PROMOTING CAMEL THORN GROWTH AND HEALTH THROUGH BENEFICIAL MICROBIAL INTERACTIONS

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Introduction

The Camel thorn tree, *Vachellia erioloba*, (previously known as *Acacia erioloba*), is a tree that is native to the southern part of Africa (Orwa *et al*., 2009; Kyalangalilwa *et al*., 2013). The Camel thorn tree forms part of two natural forests situated near the town of Kathu in the Northern Cape Province (Powell, 2005). The tree has great economic and agricultural importance when it has grown to a large size (Barnes, 2001). In the early 1990s it was observed that the forest near Kathu was declining, but no cause was identified. In 2008 an investigation was done and Cerambicid beetles and fungi were identified as the cause of the decline (Internet 1). These attacks and the removal of the trees due to mining and building activities has also led to the decline in the population. Mining activities have led to the concern of heavy metal contamination and depletion of the groundwater table (SA Forest Magazine, 2011).

Mycorrhizal fungi are common soil fungi which promote the growth and health of their plant host by interacting with the roots and forming a secondary fungal network (Willey *et al*., 2011). This network facilitates the uptake of nutrients and moisture from the soil, which are exchanged for carbohydrates formed by the host plant's photosynthetic activities. Microorganisms have also been identified that are beneficial to the symbiotic relationship between the mycorrhizal fungi and the host plant (Garbaye, 1994; Hayes and Krause, 2011). These organisms are found within the rhizospheric soil surrounding the plant, which can contribute to the health and growth of the plant (Hayes and Krause, 2011). This study aims to determine the role of mycorrhizal fungi and associated bacteria in improving the growth and health of *V. erioloba* seedlings.

Methods

Soil and root samples were collected at three locations in the area of Kathu, Northern Cape province, South Africa. Soil samples will be sent for nutrient analysis. Mycorrhizal spores were extracted from the soil using the wet sieving and decanting method. The roots were separated from the soil, stained and examined under a microscope for mycorrhizal colonisation. Serial dilutions were made from the collected soil and rhizospheric bacteria

were isolated using selective media to ensure isolation of *Bacillus*, pseudomonads and actinobacteria. The serial dilutions were also plated on nutrient agar. Single colonies were gram-stained and discontinuously streaked until pure cultures were obtained. The cultures were then tested for nitrogen fixation, phosphate solubilisation, siderophore production and indole acetic acid production. DNA extraction was performed for all the isolates, followed by PCR with rp2 and fD1 primers. PCR products will be sent to Inqaba Biotechnologies for sequencing, which will be submitted to GenBank for comparative identification. After the bacterial isolates have been identified, selected isolates will be used in a pot trial with Camel thorn seedlings to determine the effects of the rhizobacterial isolates, with and without mycorrhizal fungi, on seedling growth. An initial pot trial was conducted to determine the effects of different cadmium (Cd) concentrations on camel thorn seedling development. Cd concentrations in the biomass will be determined using a nitric acid extraction protocol and analysis by ICP-EOS using a Thermo ICAP-6300 spectrometer.

Current progress

A total of 57 bacterial isolates were obtained. These were tested for nitrogen fixation, phosphate solubilisation, siderophore and indole acetic acid production. All the isolates produced siderophores and indole acetic acid. A total of 15 isolates showing positive results for all tests coupled with production of siderophores and indole acetic acid in high concentrations were selected for identification. Selected bacterial isolates are in the process of being molecularly identified. Roots are being assessed for mycorrhizal colonisation. Table 1 provides some preliminary seedling growth data when subjected to Cd application. The roots developed faster than the shoots, which could be due to the stress caused by the heavy metals. The roots of the un-inoculated seedlings that were treated with Cd had a higher biomass than the roots of inoculated (mycorrhizal treatment) seedlings, except for the un-inoculated seedlings that did not receive Cd. Further analysis of this data is currently in progress. Figure 1 shows mycorrhizal structures within roots.

Table 1: The root:shoot dry weight ratio of the Camel thorn seedlings at different cadmium concentrations

Cd concentrations (ppm)	Mycorrhizal Inoculated	Un-inoculated
Control	1.06 : 1	1 : 1.7
100	1.03 : 1	1 .37 : 1
50	1.26 : 1	1.39 : 1
25	1 : 1.16	1.17 : 1

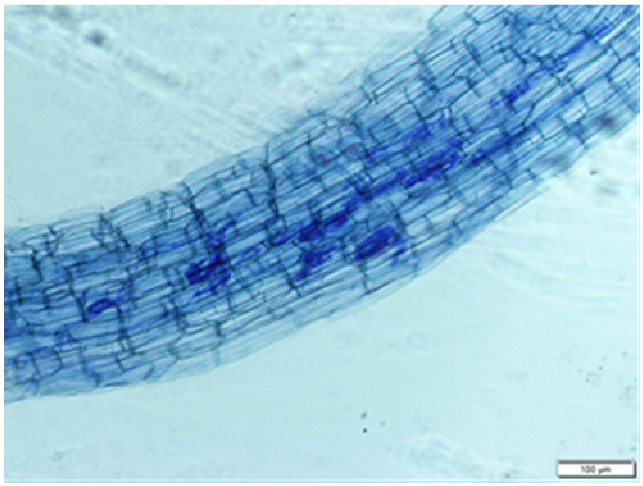

Figure 1: Mycorrhizal colonisation structures within roots of *V. erioloba*

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