Research article

A survey for plant-growth-promoting rhizobacteria and symbionts associated with crop plants in the Okavango region of Southern Africa

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Abstract: Regions in the Okavango catchment and delta such as highlands of Angola, the Kavango region of Namibia, and the Okavango Delta region of Botswana, although rich in plant diversity and density, have not produced significant yields when cropped by small scale farmers in the region. This phenomenon may be due to many factors among which is low nitrogen and other crop nutrients availability. This region due to its richness in flora may harbour bacteria which play a major role in plant nutrient availability. However, some of these rhizobacteria can be isolated and re-inoculated on crop plants to improve crop yields. Thus a survey on root nodulation of local pulses such as *Vigna unguiculata, V. subterranea*, and *Phaseolus vulgaris,* in the Chitembo area of Angola and the Kavango region of Namibia was carried out. Nodulated plants and putatively symbiotic bacteria were detected from a range of sites for all species. In Namibia, isolation of putative plant-growth-promoting rhizobacteria (PGPRs) was done on cereal crops and from other indigenous plants on farmland and from pristine areas. In Botswana, phosphate solubilizing bacteria were isolated from the roots of grasses in the floodplains and assayed for their ability to solubilize soil phosphate with the intention of using them to increase yields in sorghum (*Sorghum vulgare*). In total, 46 bacterial strains were isolated from nodules of legumes from Namibia, while 37 strains were isolated from Angola. Additionally, 32 strains of plant associated rhizobacteria were obtained from cereals or natural plants from the Kavango region in Namibia, and further ten isolates were selected from the Seronga region in Botswana. The large number of bacteria generated by this survey may contain some bacteria that may promote plant growth and improve soil fertility.

Keywords: bambara groundnut; cowpea; nodule; *Pennisetum glaucum*; phosphate solubilizing bacteria; *Rhizobium*; sorghum.

Pesquisa sobre rizobactérias promotoras do crecimento de plantas e simbiontes associados com cereais, na região do Okavango no sul da África

Resumo: Regiões na bacia e no delta do Okavango, como os planaltos de Angola, a região de Kavango da Namíbia e a região do delta do Okavango de Botsuana, embora ricas em diversidade e densidade de plantas, não produziram safras significativas quando cultivadas por agricultores de pequena escala na região. Este fenômeno pode ser devido a vários fatores, entre os quais a baixa disponibilidade de nitrogênio e outros nutrientes agrícolas. Essa região, devido à sua riqueza em flora, pode abrigar bactérias que desempenham um papel importante na disponibilidade de nutrientes nas plantas. No entanto, algumas destas rizobactérias podem ser isoladas e reinoculadas em culturas para melhorar seu rendimento. Assim, foi realizado um estudo sobre a nodulação das raízes de plantas locais, como a "*Vigna unguiculata*", "*V. subterranea*" e "*Phaseolus vulgaris*", em Chitembo na área Angolana e no Kavango na região da Namíbia. Plantas noduladas e bactérias putativamente simbióticas foram detectadas a partir de uma variedade de locais para todas as espécies. Na Namíbia, o isolamento de rizobactérias putativas promotoras do crescimento de plantas (PGPRs) foi feito em culturas de cereais e de outras plantas indígenas em terras agrícolas e em áreas intocadas. Em Botsuana, bactérias solubilizadoras de fosfato foram isoladas das raízes de gramíneas nas várzeas e analizadas por sua capacidade de solubilizar fosfato do solo, com a intenção de usá-las para aumentar a produtividade na cultura do sorgo (*Sorghum vulgare*). No total, 46 cepas de bactérias foram isoladas de nódulos de leguminosas na Namíbia, enquanto 37 foram isoladas em Angola. Além disso, 32 cepas de plantas associadas a rizobactérias foram obtidas a partir de cereais ou plantas naturais da região de Kavango na Namíbia e outras 10 cepas isoladas foram selecionadas da região de Seronga em Botsuana. O grande número de bactérias geradas por essa pesquisa pode conter algumas bactérias que podem promover o crescimento das plantas e melhorar a fertilidade do solo.

Palavras-chave: Amendoim bambara; bactérias de solubilização de fosfato; feijão-caupi; milheto; nódulo *Rhizobium*; *Pennisetum glaucum*; sorgo.

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Introduction

The predominant agriculture paradigm based on improved varieties of common staple crops in high-input systems has not succeeded addressing food insecurity and malnutrition e.g. in Sub-Saharan Africa (Rudebjer et al. 2013), where over 200 million people (28% of the population) were undernourished in 2005-2007 (FAO, 2010). Yield variability and risk for crop failure in Africa's rainfed agriculture systems contribute to factors that explain difficulties in adopting new technologies (Ogada et al. 2010). These risks are predicted to increase in many areas in Southern Africa due to climate change, as projections suggest a decline in land area suitable for cultivation of crops (Lane & Jarvis 2007). On the other hand, demand for staple crops like maize is currently increasing in Africa due to changes in eating habits, with limited production and increasing imports.

Low crop productivity is often a problem faced in smallholder farming systems in Sub-Saharan and Southern Africa. Subsistence agriculture with low agrochemical inputs is widespread in the greater Okavango catchment regions of the Okavango basin in Angola, Namibia and Botswana. For example in smallholder's farms of the Kavango region of Namibia, fields are not irrigated, herbicides as well as pesticides and fertilizers are not used, and crop yields are very low (Pröpper et al. 2010).

Low yields are often associated with declining soil fertility and low input by biologically fixed nitrogen (see below), depending on biological and environmental factors (Dakora & Keya 1997). The availability of nitrogen affects the productivity of crops and cereals worldwide in all ecosystems. Nitrogen fertilizer is the most widely used resource, of which one third is lost through emission of greenhouse gasses and leaching, causing adverse environmental impacts. Unfortunately, the majority of African small farmers are not able to afford the high mineral fertilizer prices (Yanggen et al. 1998) albeit there is a growing need for mineral N fertilizers (World bank, 2008). Low cost and sustainable technical solutions compatible with the socioeconomic conditions of small farmers are needed to solve soil fertility and yield problems (Chianu et al. 2011).

Nitrogen is unique among the other essential elements because N_2 from the atmosphere can be fixed by biological nitrogen fixation (BNF), exclusively carried out by prokaryotes that possess the enzyme nitrogenase. This potential can be employed in nitrogen-fixing symbioses between Fabaceae and rhizobia, but also in other crop systems for a more sustainable agricultural practice. The most important N_2-fixing agents in agricultural systems are the symbiotic associations between crop and forage/fodder legumes and rhizobia forming root nodule symbioses. Estimates of N fixed annually are > 100 kg N/ha/year in good-practice farmer's fields (Herridge et al. 2008). However, there is only little published knowledge on rhizobial symbionts of crops from Namibia, Angola, or Botswana (Pule-Meulenberg & Dakora 2007). Given the high cost of fertilizer in Africa and the limited market infrastructure for farm inputs, current research and extension efforts need to be directed to integrated nutrient management, in which legumes play a crucial role. Inoculation with compatible rhizobia resistant to harsh environmental conditions can make BNF a key source for farmers with little income (Smaling et al. 2008).

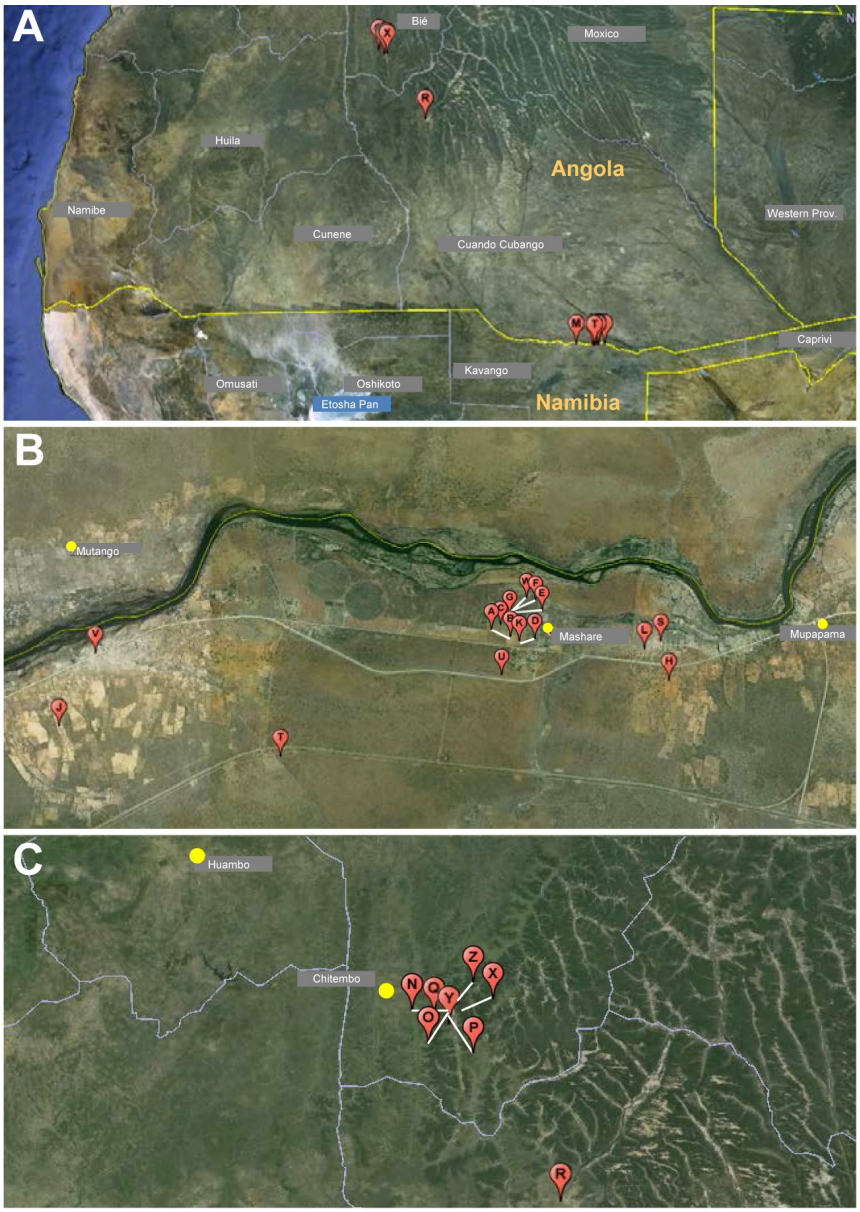

Fig. 1: Overview of the areas of the survey for nodulated grain legumes in Southern Africa. (A) Overview of sampling areas in Angola and Namibia. (B) Close-up of the sampling areas in the Kavango province of Namibia, near Kaiango, Mashare and Mupapama. (C) Close-up of the sampling area in the province of Bié near Kuseke in Angola.Flowchart of the implemented analysis scheme.

Additionally, roots support the growth of a variety of microorganisms that may have strong effects on growth or health of plants, not based on BNF. These beneficial bacteria (plant-growth-promoting rhizobacteria, PGPR) may enhance root or plant growth, nutrient uptake, stress resistance or resistance to pathogens by a variety of mechanisms (Adesemoye & Kloepper 2009, Desbrosses et al. 2009). Studies on or inoculants for traditional cereal crops in the Kavango area are not available. In a first survey, we isolated and identified endophytes from roots of Namibian maize, *Sorghum bicolor* and *Pennisetum glaucum*, which are affiliated to known PGPRs or might even represent new species (Grönemeyer et al. 2012).

Here, we report on a survey for putative symbionts of grain legumes and putative PGPRs of local cereals in the Okavango region, with focus on Namibia and extension to Botswana and Angola. In Botswana where phosphate is one of

Table 1. Survey of grain legumes for nodulation by rhizobia in Namibia and Botswana. Locations represent sites shown in Figure 1. Samples labeled with "soil" represent trapping experiments from collected soil samples, using the plant species mentioned. NAM, Namibia; ANG, Angola. MADI: Mashare Agricultural Development Institute, test fields for varieties.

Country	Location	Coordinates	Plant species	Land use form	Plant number	Isolates
NAM	A	S17.895486 E20.211047	*Arachis hypogaea*	MADI, not irrigated, local race	1	1
NAM	B	S17.895126 E20.210972	*Arachis hypogaea*	MADI, not irrigated, local race	2	1
NAM	C	S17.893389 E20.209500	*Vigna unguiculata*	MADI, irrigated, local race	3, 4	2
NAM	E	S17.893125 E20.210531	*Vigna unguiculata*	MADI, not irrigated, race from Kenia	6	1
NAM	F	S17.892806 E20.210467	*Vigna unguiculata*	MADI, not irrigated, dryland conditions, race as 6	7	2
NAM	G	S17.892661 E20.210467	*Arachis hypogaea*	MADI, field as 7, race as 1	8	0
NAM	H	S17.90074 E20.23303	*Vigna subterranea* *Vigna unguiculata*	Subsistence farmer`s field, dryland agriculture, Kalahari sands	9 10	1 1
NAM	I	S17.89546 E20.33150	*Vigna unguiculata*	Subsistence farmer`s field, dryland agriculture, old floodplain soils	14	1
NAM	J	S17.90719 E20.14718	*Arachis hypogaea*	Subsistence farmer`s field, dryland agriculture, Kalahari sands	16	1
NAM	K	S17.895881 E20.212164	*Vigna unguiculata* *Arachis hypogaea*	MADI, dryland conditions, local race, Kalahari sands	20-25 26-29	2 4
NAM	H	S17.90074 E20.23303	*Vigna unguiculata* *Vigna subterranea*	Subsistence farmer`s field, dryland agriculture, Kalahari sands	34, 35 36-38	5 5
NAM	M	S17.898225 E19.900658	*Vigna subterranea* *Arachis hypogaea* *Vigna unguiculata*	Subsistence farmer`s field, dryland agriculture, old floodplain soil, field legumes only	54-56, 60 57-59, 61 62	6 2 0
ANG	N	S13.69958 E17.06752	*Vigna unguiculata*	Subsistence farmer`s field, dryland agriculture	40, 41	4
ANG	O	S13.70122 E17.06739	*Phaseolus vulgaris*	Subsistence farmer`s field, dryland agriculture	42	2
ANG	P	S13.71203 E17.06544	*Phaseolus vulgaris* *Vigna unguiculata*	Subsistence farmer`s field, dryland agriculture	43, 44 45, 46	4 5
ANG	Q	S13.640244 E16.983867	*Vigna unguiculata* *Phaseolus vulgaris*	Subsistence farmer`s field, dryland agriculture Novel field, next to above, older field	47-48, 50-51 49	5 3
ANG	R	S14.662819 E17.665467	*Phaseolus vulgaris*	Small field in Menongue	52, 53	4
NAM (soil)	H	S17.90073 E20.23300	*Vigna subterranea*	Subsistence farmer`s field, dryland agriculture, Kalahari sands		4
NAM (soil)	S	S17.89518 E20.23199	*Vigna subterranea* *Vigna unguiculata*	Subsistence farmer`s field, dryland agriculture, old floodplain soil		1 1
NAM (soil)	T	S17.91146 E20.17834	*Vigna subterranea*	Bushveld, Kalahari sands		4
NAM (soil)	U	S17.89968 E20.20953	*Vigna unguiculata* *Vigna subterranea*	Bushveld, Kalahari sands		1 2
NAM (soil)	V	S17.89690 E20.15219	*Vigna unguiculata*	Subsistence farmer`s field, dryland agriculture, old floodplain soil		2
NAM (soil)	W	S17.89274 E20.21068	*Vigna subterranea*	Irrigation agriculture, old floodplain soil, maize field		1
ANG (soil)	X	S13.70896 E17.11225	*Vigna subterranea*	Fallow after subsistence farming		2
ANG (soil)	Y	S13.69081 E17.0633	*Vigna subterranea*	Subsistence farmer`s field, dryland agriculture		1
ANG (soil)	Z	S13.68787 E17.101756	*Vigna subterranea*	Pristine grassland / bush		2

the major limiting elements in the cultivation of sorghum, the staple crop, a study was carried out to isolate phosphate solubilizing bacteria from the rhizosphere of Seronga floodplain grasses.

Materials and Methods

Time and location of sampling campaigns

A survey for nodulated grain legumes was carried out in Namibia and Angola in the rainy season. Samples were obtained mainly in the Mashare area during 23.03.-24.03.2011. A second campaign in this area was from 25.03.-27.04.2012 and 03.04.2012. Inspection of roots for nodules was also carried out between 30.12.2012-03.01.2013. In Angola, sampling was carried out in the Kuseke area south of Chitembo between 30.03.-01.04.2012. Soils for trapping of rhizobia were also collected in the March –April 2012 field trips on the sampling sites. The coordinates of these sampling sites are given in Table 1, and the locations are shown in Figure 1.

Sampling of plants for PGPR isolation was carried out on selected sites indicated in Table 2 along the Kavango riverine agro-ecology zone in Namibia.

In Botswana, dominant flood plain grasses in Seronga flood plains were selected. The coordinates of the sampling sites are shown in Table 3.

Table 2. Source of bacterial isolates from Namibia with potential for plant growth promotion. Latin name for Mahangu is *Pennisetum glaucum*.

Country	GPS Coordinates	Plant species	Land use form	Isolates
Namibia	17°53`43.80 S 20°14`05.26 E	Mahangu	Subsistance farming	8
Namibia	17°53`49.75 S 20°09`07.07 E	Mahangu	Subsistance farming	3
Namibia	17°55`00.13 S 20°06`16.14 E	Mahangu	Subsistance farming	2
Namibia	17°54`04.40 S 20°14`14.34 E	Mahangu	Subsistance farming	3
Namibia	17°53`43.80 S 20°14`05.26 E	*Sorghum bicolor*	Subsistance farming	2
Namibia	17°55`00.13 S 20°06`16.14 E	*Sorghum bicolor*	Subsistance farming	4
Namibia	17°53`38.49 S 20°09`08.97 E	*Sporobolus sp.*	Pristine	2
Namibia	17°53`33.62 S 20°14`56.13 E	*Sporobolus sp.*	Pristine	3
Namibia	17°52`30.82 S 20°15`21.88 E	*Phragmites australis*	Pristine	1
Namibia	17°53`33.62 S 20°14`56.13 E	*Vetiveria nigritana*	Pristine	1
Namibia	17°53`38.49 S 20°09`08.97 E	*Vetiveria nigritana*	Pristine	1
Namibia	17°53`33.62 S 20°14`56.13 E	Ngwena (Local name)	Pristine	2

Sampling of nodules and isolation of bacterial symbionts

Grain legumes (Table 1) from farmer's fields were inspected for root nodules at their root systems. If nodules were present, several nodules per plant were cut off including some adjacent root tissue. They were stored in 2 ml glass vials with silica gel desiccant at room temperature during the campaign, and at 4°C upon arrival in Bremen. The cultivation of root nodule bacteria was carried out in the following way: desiccated root nodules were rehydrated in 2 ml of sterile water for four hours at room temperature. They were then surface sterilized by immersion in 70% ethanol for 30 seconds followed by a washing step in sterile water and immersion in 5% sodium hypochlorite for two minutes. After six further washing steps, surface sterilized nodules were homogenized with mortar and pestle in 100 to 500 µl (depending on nodules size) of sterile tap water, and a portion was streaked on modified arabinose gluconate medium (van Berkum 1990). The plates were incubated at 28°C for up to 14 days, and pure cultures were obtained by repeated streaking of single bacterial colonies on fresh agar plates.

To confirm the isolates` ability to fix atmospheric nitrogen, a polymerase chain reaction (PCR) based approach targeting the nifH marker gene was carried out. A portion of a bacterial colony was added to 10 µl Lyse and Go PCR Reagent (Thermo Scientific, Rockford, USA), and genomic DNA was released following the manufacturer`s instructions. 2 µL of the lysate served as a template in a PCR consisting of 2.5 U Taq DNA polymerase (Molzym, Bremen, Germany), 5 µl 10X PCR Buffer, 50 µM of each dNTP, and 0.5 µM of each primer FGPH19 and PolR (Poly et al. 2001) in a total volume of 50 µl. DNA was amplified in a Biometra TProfessional thermocycler under the cycling conditions described by Demba-Diallo et al. (2004). The presence or absence of the 429 bp PCR product was determined on a 1.8% agarose gel using PstI-digested DNA of Lambda Phage as a size marker.

Sampling of roots and isolation of PGPRs

Endophytic bacteria were carefully isolated repeatedly from root samples of various plants on selected sites along the Kavango River in Namibia (see Table 2). Pure cultures were obtained and immediately put to long-term storage while further characterization on the isolated bacteria continued. The isolated bacteria were tested for various plant-growth-promoting features including IAA production, phosphate solubilizing potential and bio-control properties.

In Botswana (Table 3), sampling was done by digging out the entire root systems of the selected plants and then

Table 3. Source of phosphate solubilizing isolates to be used as PGPR.

Country	Isolate number	GPS coordinates	Plant species	Land use form
Botswana	S1	18°49`35.48 S 22°25`55.71 E	*Eulesine africana*	Flood plain grassland
Botswana	S2	18°49`35.48 S 22°25`55.71 E	*Imperata cylindrica*	Flood plain grassland
Botswana	S3	18°49`35.48 S 22°25`55.71 E	*Imperata cylindrica*	Flood plain grassland
Botswana	S4	18°48`58.46 S 22°24`53.53 E	*Sesbania seban*	Flood plain grassland
Botswana	S5	18°49`35.48 S 22°25`55.71 E	*Panicum maxinium*	Flood plain grassland
Botswana	S6	18°48`58.46 S 22°24`53.53 E	*Cyperus sp.*	Flood plain grassland
Botswana	S8	18°47`38.4 S 22°24'19.7 E	*Cynodon dactylon*	Cattle grazing Flood plain
Botswana	S9	18°49`35.48 S 22°25`55.71 E	*Urochloa decumbens*	Flood plain grassland
Botswana	S10	18°49`35.48 S 22°25`55.71 E	*Urochloa trichophus*	Flood plain grassland

shaking off the soil to only retain the rhizosphere soil. Each plant was placed in a separate zip lock bag. All the plants were then placed in a cooler box and transferred to the laboratory. Once at the laboratory the roots were excised at the crown and the shoots were sent to the herbarium for identification, while the roots were retained for isolation of phosphate solubilizing bacteria.

Isolation of phosphate solubilizing bacteria

For the isolation of phosphate solubilizing bacteria (PSB) sterile calcium phosphate agar amended with 0.125 g of cyclohexamide/L was used (Nautiyal, 1999). Ten gram of each root sample were placed in a sterile conical flask containing 100 mL of sterile tap water. The mixture was left standing for 15 minutes, with shaking at intervals. A 1 ml aliquot was used to prepare serial dilutions to 10-4. The dilutions were then spread-plated on the solidified calcium phosphate agar and left in an incubator at 25°C. The plates were checked daily for any phosphate solubilizing ability.

After 5 days, halo zones around certain colonies were observed in some of the plates. The colonies with clear halo zones were considered to be PSB and these colonies were picked up using a sterile inoculation loop and streaked onto fresh calcium phosphate agar plates for purification. The purified cultures inoculated onto calcium phosphate agar slants and stored in the refrigerator (4°C).

To determine the phosphate solubilizing ability of the isolates, calcium phosphate agar plates were inoculated with the isolates at the centre using a sterile inoculation loop. The plates were incubated at 25°C for 20 days. These plates were also sealed with parafilm to prevent dehydration and contamination. The zones of clearance (i.e. the halozone) produced by the different isolates and diameter of the colonies were measured at 4 day intervals using a vernier calliper. These measurements were used to calculate the solubilization index (ref) of each isolate.

The solubilizing ability of the isolates was also assessed on other phosphate media i.e., magnesium, aluminium, potassium and iron phosphate.

Results and Discussion

Grain legumes as crops for smallholders in the Northern Kavango region of Namibia and the Chitembo area in Angola

In the Kavango region of Namibia, agriculture is largely dominated by smallholder farms with low crop yields and little developed market chains and food processing. Here as in many African regions, grain legumes can be regarded as "meat for the poor", due to their rich protein content and the relatively low prices of pulses in comparison to meat (Chianu et al. 2011). In our surveys of smallholder farms, cowpea (*Vigna unguiculata*, local name in Kavango makunde) was the main grain legume grown by farmers (Fig. 2A). In Namibia, also Bambara groundnut (*Vigna subterranea*, local name nongomene) is planted (Fig. 3A, B), albeit to a lesser extent. It can be regarded as part of neglected and underutilized species which are often local crops that remain important to poor communities' livelihood but are not exploited to their full potential (Rudebjer et al. 2013). Occasionally, peanuts (*Arachis hypogaea,*

Fig. 2: Intercropping in smallholder farms in the Mashare area in the Kavango region of Namibia. (A) Mahangu and cowpea intercropping. (B) Sampling on a plot for field tests on a traditional smallholder farm.

local name nongongo) were also found. In the Chitembo area, in addition to cowpea, the common bean (*Phaseolus vulgaris*) is utilized as well (Fig. 3F).

Common practice was intercropping of grain legumes with local cereals such as mahangu *(Pennisetum glaucum*), sorghum (*Sorghum bicolor*), or maize (Fig. 2), where legumes were interspersed in an irregular pattern (Fig. 2A). Farmers mostly used local varieties which they propagated themselves and stored grains for the following season. This dryland agriculture system is rainfed, with planting when the rainy season starts, typically November to December. Smallholders did not use chemical fertilizers or manure or any other input. Thus, nitrogen fixation by legume nodule symbiosis would provide an important input of nitrogen into the farming system.

Survey of grain legumes for root nodulation

In the wet season of 2011 and 2012, we undertook a survey of grain legumes and their root nodulation in Namibia and Angola. In Namibia, the North of the Kavango province was targeted, particularly the region around Mashare which belongs to the core sites of the TFO (The Future Okavango) project (Fig. 1A, 1B). The region is characterized by different forms of land use: few pristine woodlands were present; bushveld sites were woody areas used for occasional grazing of cattle; dryland agriculture was the typical form of smallholder farming (see above), and some of these fields had been abandoned (fallows); in small areas near the river, irrigation agriculture with fertilizer inputs was conducted by commercial farmers. In a parallel survey of different land use types and different soils (see Gröngröft et al. 2013), two categories of landscapes and soil types (old flood plains and Kalahari sands) were considered. Soils for trapping (see below) originated from different land use and soil types (Table 1). In Angola, the area for the survey was also one of the core sites of TFO, a rural area dominated by smallholder farms south of Chitembo (Fig. 1, Table 1). Land use types were typically forests and dryland agriculture fields on the summits of hills, horticulture in the wetlands of the valleys, and grassland at the slopes.

Inspection of root systems of pulses in fields of subsistence farmers in the Kavango region often showed a relatively

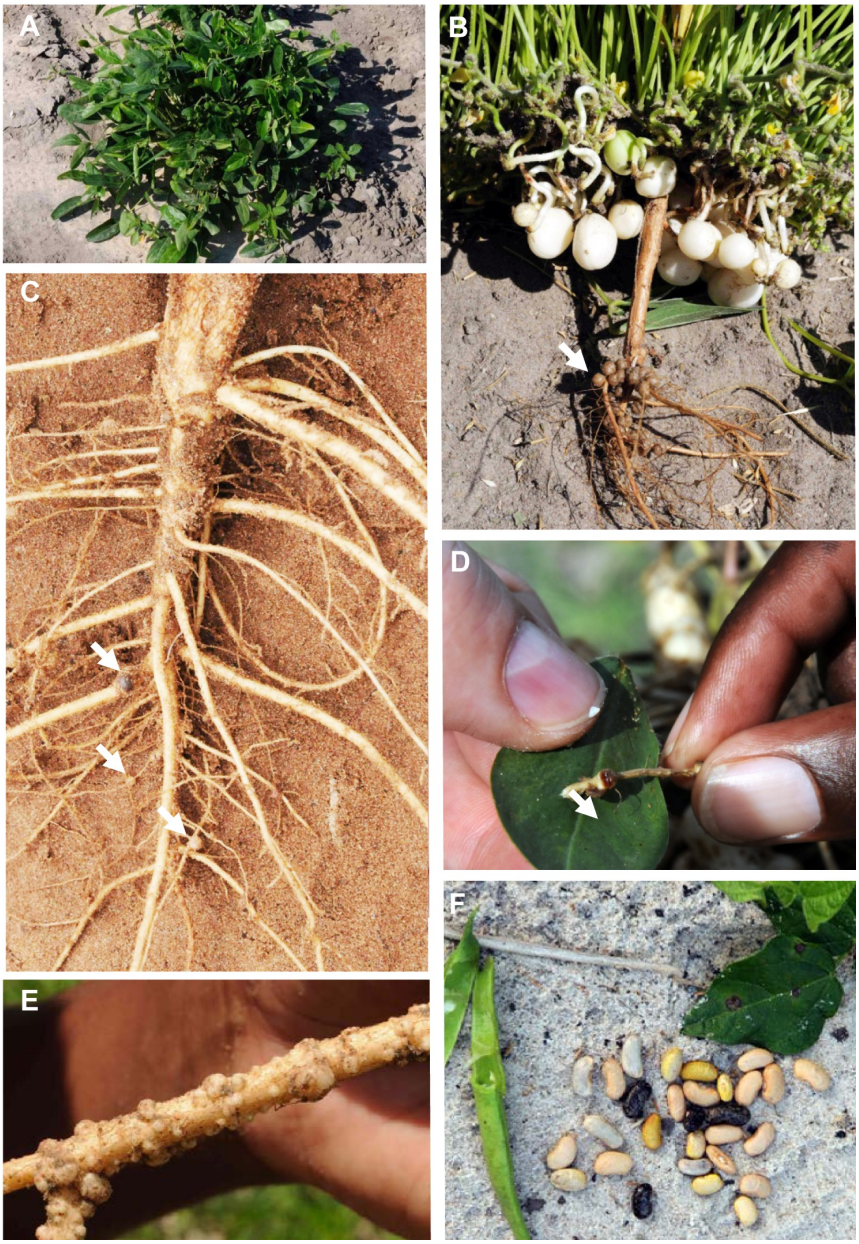

Fig. 3: Examples of nodulated grain legumes detected during the survey in Namibia. (A) Bambara groundnut (*Vigna subterranea*) plant and (B) well-nodulated root system with groundnuts. (C) Cowpea (*Vigna unguiculata*) root system and (D) detached root nodule cut open. (E) Well-nodulated roots of cowpea. Arrows point towards examples of root nodules. (F) Beans (*Phaseolus vulgaris*) cultivated in smallholder farms in Angola.

low degree of nodulation (Fig. 3 C, D), similar observations were made at the Angolan site. Therefore, a survey of traditional legume crops was undertaken for detection of nodulated plants, in order to isolate putatively efficient bacterial symbionts adapted to the soil and climatic conditions. Surveys were carried out in Namibia in the rainy season in December/January or March/April. Generally, nodules were more abundant and less senescent earlier in the season. Most samples originated from the Mashare area in the Kavango region (Fig. 1 B, Table 1). The widest range of grain legumes was found at the MADI (Mashare Agricultural Development Institute), where test fields were under irrigation or under rainfed (dryland) conditions typical for smallholders` farms. Here, cowpea, Bambara groundnut, peanut, and *Lablab purpureus* were cultivated. Several of these plants were nodulated, albeit often poorly. However, from several plants and species, diazotrophs could be isolated

from nodules (Table 1). On some fields of subsistence farmers, nodulated plants appeared to be relatively rare: for example, only from 3 out of 10 inspected individual plants of Bambara groundnut on site H, root nodules could be detected. On the other hand, in a smallholder farmer's field where mainly grain legumes were grown since several years (site M), some root systems were intensely nodulated (Fig. 3 E). Here an enrichment of compatible rhizobia might have occurred over the years.

In total, we could isolate diazotrophs from nodules collected in Southern Africa from several different grain legumes and from different sites: for cowpea, from 9 sites in Namibia and 3 sites in Angola; for Bambara groundnut, from 2 sites in Namibia; for peanut, 5 sites in Namibia; for *Phaseolus vulgaris*, from 4 sites in Angola (Table 1).

In order to increase the diversity of isolates from the underrepresented crop Bambara groundnut, we carried out trapping experiments. Soil was collected from different regions and land use forms (dryland and irrigated agriculture, bushveld in Namibia; fallow, dryland agriculture and pristine grassland in Angola) and transported to Germany with cooling. Surface sterilized grains of Bambara groundnut or occasionally cowpea were transplanted into the soils under aseptic conditions, and the root systems inspected for nodulation of root systems. This approach was also successful for isolation of diazotrophs (Table 1). Initial characterization of isolates indicated that the most diazotrophs, except for those isolated from *Phasaeolus* nodules, belonged to *Bradyrhizobium spp.*.

Survey of cereals and native plants for putatively plant-growth-promoting rhizobacteria and phosphate-solubilizing bacteria

In the Kavango region of Namibia, roots of local cereals like mahangu and sorghum as well as native plants were used for isolation of root-associated bacteria. From 12 different plant samples, 32 isolates were obtained (Table 2).

From roots of grass samples in the Seronga region of Botswana (Table 3), also numerous bacteria were isolated. Many isolates showed different abilities to solubilize phosphate on the different media. Of the many isolates obtained, only nine showed the ability to solubilize phosphate on all the phosphate agar media used. Maximum solubilization was observed on potassium phosphate agar medium. Table 4 shows the solubilization ability of the different isolates on potassium phosphate agar.

Table 4. Diameter of zone of clearance for the different isolates with time (days) on potassium phosphate agar. Values are millimeters. Means followed by the same letter in the same column are not significantly different from each other at 5% according to the Tukey-Kramer test.

Isolate	Grass source	12 d	17 d	19 d	23 d	24 d	27 d
S1	*Eulesine africana*	4b	4.5b	4.6b	5.4b	5.6b	9c
S2	*Imperata cylindrica*	1a	2a	2a	2a	2a	2a
S3	*Imperata cylindrica*	1a	2a	2a	2a	2a	2a
S4	*Sesbania sesban*	1a	2a	2a	2a	2a	2a
S5	*Panicum maximum*	0.6a	2a	2a	2a	2a	2a
S6	*Cyperus sp.*	0.4a	1a	2a	2a	2a	2a
S8	*Cynodon dactylon*	4b	5b	7b	8c	8c	8c
S9	*Urochloa decumbens*	2a	5b	6b	6bc	7c	7bc
S10	*Urochloa trichophus*	2a	4b	5b	5b	6bc	6bc

Botswana soils due to the low rainfall are often slightly alkaline and contain cations which form complexes with phosphorus. Such P deficiencies are very common in most Botswana soils. Phosphorus also forms insoluble complexes with other cations such as aluminium, magnesium, iron and potassium in soil (Thompson & Troeh 2005). It is, therefore, very crucial that any isolate that is intended to be used in the production of a phosphate solubilising biofertilizer should be able to solubilise different phosphate complexes. As such in this study the solubization ability of the isolates was also tested on different agar media i.e., potassium phosphate, magnesium phosphate, iron phosphate, and aluminium phosphate. And only those which showed the ability to solubilize all the phosphates will be selected for further studies.

Conclusions

Our survey has generated a large number of bacteria which may contribute to promote plant growth and soil fertility. Inspection of root systems of grain legumes in smallholder's fields showed large variations in root nodule symbioses, ranging from poorly or rarely to intensely nodulated. Isolation of putatively symbiotic bacteria may help to develop inoculants adapted to the crops and the harsh environmental conditions. Considering that the soil nutrient status in these areas is generally poor and especially low in N content, the role microorganisms in enhancing crop production cannot be ignored or overstated. More work is thus required in this regard to design bioformulations that can be deployed in the areas to boost crop production. Moreover, indigenous grasses in Seronga flood plains flourish in environments where crops show serious P deficiencies, probably because they habour P-solubilising bacteria which they use in acquiring P. In future research, the isolates' ability to solubilise phosphate complexes could be exploited for crop growth.

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