Efficacy of rhizobia strains on growth and chemical composition of cancer bush (Sutherlandia frutescens)

Tsobedi Absalom Masenya, Phatu William Mashela and Kgabo Martha Pofu

Green Biotechnologies Research Centre of Excellence, University of Limpopo, Sovenga, South Africa

ABSTRACT
Cancer bush (Sutherlandia frutescens), which is facing extinction, can be conserved through cultivation using best agricultural practices that include nodulation with effective rhizobia bacteria. The objective of the study was to compare the efficacy of commercial and native nodulation bacteria on productivity of S. frutescens over two seasons. Seasonal interaction on productivity variables was not significant (P < 0.05), with pooled data (n = 75) subjected to analysis of variance. Treatments had a significant effect on plant variables, contributing from 58 to 91% in total treatment variation (TTV) of the variables. Relative to untreated control, commercial strains significantly increased plant variables from 31 to 44%, whereas wild strains increased the variables from 17 to 195%. Similarly, both commercial and native strains significantly increased nitrogen, protein and symbiotic efficiency, with magnitudes of native strains being significantly higher than those of commercial strains. Treatments had no significant effects on K and protein in leaf tissues of S. frutescens. In conclusion, the native strains as investigated in the current study have the potential for use in the husbandry of cancer bush.

Introduction

Sutherlandia frutescens (L.) r.br. (syn. Lessertia frutescens (L.) Goldblatt and J.C. Manning) is an indigenous medicinal plant extensively used in South Africa. This leguminous plant is fairly widespread, drought-tolerant and grows widely in the Western, Eastern, and Northern Cape Provinces in South Africa, along with in certain parts of KwaZulu-Natal, varying in its chemical and genetic makeup across these geographic areas (Aboyade et al. 2014). Sutherlandia is found in the Fynbos Biome, home to the largest variety of plant species. Sutherlandia frutescens belongs to the class Magnoliopsida and order Fabales (Aboyade et al. 2014). Cancer bush (Sutherlandia frutescens) has a wide range of pharmacological activities against human diseases such as cancers, fever, diabetes and HIV/AIDS (Aboyade et al. 2014; Makgato et al. 2020). The plant is a legume, with unknown nodulating bacteria species. Generally, the two commonly cited nitrogen-fixing bacteria include Rhizobium and Bradyrhizobium species, each with a wide range of associations from the wild (Gerding et al. 2012). Several studies have shown that Rhizobium, a gram-negative N-fixing soil bacterium has a positive impact on most legume species (Laguere et al. 2007).

Bradyrhizobium, a gram-negative bacterium, is one of the most cosmopolitan and diverse bacterial group nodulating a variety of legume species in Africa. The efficacy of the Rhizobial strains depends mainly on the host genotype and the location of cultivated field.

The first step in assessing the suitability of indigenous nodulation bacteria is comparing their efficacy with the existing commercial strains on plant growth variables and biosynthesis of nitrogen and proteins. Koskey et al. (2017) carried out greenhouse and field experiments to evaluate symbiotic efficiency, compare the effect of native rhizobia and commercial inoculant on nodulation, growth and yield parameters of mid-altitude climbing bean (MAC 13 and MAC 64) varieties. Results demonstrated a significant improvement in nodule dry weight and seed yields of MAC 13 and MAC 64 climbing bean varieties upon rhizobia inoculation when compared to the non-inoculated controls (Koskey et al. 2017). Ndusha et al. (2019) carried out greenhouse and field experiments at Kalambo station of International Institute of Tropical Agriculture (IITA), evaluating symbiotic efficiency, comparing the effect of native rhizobia (Bradyrhizobium japonicum NAC17, NAC 22, NAC 37, NAC 42, NAC 46 and NAC 78) and commercial inoculant...
(Bradyrhizobium elkanii SEMIA5019) on yield of soybean plants. The best inoculation treatments across all experiments were native strains NAC46 and NAC17 which nodulated equally or better than the commercial strain USDA 110 (Ndusha et al. 2019). The symbiotic efficacy comparisons between native rhizobia and commercial inoculant on nodulation, growth and yield variables is a silver bullet in development of cost effective and environment-friendly inoculants. However, further characterisation and mapping of the native isolates would be imperative in development of effective and affordable commercial inoculants for the test plant species (Koskey et al. 2017). Similar comparative studies would be important in cancer bush since there is interest in the cultivation of this plant in smallholder farming systems (Makgato et al. 2020). The development of an effective nodulation bacteria would provide a cheap inoculant and reduce the use of inorganic nitrogen fertilisers, which are costly and environment un-friendly (Zuluaga et al. 2020). The objective of this study, therefore, was to investigate whether the efficacies of natural S. frutescens, nodulation bacteria would be similar to those of commercial nodulation bacteria on yield, growth, macronutrients and protein content of S. frutescens.

**Material and methods**

**The study location and preparation of materials**

Nodulated roots of S. frutescens plants were collected from two locations during spring 2018 and 2019 at Tubatse (24°63′52.5″S; 30°16′4.28″E) in the Sekhukhune District Municipality and Sebayeng (23°88′92.5″S; 29°17′8.38″) in Capricorn District Municipality with mean annual rainfall of less than 600 and 500 mm, respectively. The two sites have minimum/maximum average temperatures of 7/28°C and 13/30°C, respectively. Plant roots were collected using a spade, placed in cooler boxes and transported to Limpopo Agro-food Technology Station (LATS), University of Limpopo, South Africa (23°53′10″S, 29°44′15″E). Roots were rinsed in tapwater and then in distilled water. Healthy, undamaged, firm and pink nodules were detached from roots and then sterilised in 0.1 mercuric chloride (HgCl₂) for 30 s to break the surface tension (Hamza et al. 2017). Thereafter, nodules were rinsed 10 times in pasteurised distilled water to remove traces of HgCl₂.

**Rhizobia strain culture and inoculant preparation**

The sterilised nodules from the two locations were shade-dried to allow for gradual water loss and for the bacteria to enter the dormant survival phase. Dried materials were ground separately. The Tubatse strains (Raoutella ornithinolytica and Enterobacter cloacae species), Sebayeng isolates (Sphingomonas paucimobills, Raoutella ornithinolytica and Enterobacter cloacae species dissolvens), isolated previously in our project and two commercial strains (Bradyrhizobium and Rhizobium species) from Sygroy Company (Potchefstroom, South Africa), were each streaked on Yeast Extract Mannitol broth (YMB) (Ndusha et al. 2019) and incubated at 25°C for the preparation of inoculants (Ndusha et al. 2019). The final Rhizobium cell density was \(2 \times 10^2\) FCU/ml for rhizobia strains and then uniformly mixed with pre-autoclaved low peat soil at 12.5 ml/100 g peat soil using the two-step inoculation method (Chao and Alexander 1984; Burgos et al. 1990; Woomer et al. 2011; Thilakarathna et al. 2019). Prior to inoculation, seeds were surface sterilised by immersing in 70% ethanol for 10 s (Koskey et al. 2017) and then immersed in 3% sodium hypochlorite solution for 3 min in an Erlenmeyer flask and rinsed six times in pasteurised distilled water (Ndusha 2011). Seeds were left in the final rinsing water for 24 h for imbibition. Seeds were then air-dried for 30 min prior to manually sowing in 200-cone seedling trays, containing Hygromix-T (Hygrotech, Pretoria, South Africa) and placed on benches in the greenhouse. The 4-week-old seedlings, at four-leaf stage, were hardened-off outside the greenhouse using intermittent withdrawal of irrigation water and when 50% seedlings have wilted, they were taken to shade and irrigated to full capacity, with the ritual performed for two weeks. Thereafter, uniform seedlings were transplanted into 20-cm-diameter plastic pots, filled with approximately 2700 ml steam-pasteurised loam soil (at 250°C for one hour) and Hygromix at 3:1 (v/v) ratio. The soil comprised Hutton form (65% sand, 30% clay and 5% silt), containing 1.6% organic C, with EC at 0.148 DS/m and pH (H₂O) at 6.5. Microplots were established by inserting 20-cm diameter pots in 15-cm deep holes at 0.6 × 0.6 m spacing.

**Experimental design and cultural practices**

The five treatments, namely, Bradyrhizobium spp. (Arachis) strain, Rhizobium leguminosarum strain, Tubatse strain, Sebayeng strain and untreated control, were laid-out in a randomised complete block design, with seven replications during the first season (Experiment 1) and with eight replications during the second season (Experiment 2). Blocking was done for shading by windbreak trees in the morning and in the afternoon. Three days after transplanting, seedlings were fertilised with 2.5 g of NPK 2:3:2 (22) fertiliser mixture per plant to provide 186 N, 126 K
and 156 P mg/ml water and 2 g NPK 2:1:2 (43) Multifeed (Nulandies, Johannesburg) fertiliser to provide a total of 0.35 N, 0.32 K and 0.32 P, 0.9 Mg, 0.75 Fe, 0.075 Cu, 0.35 Zn, 1.0 B, 3.0 Mn and 0.07 Mo mg/ml water (Tseke and Mashela 2017). Control plants were irrigated weekly with 0.05% KNO₃ solution to supply nitrogen (Koskey et al. 2017). Plants were irrigated every other day with 250 ml chlorine-free tap-water to avoid leaching. Scouting and monitoring for insect pests were performed daily and none were observed.

Data collection

At 110 days after transplanting, plant height was measured from the crown to the tip of the flag leaf. Branch numbers were counted and shoots severed from roots, with stem diameter measured using a digital Vernier caliper at 5-cm above the severed end. Roots were removed from pots, placed in polythene bags in cooler boxes and taken to the processing station where they were placed on a 75 mm opening sieve and rinsed with running tap-water to remove soil particles. Root nodule position on the root system was recorded, with healthy nodules removed and placed in sterile vials to record nodule number and nodule colour using the nodule colouration scale (Yates et al. 2016). Nodules and healthy mature leaves were oven-dried at 60°C for 24-h for the determination of dry nodule mass.

Approximately 0.40 g dried leaf material was digested in 40 ml 5% nitric acid (HNO₃) solution, followed by placing the container on a vortex to allow for complete wetting of the mixture. The material was magnetically stirred, thereafter incubated in a 95°C water-bath for 60 min. The samples were allowed to cool down at room temperature, filtered and then decanted into 50 ml tubes covered with a foil. Inductively Coupled Plasma Emission (ICPE-9000) was used to measure K and P in leaf tissue. Leaf tissues were analysed for N and protein (%) using the DUMAS Protein Content Analyser/LECO Nitrogen Analyser. Symbiotic efficiency (SEF) was calculated by comparing shoot dry mass of inoculated plants with shoot dry mass of non-inoculated control plants that were supplemented with nitrogen, and then multiplied by 100% (Table 4) (Koskey et al. 2017).

Data analysis

The seasonal interactions (Experiment 1 × Experiment 2) on plant and nutrient elements were not significant (P ≤ 0.05) and data for the two seasons were pooled (n = 75). Data for branch number and nodule number were transformed using log₁₀ (x + 1) and subjected to analysis of variance. The Shapiro–Wilk test was performed on each dataset to determine the normality of distribution of the recorded data (Shapiro and Wilk 1965; Ghasemi and Zahediasl 2012), with the data depicting normal distribution. Data for each variable were subjected to analysis of variance using Statistix 10 software. Mean sum of squares (MSS) were partitioned to establish the total treatment variation (TTV) on each variable (Little 1981). Mean separation was accomplished using Fisher’s Least Significant Difference (LSD) test at the probability level of 5%. Unless otherwise stated, only means which were significant at the probability level of 5% were discussed.

Results

Treatments had highly significant effects (P ≤ 0.01) on plant height, root length, dry shoot mass, dry nodule mass and nodule number, contributing 87, 58, 66, 71 and 91% in TTV of respective variables, respectively (Table 1), but had no significant effects on stem diameter, branch number, nodule position. Relative to untreated control, *Bradyrhizobium* strain, *Rhizobium* strain, Tubatse strain and Sebayeng strain increased plant height, root length and dry shoot mass by 31, 33, 44 and 40%, 30, 41, 40 and 42% and 48, 195 and 17%, whereas dry nodule mass and nodule number were reduced by 97, 98, 98 and 98% and 88, 89, 91 and 89%, respectively (Table 2). In some instances, the effects of native strains on plant growth variables were significantly better than those of the commercial strains. Although not statistically different from Sebayeng strain, Tubatse strain recorded the highest plant height and higher dry shoot mass relative to the

| Table 1. Source of variation affecting plant height, root length and dry shoot mass of *S. frutescens* at 110 days after rhizobia inoculation under microplot conditions (n = 75). |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Plant height    | Root length     | Dry shoot mass  | Dry nodule mass | Nodule number   |                |                |                |                |                |                |
| Source         | Df              | MS              | %               | MS              | %               | MS              | %               | MS              | %               | MS              | %               |
| Rep            | 14              | 150.1           | 9               | 59.582          | 27              | 267.22          | 24              | 1.090           | 15              | 0.032           | 5               |
| Trt            | 4               | 1431.9          | 87***           | 127.24          | 58***           | 733.85          | 66***           | 5.809           | 77***           | 0.566           | 91***           |
| Error          | 56              | 65.03           | 4               | 33.36           | 15              | 108.34          | 10              | 0.624           | 8               | 0.026           | 4               |
| Total          | 74              | 1647.03         | 100             | 220.18          | 100             | 1109.41         | 100             | 7.523           | 100             | 0.624           | 100             |

***Highly significant at P ≤ 0.01.
control and commercial strains in the two seasons (Table 2). Relative to the untreated control, *Rhizobia* strains increased root length, but the strains did not have significant differences (Table 2).

Treatments had highly significant effects on N, protein and SEF, contributing 84, 74 and 31% in TTV of the respective variables (Table 3). However, the treatments did not have significant effects on K and P in leaf tissues. Relative to untreated control, Tubatse, Sebayeng, *Rhizobium* and *Bradyrhizobium* strains increased N, protein and SEF by 7, 25, 80 and 13%, 10, 24, 69 and 13% and 31, 133, 292 and 82%, respectively (Table 4). Relative to the untreated control and other rhizobia inoculates, Tubatse strain had the highest N in leaf tissues, while the lowest N was in leaf tissues of non-inoculated plants (Table 4). Relative to commercial strains and the control, plants inoculated with Tubatse strain had the highest SEF values with the relative mean value of 391%, but which was not significantly different from *Rhizobium* strain, whereas SEF from Sebayeng strain was also not significantly different to that of *Bradyrhizobium* strain (Table 4).

**Discussion**

The rhizobia inoculates varied in impact on growth of *S. frutescens* (Table 1). The differences among the treatments on different plant variables had also been encountered in other studies where indigenous nodulation bacteria were compared with the commercial strains (Gicharu et al. 2013; Ouma et al. 2016). The increased plant height in plants inoculated with Tubatse strain relative to commercial strains were consistent with observations elsewhere (Table 2), where native strains outperformed commercial strains on increasing plant height in three climbing bean cultivars (Gicharu et al. 2013). Kawaka et al. (2014) also observed similar results when comparing four native strains with two commercial strains (*Rhizobium tropici* CIAT 899 and *Rhizobium leguminosarum* Strain 446) on plant growth of beans. Apparently, in the current study and others

| Table 2. Effect of rhizobia inoculation on plant variables of *Sutherlandia frutescens* at 110 days after rhizobia inoculation under microplot conditions (*n* = 75). |
|---|---|---|---|---|---|
| Strains | Plant height (mm) | Root length (cm) | Dry shoot mass (g) | Nodule number | Nodule mass (g) |
| Control | 56.22 c ± 1.63 | 16.42b ± 1.23 | 8.38b ± 1.89 | 0.03 b ± 0.02 | 0.05c ± 0.02 |
| Bradyrhizobium strain | 73.64 b ± 2.55 | 21.39 a ± 1.15 | 23.16 a ± 2.16 | 7.76 a ± 1.40 | 99 |
| Rhizobium strain | 74.68 b ± 2.44 | 23.18 a ± 1.16 | 7.76 a ± 1.40 | 99 |
| Tubatse strain | 81.05 a ± 2.0 | 23.01 a ± 1.59 | 7.76 a ± 1.40 | 99 |
| Sebayeng strain | 78.68 ab ± 2.81 | 23.27 a ± 1.64 | 7.76 a ± 1.40 | 99 |

**Table 3.** Source of variation affecting shoot nitrogen, shoot protein and Symbiotic efficiency of *Sutherlandia frutescens* at 110 days after rhizobia inoculation under microplot conditions (*n* = 75).

| Source | Df | MS | %MS | %MS | %MS |
|---|---|---|---|---|---|
| Rep | 14 | 1.408 | 9 | 85.15 | 17 | 10.152 | 16 |
| Trt | 4 | 13.35 | 84*** | 370.04 | 74*** | 19.695 | 31*** |
| Error | 56 | 1.134 | 7 | 42.98 | 9 | 33.645 | 53 |
| Total | 74 | 15.89 | 100 | 498.17 | 100 | 63.492 | 100 |

***Highly significant at *P* ≤ 0.01.
Karaca and Uyanöz (2012; Ouma et al. 2016), the differences in plant height and root length (Karaca and Uyanöz 2012) were due to disproportionate accumulation of plant growth regulators which were induced by the native strains.

Although all roots had nodules, the results showed that treatments decreased nodule number and dry nodule mass, as observed by others (Arafa et al. 2018). The improved dry mass on plants inoculated with native strains suggested that there was better combining and symbiotic relationship between native strains and *S. frutescens*, as described in another related study (Ndusha 2011). Nodulation is an important symbiotic trait for effective symbiosis between *Rhizobium* species and legume host plants. Observations in the current study agreed with those of others; Ouma et al. (2016), where rhizobia inoculates had similar effects on growth of soybean. In the current study, dry shoot mass was significantly increased in plants inoculated with Tubatse strain than other rhizobia strains and the untreated control. Koskey et al. (2017) observed similar results, were climbing beans inoculated with native isolate ELM3 had the highest dry shoot mass when compared with commercial strains and control treatments. Generally, dry matter can be used as an indicator for symbiotic effectiveness of nodulation isolates since the variable is significantly correlated with nitrogen fixation (Arafa et al. 2018).

The influence of rhizobia inoculants on macronutrient and protein contents of *S. frutescens* relative to the control exhibited increased N and protein content in leaf tissues of plants inoculated with Tubatse strain (Table 4). In other studies (Ouma et al. 2016; Koskey et al. 2017), native strains also outperformed commercial strains on assimilation of certain nutrient elements in leaf tissues of test plants.

According to the rating scale developed by Lalande et al. (1990), relative to untreated control, rhizobia strains used in our study increased symbiotic nitrogen fixing efficiencies (SEF > 80%). The observed high symbiotic efficiency in plants treated with Tubatse strain, concurred with observations where native strains were used in climbing beans, whereas Koskey et al. (2017) observed SEF values that ranged from 86.17% to 123.72% when compared to those of commercial strains. Generally, the SEF values were rated as being highly effective when SEF > 80%, effective when SEF was < 80%, but > 50%, lowly effective when SEF was 35%, but < 50% and ineffective when SEF < 35% (Lalande et al. 1990).

Similarly, Kawaka et al. (2014) noted SEF values due to native strains in Western Kenya that ranged from 67 to 170% when compared with values from commercial strains and control plants. Unfortunately, native strains are not as resilient as commercial strains under different conditions and could fail to compete successfully with commercial *Rhizobia* species in certain plant species (Kebede et al. 2020). The study demonstrated the potential superior presence of effective native nodulation strains that compared favourably with commercial strains in nodulation, symbiotic efficiency, macronutrients, protein content and growth performance of the test plants. Native strains have great potential of being further developed to provide cheap and efficient inoculum in the study area and beyond. Such native isolates could have an added advantage of being more adapted to the soils than the commercial inoculant strains. Future studies should be explored to assess the adaptability of the test native strains under various conditions.

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Notes on contributors

Tsobedi William Mashela is a PhD student at the University of Limpopo.

Prof PW Mashela is involved in innovation of botanical products with reference to plant protection.

Dr KM Pofu is involved in management of plant nematodes using climate-smart strategies that exclude the use of synthetic chemical nematicides.

ORCID

Phatu William Mashela http://orcid.org/0000-0002-9759-2625

Kgabo Martha Pofu http://orcid.org/0000-0002-1575-5962

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