



Review

Rhizobial diversity and function in rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia* spp.) plants: A review

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ABSTRACT

Aspalathus linearis (rooibos) and *Cyclopia* spp. (honeybush) are leguminous plants endemic to the fynbos region of South Africa. These plants have become popular for their use as herbal teas and thrive in the acidic, nutrient poor soils of the fynbos region. Plants in this region rely on rhizobia to fix nitrogen and supply them with other important nutrients such as phosphorous, in exchange for a carbon source. This review focuses on the species diversity of rhizobia associated with *A. linearis* and *Cyclopia* spp. plants. Genera that are regularly isolated from rooibos and honeybush root nodules include *Rhizobium*, *Burkholderia*, *Mesorhizobium* and *Bradyrhizobium*. These species produce bioactive compounds that directly or indirectly affect growth of leguminous plants.

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1. Introduction

The Cape Floristic Region (CFR) is endemic to the south-western and western regions of South Africa and is characterized by a high floral diversity. Legumes in the CFR contribute 10% of the endemic plant species and include two commercially important species, *Aspalathus linearis* (rooibos) (Fig. 1) and *Cyclopia* spp. (honeybush) (Fig. 1) (Garau et al., 2009; Howieson et al., 2013; Lemaire et al., 2015a). These two legumes

belong to the family Fabaceae, where *Aspalathus* belongs to the Crotalariaeae tribe and *Cyclopia* to the Podalyrieae tribe (Joubert et al., 2008). *Aspalathus linearis* and some *Cyclopia* spp. (*C. subternata*, *C. intermedia*, *C. sessifolia* and *C. longifolia*) are used to produce herbal teas known as Rooibos and Honeybush, respectively. One of the main reasons for the popularity of these teas is that it is free of caffeine and low in tannins (Joubert et al., 2007).

Aspalathus linearis is indigenous to the Cederberg region in the Western Cape of South Africa (Fig. 2). This region has winter rainfall and only receive an average of 200 mm rain per year, and is, therefore, classified as an arid region (Hoffman et al., 2009). *Cyclopia* spp. can be

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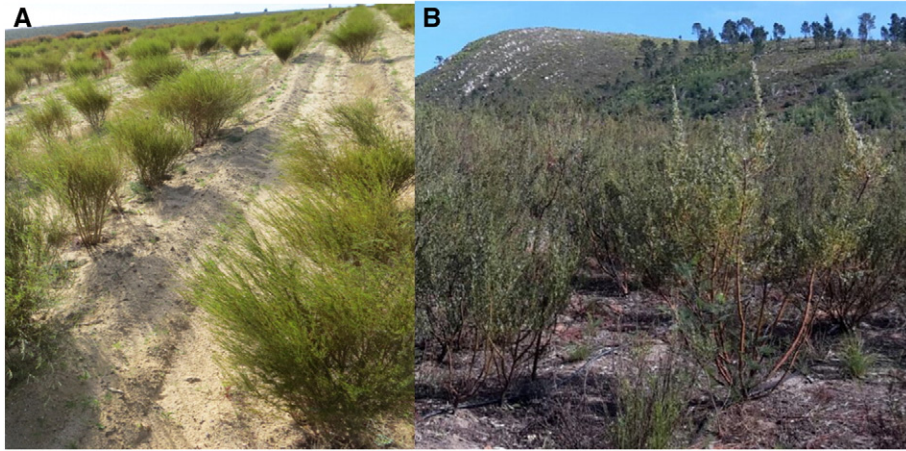


Fig. 1. (A) *Aspalathus linearis* (rooibos) and (B) *Cyclopia subternata* (honeybush sp.) in plantations (photo credit: Jonathan Kriel and Armand van Wyk).

found on the southern slopes of mountainous and coastal regions of the Eastern and Western Cape provinces of South Africa (Fig. 2). Some honeybush species occur in a widespread region, while others can only be found in a small geographical area. Honeybush that prefer the mountainous region can be found on the wetter, cooler southern slopes. Honeybush plants have successfully adapted to the natural occurring fires associated with the fynbos biome through developing two survival strategies, namely resprouters and seeders (Joubert et al., 2011). This differs from the commercially used Rooibos which is a seeder (Van Heerden et al., 2003). Power et al. (2011) suggest that the seeders are likely to have higher nutrient requirements than resprouters, due to more resources allocated for reproduction. The seeder plants have a hardened seed coat, consequently scarification or fire is usually required for effective germination to take place (Joubert et al., 2011).

Soils in which *Aspalathus* and *Cyclopia* species grow are acidic and low in nutrients, especially in nitrogen and phosphorus concentrations (Richards et al., 1997; Muofhe and Dakora, 2000; Power et al., 2010; Maistry et al., 2013). The availability of nutrients is one of the limiting factors for plant growth in this region (Witkowski and Mitchell, 1987; Vardien et al., 2014). To overcome this limitation, efficient nutrient exchange between plant roots and the soil environments as well as interactions with microorganisms occur in the rhizosphere (Dakora and Phillips, 2002). In this environment, microorganisms that form symbiotic interactions with plant roots play a critical role in nutrient uptake for plants (Richards et al., 1997; Vardien et al., 2014). Bacteria and fungi can

provide plants with important nutrients such as nitrogen and phosphates. Some Gram-negative, rod shape bacteria, collectively known as rhizobia, have the ability to form root nodules with some legumes (Prell and Poole, 2006; Peix et al., 2015). These bacteria aid in nitrogen fixation for the plant in exchange for carbon sources (Ashraf et al., 2013). Apart from the positive effect rhizobia have on plant growth through nitrogen fixation, some members of this group of bacteria also produce plant growth promoting compounds. Rhizobia often have a positive effect on plant growth and are known as plant growth-promoting rhizobacteria (PGPR). Although some PGPR form root nodules with the host plant, the majority are free-living soil bacteria and do not form root nodules (Hayat et al., 2010). Some rhizobia are able to form root nodules, but do not produce plant growth-promoting compounds. There are many different species of PGPR that produce plant growth promoting factors, albeit in different concentrations (Rosenblueth and Martínez-romero, 2006). Consequently, rhizobia that usually produce the highest concentrations of plant growth-promoting factors, are often used in seed inoculations to improve growth of commercial crops (Akladios and Abbas, 2014; Bashan et al., 2014). Some rhizobia can have non-nodulating and often endophytic relationships with non-leguminous plants. These associations can have a positive effect on plant growth and nutrient uptake (Biswas et al., 2000; Mishra et al., 2012). However, the production of metabolites by rhizobia and their ability to colonization plant roots, are significantly influenced by various abiotic and biotic factors (Ciccillio et al., 2002).

The most abundant and frequently isolated rhizobial species in this region include members from the genera *Burkholderia* (Garau et al., 2009; Gyaneshwar et al., 2011; Beukes et al., 2013; Howieson et al., 2013; Lemaire et al., 2016a, 2016b) *Rhizobium* (Lemaire et al., 2015a) and *Mesorhizobium* (Lemaire et al., 2015a). All legume nodulating bacteria belong to the phylum Proteobacteria, although they do not form a monophyletic group (Williams et al., 2010; Williams and Kelly, 2013; Peix et al., 2015). Initially, rhizobia was only considered to belong to the class α -Proteobacteria (Peix et al., 2015). This changed in 2001 when Moulin et al. (2001) detected nodule-like structures that did not fix nitrogen on the roots of *Macroptilium atropurpureum* after their inoculation with two strains of *Burkholderia*, and when Chen et al. (2001) reported the isolation of putatively nodulating strains of *Ralstonia* (Cupriavidus) from *Mimosa pudica* nodules in Taiwan. This was followed by their study in 2005 that proved the ability of *Burkholderia* to form symbiotic relationships with legumes in their studies on *Mimosa* species (Chen et al., 2005a, 2005b). Some rhizobia species from subgroups α - and β -Proteobacteria have megaplasmids that contain the symbiosis genes. Phylogenetic analysis showed that there is a smaller difference in the *nod* genes of α - and β -Proteobacteria than the difference between the 16S rRNA genes from α - and β -Proteobacteria (Moulin et al., 2001).

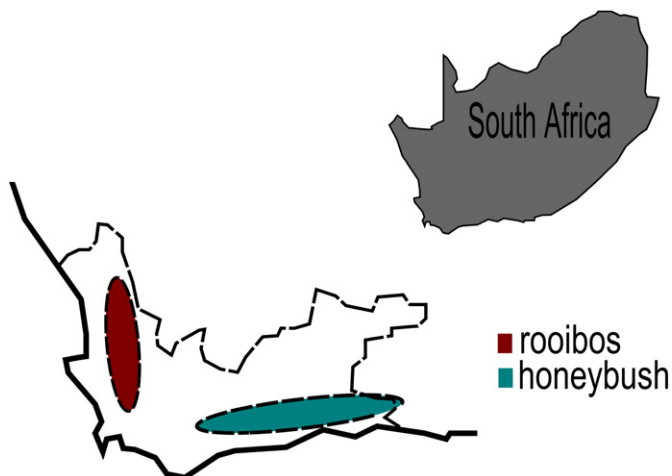


Fig. 2. Map of geographical distribution of rooibos (red) and honeybush (green) of the Eastern and Western Cape.

The presence of *nod* genes in α - and β -Proteobacteria suggests that it probably occurred through horizontal gene transfer. This is, however, only true for papilionoid-nodulating *Burkholderia* spp. In contrast, the *nod* genes from the *Mimosa* nodulating *Burkholderia* are different to the genes of α -rhizobia. This suggests that the symbiosis genes diverged over a long period of time within *Burkholderia* (Bontemps et al., 2010).

Agricultural practices usually result in a decline in microbial diversity and structure as observed in other crops (Griffiths et al., 2001). However, it was found that the bacterial diversity and species richness in the rhizosphere of commercial and wild honeybush plants are highly similar. This is most likely because land is not cleared for honeybush plantations as is the case with other cropping systems (Postma et al., 2016). A very similar picture emerged from studies on the rooibos rhizosphere communities (Postma et al. submitted). On the other hand, a significant seasonal effect was found and the wet and dry seasons had different community structures in both the honeybush and rooibos systems (Postma et al., 2016). These seasonal changes in the microbial community suggest that bacteria react differently to environmental changes (Postma et al., 2016). In this review, we explore the current knowledge on the biodiversity and bioactivity of rhizobia that form root nodules with the two commercially important fynbos plants, rooibos and honeybush.

2. Biodiversity

Due to the high diversity of legumes that grow in the CFR, it is hypothesized that South Africa can potentially be considered as one of the diversity hotspots for rhizobial species as soils of the CFR may have a high diversity of rhizobial species. This diversity could then possibly be attributed to the high diversity of legumes that grow in different environmental conditions in the CFR (Slabbert et al., 2010; Lemaire et al., 2015a). Globally, the biodiversity of rhizobia species is enormous. However, identifying these microorganisms is a complicated process as the identification of rhizobia cannot be based solely on 16S rRNA sequence analysis. Housekeeping genes such as *recA* and *atpD*, as well as the symbiosis genes *nodA* and *nifH* needs to be analyzed to accurately identify rhizobia (Lemaire et al., 2015a). Lemaire et al. (2015a, 2016a) found that the *nod* gene of *B. tuberum* STM678 is ubiquitous in *Burkholderia* and this strain is able to form root nodules with a wide range of legumes in the CCR, including honeybush. These housekeeping genes are used to determine their phylogenetic relationships whereas symbiosis genes are used to study symbiotic traits and evolution (Lemaire et al., 2015a). The range of legumes that some rhizobia are associated with is also expanding as knowledge becomes available.

The most important genes that distinguish rhizobia species from other soil bacteria is the presence of *nod* and *nif* genes. The *nif* genes encode for the nitrogenase reductase enzyme that is involved in fixing atmospheric nitrogen. This gene is also found in free-living nitrogen fixing bacteria (Gaby and Buckley, 2012). The *nod* genes encode for Nod factors that are responsible for root nodule formation. The *nodA* gene is one of the most studied genes of rhizobia, because it determines the structure of the Nod factor and it can determine host specificity (Gerding et al., 2012). These genes can also be used for phylogenetic analysis to distinguish between the different bacterial species. It is found that most fast-growing *Rhizobium* spp. have megaplasmids (pSyms) that contain the *nod* and *nif* genes. However, not all rhizobia have these plasmids but carry the symbiotic genes on their chromosome, such as the slow-growing *Bradyrhizobium* and *Mesorhizobium* (Nap and Bisseling, 1990; Van Rhijn and Vanderleyden, 1995). A study done by Martínez-Aguilar et al. (2008) found that some *Burkholderia* species also have the *nifH* gene on plasmids, while other species have the gene on their chromosome. De Meyer et al. (2016) found that mimosoid-nodulating *Burkholderia* have *nod* genes on a symbiotic plasmid and that the *nod* genes differ from the papilionoid-nodulating *Burkholderia* which have the *nod* genes in the chromosome. This suggests that the mimosoid-nodulating and papilionoid-nodulating

Burkholderia did not obtain the symbiosis gene from the same source which can also be observed in the *nod* gene sequences.

2.1. Fynbos rhizobia diversity

Leguminous tribes in the fynbos have a high preference to form symbiotic relationships with specific rhizobia. The tribe Psoraleeae has a preference for *Mesorhizobium*, while Podalyrieae has a preference for *Burkholderia* (Lemaire et al., 2015a). *Bradyrhizobium* and *Rhizobium* spp. have also been isolated from the fynbos legumes (Kock, 2004). A few novel *Burkholderia* species have recently been isolated from fynbos legumes. These novel species include: *B. rhynchosiae*, *B. sprentiae*, *B. kirstenboschensis*, *B. dilworthii* and *B. aspalati* (De Meyer et al., 2013a, 2013b, 2014; Mavengere et al., 2014; Steenkamp et al., 2015). However, an increased sampling effort is needed to fully determine the rhizobial diversity in the fynbos region.

2.2. *Aspalathus* rhizobia diversity

Most rhizobia associated with rooibos root nodules belongs to *Mesorhizobium* (Elliott et al., 2007). *Mesorhizobium* is also a PGPR that can produce indole acetic acid (IAA), ACC deaminase and fix nitrogen for the plant (Verma et al., 2013; Lemaire et al., 2015b). Lemaire et al. (2015a) showed that *Mesorhizobium* spp. can co-exist with *Burkholderia* spp., because both genera occur in similar soil types with a low pH. Furthermore, Elliott et al. (2007) conducted a study to determine whether selected species of *Aspalathus* and *Cyclopia* could be nodulated by the same *B. tuberum* STM678 strain. The results showed that *B. tuberum* was only able to nodulate the *Cyclopia* spp., and could not nodulate any of the five *Aspalathus* spp. tested, but in a recent study by Lemaire et al. (2016a) this same strain has been shown to nodulate *A. carnosa*.

2.3. *Cyclopia* rhizobia diversity

In contrast to *Aspalathus* spp. that are associated with a diverse range of rhizobia, the tribe Podalyrieae (that include *Cyclopia* spp.) has a distinct preference to form root nodules with *Burkholderia* spp. (β -Proteobacteria) (Elliott et al., 2007; Gyaneshwar et al., 2011; Beukes et al., 2013; Lemaire et al., 2015a). Only a few α -Proteobacteria were isolated and identified as *Bradyrhizobium* species (Kock, 2004). A study done by Postma et al. (2016) also found that Proteobacteria dominated the rhizosphere of *Cyclopia* plants with Rhizobiales and Burkholderiales the most abundant orders in the Proteobacteria.

Burkholderia is a rod shaped, Gram-negative, non-sporulating bacterium (Sessitsch et al., 2002). This genus contains pathogenic species that can infect humans, animals and plants. Other *Burkholderia* spp. are able to associate with plants and can be beneficial for plant growth (Suárez-Moreno et al., 2012). *Burkholderia* species can produce IAA, ACC deaminase and it is also able to solubilize phosphates (Palaniappan et al., 2010). One reason for the dominance of *Burkholderia* species in fynbos soil is most likely due to their adaptation to nutrient poor and acidic soils (Garau et al., 2009). This finding was confirmed by Howieson et al. (2013) and Lemaire et al. (2015a).

A study done by Beukes et al. (2013) found that the *Burkholderia* that are associated with *Cyclopia* spp. have unique *nifH* and *nodA* genes. The *nodA* gene sequences from *Burkholderia* species isolated from South Africa, was closely related to the α -Proteobacteria, *Methylobacterium nodulans*. It is thought that the distribution of nodulating *Burkholderia* species is dependent on environmental factors rather than a specific host legume. *Burkholderia* spp. isolated from the fynbos had different *nod* genes than the *Burkholderia* spp. isolated from *Mimosa* spp. in South America (Garau et al., 2009; Howieson et al., 2013; Liu et al., 2014; De Meyer et al., 2016). Studies done by Lemaire et al. (2015a, 2015b, 2016a, 2016b) showed that *Burkholderia* nodulate a wide range of legumes in the fynbos. One of the most dominant *Burkholderia* species isolated was *Burkholderia tuberum* and it was found to only

nodulate *Cyclopia* spp. and forms indeterminate nodules (Elliott et al., 2007). *Burkholderia tuberum* are able to efficiently nodulate more than one *Cyclopia* species in acidic and nutrient poor soils (Spriggs and Dakora, 2009). Different *Burkholderia* strains, however, do not have the same plant growth-promoting effect on the same *Cyclopia* species, but inoculation of *Cyclopia* seeds with *Burkholderia* strains, showed a significant increase in plant growth, compared to the un-inoculated plants (Spriggs and Dakora, 2009).

Different types of rhizobia are found in rooibos and honeybush plants, and it may seem that the diversity of rhizobia is higher for rooibos plants than honeybush plants. Either α - or β -Proteobacteria tend to nodulate *Aspalathus* spp. (Lemaire et al., 2015a). However, *Cyclopia* spp. as well as most other members of Podalyriaceae, are exclusively nodulated by *Burkholderia* spp. (Elliott et al., 2007; Gyaneshwar et al., 2011; Beukes et al., 2013; Sprent et al., 2013; Lemaire et al., 2015a, 2016a). An increased sampling effort is needed to effectively determine the species and relative abundance of bacterial species that are able to form root nodules with rooibos and honeybush roots. More than one species of rhizobia can be isolated from a root nodule. Palaniappan et al. (2010) isolated two different colony types, on yeast mannitol agar, from the same root nodule. Studies have shown that non-rhizobial bacteria have also been isolated from root nodules and may have a positive effect on plant growth (Bai et al., 2002; Rajendran et al., 2008). However, the extent and function of the non-rhizobial bacteria is still unknown.

Selected bacteria, that are isolated from rooibos and honeybush root nodules, can produce plant growth-promoting properties and are classified as PGPR (Palaniappan et al., 2010; Verma et al., 2013). Some of these bacteria are adapted to live in acidic and nutrient poor soils, that make them effective in infecting and forming root nodules in rooibos and honeybush plants (Lemaire et al., 2015a). This is however, another area of research that remains largely unexplored.

3. Bioactivity

Rhizobia produce bioactive compounds that affect plant growth. Some rhizobia species isolated from rooibos and honeybush plants such as *Burkholderia*, *Mesorhizobium* and *Bradyrhizobium* are able to produce compounds with plant growth-promoting properties (Palaniappan et al., 2010; Verma et al., 2013).

3.1. Nitrogen fixation and root nodules

Nitrogen fixing makes rhizobia unique and also has a positive effect on plant growth. Biological nitrogen fixation (BNF) is affected by abiotic factors, especially drought. During drought, there is less infection of the legume by rhizobia and the nodule development and growth is affected negatively. This directly affects nodule function (Arrese-Igor et al., 2011) and the growth of rooibos and honeybush plants. Because the plants are rain-fed, it receives little water during the summer months. This corresponds with a study done by Lotter et al. (2014) where the rooibos growth rate declined by 50% during drought. This decline in growth is due to the negative effect on BNF and the plants have to rely on the available nitrogen in the soil. During drought, the C:N ratio

increases in nutrient poor ecosystems such as the fynbos. The drought had direct effects on the functioning and activity of the root nodules (Lotter et al., 2014). The reason for the lower levels of BNF is that the water limitation decreases photosynthesis and lowers the level of photosynthates needed by the bacteria for nitrogen fixation.

Studies have been done to determine the percentage of nitrogen derived from atmospheric nitrogen, through nitrogen fixation, found in rooibos and honeybush plants. The amount of nitrogen fixed has also been determined (Table 1). Interestingly, the natural honeybush plants had more consistent values of nitrogen derived from atmospheric nitrogen compared to the managed plants. Some species had similar values in the managed plants and the natural sites, but there is variation of values at different sites (Muofhe and Dakora, 1999; Spriggs and Dakora, 2009). Maseko and Dakora (2015) found that annual harvesting of the plants led to a decrease in N_2 fixation. Removal of the shoots affects the oxygen diffusion to the root nodules and leads to a decrease in nitrogenase activity (Maseko and Dakora, 2015).

3.2. Determinate and indeterminate root nodules

Root nodules differ in shape and size based on the bacteroid population and can be classified into two groups, indeterminate (Fig. 3) or determinate nodules (Sprent et al., 2013). Indeterminate nodules contain a heterogeneous population of bacteroids. These nodules undergo continuous cell division and this results in different developmental stages as the root nodules elongate. Determinate nodules contain a homogeneous population of bacteroids as differentiation of the infected cell is synchronized and the nodules have a short live-span of only a few weeks. Determinate root nodules are often more rounded in shape than indeterminate root nodules (Ferguson et al., 2010). Although both rooibos and honeybush plants form indeterminate root nodules (Elliott et al., 2007; Gyaneshwar et al., 2011; Lemaire et al., 2015a), rooibos root nodules are branched whereas honeybush root nodules are less branched (Sprent et al., 2013). Although both rooibos and honeybush nodules are generally indeterminate in their morphology, they differ in their internal structure. *Cyclopia* nodules are typically indeterminate with an infected zone containing both infected and uninfected (interstitial) cells (Elliott et al., 2007), whereas *Aspalathus* nodules are crotalaroid, and so their infected zone does not contain any uninfected cells (Lemaire et al., 2015a, 2015b).

3.3. The effect of pH on rhizobia and plants

The soil pH also has an effect on root nodule functioning. Morón et al. (2005) found that an acidic environment lead to increased production of Nod factors by *R. tropici*. Higher soil pH is often associated with lower concentrations of Nod factors that are produced. The optimum pH for rhizobia is 5.5, which showed significant differences in Nod factors and plant growth when compared to higher pH. Rhizobia strains that are sensitive to pH, might be unable to regulate their internal pH. The regulation of cytoplasmic pH might be a requirement for growth under acidic conditions. This may be beneficial for rhizobia that prefer an acid growth environment. Low pH affects the interaction between the micro-symbiont and the legume host, as well as growth of the

Table 1

Amount of nitrogen fixed under field conditions and the % N derived from atmospheric nitrogen in rooibos and honeybush plant.

Species	Amount of nitrogen fixed under field conditions (g.plant ⁻¹)	Highest %N derived from atmospheric nitrogen		Reference
		Field trial	Natural sites	
<i>A. linearis</i>	3.80 ± 0.3	52.0 ± 3.0	N/A	Muofhe and Dakora (1999)
<i>C. subternata</i>	N/A	100 ± 41.1	69.7 ± 4.1	Spriggs and Dakora (2009)
<i>C. genistoides</i>	N/A	100 ± 17.5	100 ± 7	Spriggs and Dakora (2009)
<i>C. intermedia</i>	N/A	62.8 ± 16.1	100 ± 16.5	Spriggs and Dakora (2009)
<i>C. maculata</i>	N/A	89.0 ± 3.2	100 ± 42.4	Spriggs and Dakora (2009)
<i>C. sessiliflora</i>	N/A	100 ± 27.7	100 ± 17.1	Spriggs and Dakora (2009)

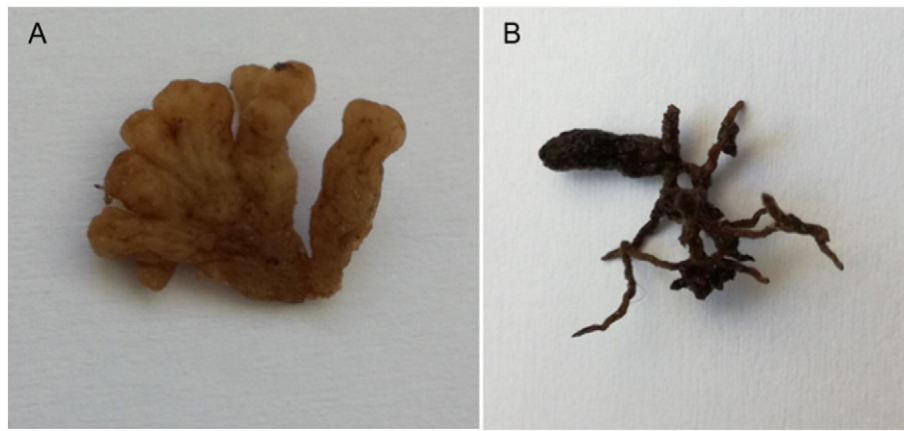


Fig. 3. Different indeterminate rooibos (A) and honeybush (B) root nodules (photo credit: Casper Brink).

plant (Dakora, 2012). Nitrogen fixing rhizobia isolated from the nutrient poor and acidic soils from fynbos are tolerant to low pH levels and this indicates that they have adapted to their environment.

Burkholderia and *Mesorhizobium* spp. have been isolated from acidic soils with the pH ranging from 3.18 to 6.7 (Lemaire et al., 2015a). Other studies also confirm that *Burkholderia* are able to grow over a pH range from 4.5 to 8 without the pH effecting growth (Howieson et al., 2013; Liu et al., 2014), while *Mesorhizobium* spp. did not grow at a pH below 5 (Howieson et al., 2013). The bacteria are tolerant to these environments and have adapted to survive in these conditions (Dakora, 2012).

Rooibos plants can control the pH of the rhizosphere and elevate the pH from 4 to 6.8 to overcome the growth inhibition of the symbiotic microorganisms (Muofhe and Dakora, 2000). During nitrogen fixation, the root nodules produce protons, resulting in a higher rhizosphere pH. At night the pH lowers in the rhizosphere. This could be because nitrogen fixing is an energy demanding process and can be linked to the photosynthetic activity of the plant. As the photosynthetic activity decreases at night, nitrogen fixation also decreases, resulting in the rhizosphere to acidify at night (Blossfeld et al., 2013). This acidification occurs through the release of soluble P from the inorganic or organic phosphorus source resulting in a significant drop in pH (Alikhani et al., 2006).

4. Challenges

To study rhizobial diversity remains a challenge due to the complex interactions between rhizobial species with each other, the host plants and their environment. In addition, most soil bacteria are unculturable and culture based methods often fall short to characterize the true diversity of this group of bacteria. This makes it difficult to use culture based methods to study rhizobial diversity. Studies on microbial diversity and community structures have moved towards using molecular based techniques such as next-generation sequencing (Mardis, 2008; Metzker, 2010; Van Dijk et al., 2014). Next-generation sequencing is used in metagenomic studies that is based on the genetic material from environmental samples (Handelsman, 2004). Metagenomics can be used to determine the microbial diversity and identify microorganisms to a certain extent (Neelakanta and Sultana, 2013). A disadvantage of metagenomics is that it is expensive and time consuming (Zhou et al., 2015).

Although molecular techniques are useful, there are also some challenges in studying rhizobia. Some rhizobial species have multiple copies of the *nodA* and *nifH* genes (Nap and Bisseling, 1990; Van Rhijn and Vanderleyden, 1995). This makes it difficult to do phylogenetic analysis, especially as the 16S rRNA genes are not sufficient in identifying these species. Furthermore, the databases of the housekeeping genes (*recA* and *atpD*) are incomplete.

There is a lack of knowledge on the rhizobia that associate with rooibos and honeybush plants. More research is needed on the plant

growth-promoting compounds, nodule functioning and nodulation kinetics of rhizobia that associate with rooibos and honeybush plants. For example, IAA and ACC deaminase production has been studied in other rhizobia and have shown to have a positive effect on plant growth (Glick, 2005; Ashraf et al., 2013). Root nodule formation and the differentiation of rhizobia into bacteroids as well as all the other metabolic compounds that have an influence on these processes in rooibos and honeybush plant still have to be studied. This has been done on other leguminous plants as shown by Desbrosses and Stougaard (2011) and Terpolilli et al. (2012). The pathway of how nitrogen is transported from the bacteroid to the plants cell also has to be determined for rooibos and honeybush.

Research in microbial inoculants for rooibos and honeybush plants, may increase the yield of the plants and increase plants resistance to environmental stress. However, the development of a microbial inoculant will take time. It will be necessary to screen and test different combinations of rhizobia, as some rhizobia can have a negative effect on each other and different strains may produce different amounts of plant growth-promoting metabolites.

5. Conclusion

Rooibos and honeybush plants are unique not only for their herbal teas, but because they are legumes that can host different species of rhizobia. The symbiotic relationship between rhizobia and legume is complex and further studies are required to fully understand the mechanisms of nodulation and host specificity. Rhizobia are ecologically important microorganisms that provide nitrogen, phosphorous and other nutrients to plants. These bacteria also produce compounds that have a positive effect on plant growth. The metabolic characteristics and function of rhizobia associated with legumes might provide insight to some of the mechanisms that are still unknown.

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