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First Report of *Alternaria compacta* on *Acacia karoo* in Namibia

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Acacia **tree species are by far the most common tree in Namibia. This is probably because, they are better adapted to the arid and semi-desert conditions that predominate in Namibia.** ***Acacia karoo***, ***Acacia mellifera***, ***Acacia hebeclada***, ***Acacia erioloba***, ***Acacia luderitzii*** **and** ***Acacia hereroense*** **are some of the commonest one. The city of Windhoek green patches are naturally well decorated by the** ***Acacia karoo*** **species. Traditional** ***Acacia*** **species are used by several indigenous people to treat minor ailments. The aim of this study is to investigate the identity of the causal agents of twig dieback that was recently observed on** ***Acacia karoo*** **species in Windhoek and other areas of Namibia. 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 belonged to the fungal genus** ***Alternaria*****. 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** ***Alternaria compacta*****. From this work we conclude that** ***Alternaria compacta*** **is associated with** ***Acacia karoo*** **dieback and that confirmation by Koch's postulates will be the next step in order to determine the virulence and pathogenicity of the** ***Alternaria*** **isolates that have been collected so far.**

Keywords: *Acacia karoo*, *Alternaria compacta*, pathogen, dieback, internal transcribed spacer region.

Acacia tree species are by far the commonest floral feature of the Namibian landscape and have economic and cultural uses. The *Acacia erioloba* is known for its beautiful umbrella shape which provides shade and can harbour large nest of weavers. These tree species have common uses both economically and a wide range of uses for indigenous people. The Kwanyama people in the north of the country use roots of the *Acacia erioloba* to treat cough, 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 are used by the Topnaar people as perfume for the body and homes and as firewood[2]. The *A. karoo* is a source of an edible gum used to cure mouth ulcers and oral thrush and bark is used in the leather industry for tanning and dying leather.

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The *Acacia karoo* species, that naturally decorate the green patches in the Windhoek city (capital city of Namibia), have recently been observed to be infected by a fungal agent that is causing twig dieback and eventual death. In general, fungi are known to cause enormous plant damage such as potato blight in Ireland in the mid-nineteenth century, which resulted in famine and death[3]. Fungal pathogens through their spores can be spread in several ways including wind, water and insect vectors[4]. The rate of spread depends on the susceptibility of the plant and the virulence of the fungal strains. Immediately when there is contract between the susceptible host and the fungal pathogen the infection starts, the effects however remain localized until the tissue have been penetrated by mycelia[3]. The nature of invasion will depend on the type of fungi causing it. Some fungi are highly invasive, colonizing all tissues and others are tissue-specific they invade xylem vessels especially vascular wilt fungi. These disrupting physiological functions of the plant and leading to eventual death. Effects of fungal diseases are usually visible and some examples of these diseases are cankers, leaf blight, needle cast, dieback[5] and rusts[6].

The objective of this study was to determine the identity of the fungal agents associated with die back of *Acacia karoo* in Namibia and in order to do that a molecular technique called ITS–PCR was used. Internal transcribed spacers[7] (ITS) are regions found in sequences of genomic ribosomal DNA of eukaryotic cells. There are several known types such as ITS1, ITS2, ITS3 and ITS4. The ITS1 and ITS2 regions are separated by the 5.8S rRNA gene. These regions are situated between 18S and 28S rRNA gene[7]. ITS sequences can be used for fungal identification[7, 8] performing BLAST searches to interrogate Gene bank databases. Basic Local Alignment Search Tool (BLAST) is a heuristic method to find the highest scoring locally optimal alignments between a query sequence and a target sequence in a database. A query sequence which is the sequence requiring identification can be compared to an existing sequence or target sequence in the international DNA databases. Variations within the sequences of the ITS regions make them ideal for further use in phylogenetic and populations genetic studies[8].

MATERIAL AND METHODS

Fungal cultures and DNA extraction

Portions of die backed *Acacia karoo* trees were collected from many locations in Windhoek and other parts of Namibia. Smaller portions of about 5mm^2 were surface sterilized and used in primary isolations. Potato Dextrose Agar (PDA) (2%) was used to make the primary isolations. Malt extract agar (MEA) 2% was used to do sub cultures and the single spore pure cultures. The pure cultures were then grown in Malt extract both (2%) for a period of 10 days at room temperature. The fungal mycelium was filtered with Whatman No.10 filter and allowed to air dry in a laminar flow hood for two days. After that the mycelia was used to extract fungal DNA used a Fermentas kit following the manufacturers' instructions.

ITS–PCR and Sequencing and Sequence Analysis

The DNA obtained from six fungal pure isolates was analysed on a 1% agarose gel stained with ethidium bromide, the concentration was estimated with a ë DNA standard. Subsequently the DNA samples from the six isolates was diluted to 10çg/µl to be used as templates in an ITS- PCR amplification. The GoGreen *Taq* Master Mix (Promega) which contains all ingredients needed for DNA amplification (dNTPs, *Taq* polymerase, $MgCl_2$, 10× PCR buffer) was used. The following reaction mixture was used: GoGreen *Taq* Master Mix 12.5 µl, 5µM ITS1 primer 2 µl, ITS4 primer 2 µl, sterile double distilled water 4.5 µl and DNA template (10çg). 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 at 30 seconds; 30 seconds at 62°C, and 1 minute at 72 °C then a final extension step of 10 minutes at 72 °C and holding at 4 °C. PCR products (5%) were analyzed on a 1% agarose gel stained with 0.5% ethidium bromide. The remainder of ITS PCR products were purified and sequenced by a commercial company in an automated sequencer using the Big-dye chemistry by a commercial company using forward and reverse primers for each of the six isolates. The forward and reverse sequence of each isolate was aligned appropriately to obtain one complete sequence using BioEdit program. The resulting six sequences were identical and only one of them was

chosen for use in BLAST searches at the NCBI Genbank.

RESULTS

Genomic DNA was successfully isolated from six fungal isolates and used in separates ITS-PCR amplifications. The DNA sequences (493bp long) obtained from the six isolates were identical and only one of them was chosen for BLAST searches. The BLAST searches and local pairwise sequence alignment revealed a 99% sequence similarity (Fig. 1) of that of the fungus *Alternaria compacta*, a rather rare pathogen known to infect castor bean[9]. The fungus isolated in this study is therefore called an isolate of *Alternaria compacta* and the ITS sequences are deposited in the Gen bank with accession number: bankit1018278 (EU159412).

This result confirmed previous findings from our lab that that fungus associated with the *Acacia karoo* dieback belong to the *Alternaria* genus from morphological examinations.[10] The current study is a first report of *Alternaria compacta* on *Acacia karoo* in Namibia. Future work with this fungus involves satisfying Koch's postulates, and conducting pathothencity and virulence trials wit the various isolates of *Acacia compacta* that have been collected so far.

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Query          37     TGAATTATTCACCCGTTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCCACCA  96
                      ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
A.compacta     77     TGAATTATTCACCC-TTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCCACCA  135
                      ************** ******************************************
Query          97     CTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAATAATTAC  156
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A.compacta     136    CTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAATAATTAC  195
                      ************************************************************
Query          157    AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA  216
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A.compacta     19     AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA  255
                      ************************************************************
Query          217    AGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTT  276
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A.compacta     256    AGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTT  315
                      ************************************************************
Query          277    TGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTG  336
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A.compacta     316    TGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTG  375
                      ************************************************************
Query          337    TTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCT  396
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A.compacta     376    TTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTCGCCTTAAAGTAATTGGCAGCCGGCCT  435
                      ************************************************************
Query          397    ACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTA  456
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A.compacta     436    ACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTATCAGCAAAGGTCTAGCATCCATTA  495
                      ************************************************************
Query          457    AGCCTTTTTTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATA  516
                      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A.compacta     496    AGCCTTTTTTTCAACTTTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATA  555
                      ************************************************************
Query          517    TCAATAAGGGGGA  529
                      |||||||| ||||
A.compacta     556    TCAATAAGCGGGA  568
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Fig. 1. Local pairwise sequence alignment of the ITS regions showing 99% similarity to *Alternaria compacta*. Asterisks indicate identity of bases. The ITS sequence of the fungus understudy is indicated in grey.

REFERENCES

1. Von Koenen E. Medicinal, Poisonous & Edible Plants in Namibia; 2001; p.67-70.
2. Van Wyk B. and Gericke N. People's plants: A guide to useful plants in Southern Africa. 2000.
3. Webster J. Introduction of Fungi, 1994; 208-211.
4. Noble WC and Naidoo V. *Microorganisms and Man*. 1979.
5. Bester FV. Involvement of pathogenic fungi in the natural dieback of blackthorn in Namibia, Part1-6. *Spotlight on Agriculture.* 2004; 82.
6. Palmer E and Pitman N. Trees of Southern Africa, 1992; 706.
7. Henry T, Iwen PC and Hinrichs SH. Identification of *Aspergillus* species using internal transcribed spacer 1and 2. *Journal of Clinical Microbiology*, 2000; **38**(2): 1510-1515.
8. Fox RTV. Principles of Diagnostic Techniques in Plant Pathology. 1993.
9. Agriculture Handbook 165. Index of plant diseases in the United States. USGPO. Washington. 1960.
10. Uushona H. Isolation and characterization of fungal agents associated with A*cacia karoo* twig dieback in dorado valley in Windhoek, Final year Project Dissertation, 2005.