba*****,** ***Acacia luderitzii*** **and** ***Acacia hereroense*** **are some of the commonest species encountoured. The** ***Acacia erioloba*** **species (Camelthorn) is easily noticed for its large and shady canopy. Traditionally,** ***Acacia*** **species are used by several indigenous people to treat minor ailments, whereas nowadays most people grow it for its shade and it also grows to become large and beautiful scenic plant. In the recent years these trees have been attacked by yellowing dieback diseases, which is threatening their existence. The aim of this study was to investigate the identity of the causal agents of twig yellowing diseases dieback that was recently observed on** ***Acacia erioloba*** **species in southern and eastern Namibian farms. Identification of pathogenic agents is an important dedicated step to finding remedial ways to any phytopathological problem. Previous morphological work in our laboratory had shown that the causal agent was a fungus. In this study fresh diseased twig were sampled and pure single spore cultures were made. From these pure cultures DNA was extracted and used in an internal transcribed spacer (ITS) PCR. The PCR products were purified and sequenced. The sequences were used in BLAST searches to interrogate the Genbank and high sequence similarity of 99% was obtained with** ***Ampelomyces sp.*****. From this work we conclude that** ***Ampelomyces sp.*** **is associated with** ***Acacia erioloba*** **dieback and that confirmation by Koch's postulates will be the next step in order to determine the virulence and pathogenicity of the** ***Ampelomyces sp.*** **isolates that have been collected so far.**

Key words: *Acacia erioloba, Ampelomyces sp.* pathogen, dieback, internal transcribed spacer region.

Acacia species belong to the family of about 1500 dicotyledonous, herbaceous and woody plants distributed mainly in the tropics and subtropics. *Acacia* species are perennial shrubs or trees which grow to height of 12m. There are different common names for *Acacia* species depending on their indigenous communities e.g. Karroo thorn, Kikar, Cockspur thorn, and Camel thorn. The *Acacia erioloba* is known for its beautiful umbrella shape which provides shade and can harbor large nest of weaver birds.

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Acacia tree species have common uses both economically and medicinally for indigenous people. The Kwanyama people in northern Namibia use roots of *Acacia erioloba* to treat coughs, diarrhea and cold. The root is crushed then boiled and taken whilst hot. Another local tribe, the Hereros use leaves to cure bloody coughs and chest pains. In general, the *Acacia* species are also used to make remedies for headaches, pneumonia, diarrhea, eye inflammations and gynecological problems[1]. *Acacia karoo* and *Acacia erioloba* seeds are brewed and used as a substitute for coffee. A powder made from the bark of these trees is used by the Topnaar people as perfume for the body and homes and also used as firewood[2].

In recent years most of the *Acacia* trees have been observed to be infected by fungal species or agents causing disease symptoms like twig dieback[3, 4]. *Acacia* trees are a common feature of the Namibia landscape[5, 6] and are of economic, medical, industrial, and domestic uses. *Acacia* species are often found in association with fungus. Plant fungi can either be pathogenic or symbiotic. Most fungi are saprotrophic decomposers feeding on the waste products and dead remains, some fungi are parasitic and could cause serious disease symptoms and eventually death. The outcome of a host-parasite relationship depends on the pathogenicity of the parasite. The effects of fungal diseases are usually visible; some examples of the disease symptoms associated with fungal infection on *Acacia* trees are root rot. Phyllode rusts, heart rot, cankers, twig dieback and pod malformations are easily observed.

PCR technology is a relatively new technique that has many applications in recombinant DNA research. Molecular identification of fungal species can now be done easily with great rapidity and accuracy by sequencing of the specific DNA regions like the internal transcribed spacer region (ITS) of the ribosomal DNA isolated from fungi[7, 8].

The main objective of this study was to identify the fungal species associated with twig bright yellowing disease in *Acacia erioloba* that has been observed in many farms in Southern and Eastern districts of Namibia. By knowing the identity of such fungal pathogen, it enhances our understanding of the biological activities of such species thereby enabling us to develop control mechanisms to inhibit further spread of associated diseases *in vivo* and therefore conservation of *Acacia erioloba* in their natural habitats.

MATERIAL AND METHODS

Collection of samples

Fresh twigs of *Acacia erioloba* showing the yellowing symptoms (Fig. 1) were collected from Southern and Eastern districts of Namibia. The twigs were surface sterilized with a solution of 70% alcohol and rinsed three times with distilled water. The twigs were then cut into almost equal portions (4mm × 4mm) and then divided into 2 replicates of 10 portions.

Isolation and identification of fungus

The fungus was cultured from the collected twigs. Single spore of pure cultures of the fungus were grown on sterile MEA media in an incubator at 30°C for 5 days before sub-culturing. The DNA was extracted from fungal mycelium using a Fermentas DNA isolation kit.

PCR amplification of the ITS region of fungal DNA

The Go Green *Taq* Master Mix which contains all the ingredients needed for DNA amplification (dNTPs, *Taq* polymerase, $MgCl_2$, 10× PCR buffer) was used. The following reaction mixture was used: Go Green *Taq* Master Mix 12.5µl, sterile dH_2O 5.5µl, primer (working stock) for ITS 1&2 3.0µl each, and DNA solution 4.0µl. the mixture was then put in the PCR machine under the PCR amplification profile: an initial of 94°C for 4 minutes followed by 32 cycles of 94°C for 30 seconds; 30 seconds at 57°C for primers ITS1&2, 72°C for 1 minute, and then at 72°C for 10 minutes and holding at 4°C. PCR products (5µl were analyzed on a 1% agarose gel stained with ethidium bromide. The remaining PCR products were purified and sequenced by Inqaba Biotec industries in Pretoria using an automated Sequencer using BigDye Chemistry.

RESULTS AND DISCUSSION

Genomic DNA was successfully isolated from six fungal isolated and used in separates ITS-

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