Review

Assessing the biological potential of N_2-fixing Leguminosae in Botswana for increased crop yields and commercial exploitation

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The Leguminosae are a major source of food, fodder, timber, phytochemicals, phytomedicine, nutriceuticals and N fertility in Botswana. Although the country is beset by drought as a result of low rainfall (150 – 650 mm per year) and high potential evapotranspiration, the Leguminosae appear adapted to this low-nutrient environment. Conducting field inoculation trials with infective rhizobia and selecting legume genotypes or host/strain combinations for increased plant growth would help identify superior symbiotic performers under the environmental stress conditions of Botswana. Thus, selecting high N_2-fixing legume genotypes has the potential to overcome low soil fertility as a production constraint in Botswana, and increase fodder and grain production to support the growing local livestock and wildlife industries. Additionally, identifying native rhizobia in Botswana that alleviate water stress when in symbioses with legumes could be a first step to tapping the biological potential of the Leguminosae for increased yields in drier environments.

Key words: Leguminosae, N_2 fixation, crop yields, Botswana, rhizobia.

INTRODUCTION

The major constraints to exploiting the biological potential of symbiotic Leguminosae and other crop species in Botswana include inadequate water, soil degradation, low soil nutrient concentrations, and high soil temperatures. Rainfall in Botswana varies from 650 mm in the north to 150 mm in the south-western part of the country. Its occurrence is not only low and erratic, the distribution and duration are also short (Bhalotra, 1987a, b). So while natural soil moisture may be too low to support crop development over a longer period of time, collected surface water in the form of dams is also non-existent in Botswana for irrigation. Treated water from waste plants is currently used on a small scale to irrigate crops, but the high NO_3 concentration in such waste effluents can inhibit nodulation and N_2 fixation in symbiotic legumes (Streeter, 1988; Dakora, 1997; Ayisi et al., 2000), and thus reduce grain yields.

Compounded in this, are the very high ambient temperatures which usefully promote photosynthetic rates under atmospheric conditions, but drastically increase potential evapotranspiration (PET), defined as the amount of water transpired in a given time by crop, completely shading the ground, of uniform height and with adequate water status in the soil profile) in soil, thus further decreasing soil moisture needed for root growth and the survival of mutualistic soil microbes such as rhizobia and arbuscular mycorrhizal fungi. Unfortunately, in Botswana, potential evapotranspiration rates are quite high, ranging from 1500 to 2000 mm per annum, levels which far exceed annual rainfall. These high rates of water loss through evapotranspiration tend to reduce the effectiveness of natural rainfall in supporting plant growth and crop yields, a problem which underscores the need to develop drought-tolerant crop species to meet the dry conditions of Botswana. Furthermore, soil processes such as nitrification and organic matter decomposition are also reduced or inhibited because of a decrease in microbial numbers due to high temperatures. A recent study at Sebele in Botswana showed that soil concentrations of N and P were about 0.003% and 0.00003%, respectively (Pule-Meulenberg, unpub. data),

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levels too low to support growth of crop plants and their associated microsymbionts. Those data suggest that nitrification rates were probably low because the effect of the high temperatures decreased the population of nitrifying bacteria in soil, or the adverse effects of low soil pH reduced nitrifier numbers.

Furthermore, the low concentration of nutrients in the soils of Botswana is an added constraint to increased crop yields in that country (Pule-Meulenberg, 2003). Historically, many of the soils have originated from aeolian parent materials, and are therefore low in pH and mineral nutrients, with poor water retention and cation exchange capacity (Brady and Weil, 2002). The Arenosols and Regosols, which dominate in Botswana, are highly leached acidic soils (85% sand) with low nutrient concentrations, especially N, P, Ca, and Mg in both the top and subsurface soils (Table 1). Conse-quently, identifying crop species that are adapted to low nutrient conditions is critical for increased grain yield in Botswana.

This review examines the potential of the Leguminosae in Botswana for promoting increased and sustainable crop yields in a country beset by major environmental stress factors.

THE LEGUMINOSAE: THEIR DIVERSITY, DISTRIBU-TION AND UTILISATION IN BOTSWANA

The Leguminosae are unique in their ability to form N_2-fixing symbioses with members of the Rhizobiaceae (or "rhizobia", namely, *Rhizobium, Bradyrhizobium, Sinorhi-zobium, Mesorhizobium, Azorhizobium* and *Allorhizo-bium*). Inside root nodules, these rhizobial bacteria are able to reduce atmospheric N_2 into NH_3 via the GS/GOGAT (glutamine synthetase/glutamate-oxogluta-rate amidotransferase) pathway and exchange this nitro-genous solute for photosynthate from the host plant (Dakora, 1994). This mutualistic relationship between the Leguminosae and the Rhizobiaceae forms the basis for the ecological importance of legumes in natural and agricultural ecosystems in promoting increased crop yields. But as indicated previously, tapping the biological potential of these legumes in Botswana is currently constrained by environmental factors such as low soil pH, high soil temperatures, low soil moisture, and low mineral nutrients.

Fortunately, the leguminous floras of Botswana are highly rich in species diversity, and seem well adapted to growth in a wide range of habitats within the different agro-ecological zones. Of the Leguminosae, the Mimosaceae occupy about 70% of the land area of Botswana, including the Kalahari sands, followed by the Papilionaceae and the Caesalpiniaceae (Table 2). A few of these species such as *Elephantorhiza burkei*, *Acacia haematoxylon*, *Acacia hereroensis*, *Acacia burkei* and *Acacia grandicornuta* are endemic to Botswana and

areas south of the Zambezi river (Van Wyk and Van Wyk, 1997). Given the widespread distribution of the N_2-fixing Leguminosae in Botswana, biological N_2 fixation must be the major pathway for N input into the natural and desert ecosystems of that environment. The dominance of N_2-fixing Mimosaceae, especially the *Acacia* species, in these sandy low-nutrient soils of Botswana must reflect their symbiotic adaptation to this harsh environment. Yet few studies, if any, have examined the Mimosaceae of Botswana for their symbiotic performance or dependence on N_2 fixation for their N nutrition.

Although the ecological significance and biological basis of their distribution is yet to be assessed, in Botswana the Caesalpinioid legumes occur mostly in the north where the rainfall is greater than 500 mm per annum. One of the most important of these Caesalpi-nioids in Botswana is *Colophospermum mopane*. This species serves not only as a browse legume for livestock and wildlife during the winter, but also as foliage for *Imbrasia belina*'s caterpillars, which serve as a high protein food for resource-poor rural communities. The dry roasted caterpillars are not only sold at local markets in Botswana, but also exported to South Africa, Zimbabwe and Mozambique as dietary delicacy and livestock feed. Studies are therefore urgently needed to evaluate the foliage-feeding effect of these caterpillars on plant growth and N_2 fixation in *Colophospermum mopane* if this local industry is to grow and economically empower rural communities in Botswana.

Members of the Papilionaceae such as cowpea (*Vigna unguiculata*), Bambara groundnut (*Vigna subterranea*), soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), groundnut (*Arachis hypogea*) and others are also known for their role as food legumes (Sprent, 2001) and nutriceuticals. The leaves and grain are not only used as high-protein diet, but also as high-protein fodder for livestock. In Botswana, cowpea, Bambara groundnut and groundnut are the most commonly grown food legumes possibly because of their relative adaptation to this drought-prone, low-nutrient environment. Whether in rotation or intercrop, studies in Botswana have shown the beneficial effects of fixed-N from symbiotic legumes to cereal crops such as sorghum. So far, however, no studies have quantified legume N_2 fixation nor investiga-ted the indigenous rhizobial bacteria that nodulate these legume flora in Botswana. It is noteworthy that *Pterocar-pus angolensis*, a woody Papilionoid legume comercially exploited for high quality timber and furniture in Bots-wana, is currently facing extinction from over-exploitation and dieback disease (M. Obopile, pers. comm.). Collec-ting and biologically evaluating landraces, accessions and ecotypes of this legume could increase our under-standing of its biology and hence save the local furniture and timber industry.

Like the acacias within the Mimosaceae, most mem-bers of the Caesalpiniaceae are trees, which exhibit a low level of nodulation ability with rhizobia, except for a few

Table 1. Chemical properties of the topsoil and subsoil in the different agro-ecological zones of Botswana (Anon, 1990; Pule-Meulenberg, unpub. data). Ca, Mg and P are extractable concentrations.

Location	pH (CaCl$_2$)		Available P (mg/kg)		% Org. C		EC (dSm^{-1})		Ca (mg/kg)		Mg (mg/kg)		% Clay	
	Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil
Gaborone Region														
Ramotswa	5.3	4.3	11.0	3.0	0.4	0.2	0.0	0.1	0.25	0.29	0.06	0.041	14.3	18.6
Molepolole	6.8	7.8	3.0	0.25	0.6	0.3	0.1	0.2	0.73	0.85	0.02	0.046	14.0	26.0
Sebele	4.5	4.7	3.0	2.2	0.2	0.2	0.0	0.0	0.038	0.03	0.008	0.019	15.0	22.0
Ngami/Chobe region														
Panda1	4.8	4.3	12.0	2.6	0.5	0.2	0.0	0.0	0.02	0.01	0.006	0.009	5.0	11.0
Panda2	5.9	6.5	4.0	0.8	0.5	0.4	0.1	0.2	0.52	0.30	0.17	0.19	58.0	64.0
Maun	4.2	3.7	4.0	3.7	5.3	1.2	0.0	0.0	0.29	0.09	0.06	0.022	69.0	43.0
Central Region														
Dukwi	7.9	8.8	3.0	1.8	0.2	0.1	0.5	12.2	0.41	0.56	0.008	0.014	4.0	17.0
S/Phikwe	7.0	9.3	1.0	1.0	0.2	0.06	0.1	6.9	0.08	0.19	0.007	0.006	10.0	9.0
F/Town	5.3	4.3	3.0	2.5	0.4	0.1	0.0	0.0	0.04	0.018	0.006	0.004	20.0	35.0
Kalahari Region														
Tshane	5.2	6.1	0.3	0.02	0.3	0.02	0.0	0.0	0.008	0.008	0.0024	0.005	5.0	1.0
Tsabong	5.2	4.9	1.5	1.2	1.5	1.2	0.0	0.0	0.04	0.028	0.007	1980	4.0	2.0
Ghanzi	8.2	9.3	7.2	6.4	1.0	0.6	5.1	7.5	0.04	0.60	0.18	96	3.0	1.0
Southern Region														
Goodhope	6.2	6.0	1.0	0.3	0.2	0.1	0.0	0.0	0.15	0.09	0.006	0.01	20.0	35.0
Moshupa	7.6	7.6	2.0	2.0	0.6	0.6	0.1	0.1	0.10	0.10	0.15	0.15	20.0	20.0
Jwaneng	6.2	6.5	1.0	1.0	0.2	0.2	0.0	0.1	0.04	0.04	0.006	0.006	5.0	6.8

Table 2. Members of the Leguminosae found in Botswana (Van Wyk and Van Wyk, 1997; Geissler et al., 2002; Geyid et al., 2005)

Legume	Distribution	Sub-family	Uses
Trees/Shrubs			
Acacia ataxacantha	Maun, Western	Mimosaceae	Browse
A.burkei	Gaborone	Mimosaceae	Browse
A. erubescens	Maun, Central	Mimosaceae	Browse
A. fleckii	Maun, Central	Mimosaceae	Browse
A. galpinii	Maun, Central	Mimosaceae	browse
A. mellifera	Maun, Central, Gaborone, Western		

Table 2. Contd.

A. nigresences	Maun, Central	Mimosaceae	Browse
A. senegal	Central	Mimosaceae	Gum Arabia, browse
A. tortilis	Maun, Central, Gaborone	Mimosaceae	Browse, medicine
A. arenaria	Central	Mimosaceae	Browse
A erioloba	Maun, Central, Gaborone, Southern, Western	Mimosaceae	Browse, edible gum, medicine
A.gerradii	Central, Southern	Mimosaceae	Browse, twine, medicine
A.grandicornuta	Maun, Central	Mimosaceae	Browse
A. haematoxylon	Kalahari	Mimosaceae	Browse
A. karoo	Maun, Central, Gaborone, Western	Mimosaceae	Browse, tanning edible gum
A. kirkii	Maun, Central	Mimosaceae	Browse
A. newbrownii	Maun, Central, Western	Mimosaceae	Browse
A. nilotica	Maun, Central, Gaborone	Mimosaceae	Browse firewood, fencing, edible gum, medicine
A. rehmannia	Central	Mimosaceae	Rope
A. robusta	Maun, Central	Mimosaceae	Browse
A. sieberiana	Maun	Mimosaceae	browse
A. polycantha	Maun	Mimosaceae	adhesive as gum, confectionery, tanning, medicine
A hereroensis	Gaborone, Southern	Mimosaceae	Browse?
A. hebeclada	Maun, Central, Western, Central	Mimosaceae	Browse
A. schweinfurthii	Maun	Mimosaceae	Browse
Faidherbia albida (A. albida)	Maun, Central	Mimosaceae	Browse, medicine
Elephantorrhiza burkei	Maun, Central, Western, Gaborone	Mimosaceae	Medicine
Dichrostachys cinerea	Maun, Central, Western	Mimosaceae	Browse, fibre, fencing, medicine
Albizia amara	Maun, Central	Mimosaceae	Soap, medicine
Albizia anthelmintica	Maun, Central, Western	Mimosaceae	Browse, medicine (tapeworm)
Albizia harveyi	Maun, Central	Mimosaceae	Browse
Alibizia versicolor	Maun	Mimosaceae	Timber, browse, soap substitute, medicine
Baphia massaiensis	Maun, Central	Papilionaceae	Browse
Lonchocarpus nelsii	Maun, Central, Western	Papilionaceae	Browse
Lonchocarpus capassa	Maun, Central	Papilionaceae	Ornaments, browse, medicine
Mundelea sericea	Maun, Central, Gaborone, Southern	Papilionaceae	Browse, medicine
Dalbergia melanoxylon	Maun	Papilionaceae	Ornaments woodwind musical instruments, medicine
Bolusanthus speciosus	Cenrtral	Papilionaceae	Medicine
Burkea Africana	Maun, Central	Caesalpiniaceae	Tanning, medicine, edible caterpillars of *Cirina forda*, medicine
Peltroforum africanum	Maun, Central, Gaborone, Southern	Caesalpiniaceae	Browse, medicine
Colophospermum mopane	Maun, Central	Caesalpiniaceae	Furniture, firewood, browse, edible caterpillars of *Imbrasia belina*
Guibourtia coleosperma	Maun, Central	Caesalpiniaceae	Edible seeds

Table 2. Contd.

Pterocarpus angolensis	Maun	Caesalpiniaceae	Furniture, ornaments, browse, medicine
Bauhinia petersiana	Maun, Central, Gaborone	Caesalpiniaceae	Medicine, coffee substitute
Piliostigma thonnigii	Maun, Central `	Caesalpiniaceae	Twine, red dye, blue dye, browse
Baikiaea plurijuga	Maun	Caesalpiniaceae	Timber (Zimbabwe teak)
Amblygonocarpus andongensis	Maun	Caesalpiniaceae	Pods used as rattles
Erythrophleum africanum	Maun	Caesalpiniaceae	Timber, gum
Brachystegia boehemii	Maun	Caesalpiniaceae	Tanning, fibre, railway sleepers
Julbernardia globiflora	Maun	Caesalpiniaceae	Timber, fibre
Dialium engeleranum	Maun	Caesalpiniaceae	Edible fruits
Parkinsonia africana	Western	Caesalpiniaceae	Ornaments and curios
Swartzia madagascariensis	Maun	Caesalpiniaceae	Browse, insecticide
Grains			
Vigna unguiculata	Maun, Western, Gaborone, Central, Southern	Papilionaceae	Food, fodder
Phaseolus vulgaris	Maun, Western, Gaborone, Central, Southern	Papilionaceae	food
Phaseolus acutifolis	Maun, Western, Gaborone, Central, Southern	Papilionaceae	food
Phaseolus aureus	Maun, Western, Gaborone, Central, Southern	Papilionaceae	food
Phaseolus mungo	Maun, Western, Gaborone, Central, Southern	Papilionaceae	food
Glycine max	Maun, Western, Gaborone, Central, Southern	Papilionaceae	Food, fodder
Vicia faba	Maun, Western, Gaborone, Central, Southern	Papilionaceae	Food,
Pisum sativum	Maun, Western, Gaborone, Central, Southern	Papilionaceae	food
Cajanus cajan	Maun, Western, Gaborone, Central, Southern	Papilionaceae	Food, fodder
Arachis hypogea	Maun, Western, Gaborone, Central, Southern	Papilionaceae	Food, oil

species (Sprent and Parsons, 2000; Sprent, 2001). In Botswana, the symbiotic status of the Caesalpinioid genera remains unknown, even though most of these tree legumes are highly prized for their durable wood, edible fruits, chemical dyes and medicinal use (Table 2). As documented elsewhere (Dakora, 1995), African tree and shrub legumes are a major source of phytomedicine. The roots of *Acacia mellifera, Millenia ferruginea, Pilistigma thongii, Sesbania sesban, Albizia malorcophylla, Albizia coriaria, Erythrina abyssinica, Indigofera arrecta* and *Erythrina brucei* are, for example, used to treat various physiological disorders, including elephantiasis, leprosy, wet eczema, conjunctivitis, measles, intestinal worms and uterine activity in humans (Table 2; Dakora, 1995). Similarly, the bark of *Tephrosia* species is used against lymphadenopathy, while the resin of various *Acacia* species is used to treat gum bleeding and headaches (Dakora, 1995). Although many of these treatments have not yet been clinically validated, there is no doubt that the Leguminosae play a major role as phytomedicine in the healthcare system of African people. So, understanding the biology of these legume species is a first step to their conservation and cultivation for increased economic utilisation, including their medicinal application.

Outside the export of diamonds and other precious minerals, Botswana's next major income earners are ecotourism (from the development of National Parks), and livestock (through the increased production of beef cattle). In Botswana, the wild herbivores in national parks depend largely on fodder from the Leguminosae, especially the *Acacia* members of the Mimosaceae, for improved nutrition. The livestock component, which includes cattle, sheep and goats, also depend on natural grazing of the Leguminosae and indigenous grasses for increased body weight, important for meat production. Because of their ability to obtain biologically-fixed N form their root associated rhizobia, symbiotic legumes are the main source of high-protein feed for both livestock and wildlife. Assessing the Leguminosae of Botswana for N_2 fixation therefore has the potential to identify fast-growing, high N_2-fixing species and genotypes for increased protein production to support the developing livestock and wildlife industries. Whether in Botswana, South Africa, Kenya or Tanzania (where national parks are more prevalent in Africa), no study has critically evaluated the effect of wild grazers on the vegetational composition of those ecosystems, especially the Leguminosae. It is therefore not known whether grazing pressure has caused extinction of some leguminous species in national parks in Africa, nor do we currently know the effects of big grazers on plant growth, N_2 fixation and species diversity of symbiotic Leguminosae in the nationnal parks of Botswana and elsewhere in Africa.

THE RHIZOBIACEAE: THEIR DIVERSITY, NODULATION AND N_2 FIXATION IN THE LEGUMINOSAE

Bacterial geneticists have so far identified six genera within the Rhizobiaceae that have the ability to infect roots and form N_2-fixing nodules in members of the Leguminosae and in the non-legume *Parasponia* (Ulmaceae). These genera of root-nodule bacteria ("rhizobia") include *Rhizobium, Bradyrhizobium, Sinorhi-zobium, Mesorhizobium, Azorhizobium* and *Allorhizobium* (Sprent, 2001). Their ability to specifically nodulate a particular legume, but not another, is based on the conc-entration, types and profile of lipo-chito-oligosacha-rride nodulation (Nod) factors that these bacteria produce and release into the rhizosphere environment of their host plant. However, because the legume/rhizobia symbiosis involves a two-way molecular conversation, this nodula-tion specificity is also determined by the quality and quantity of chemical signal molecules such as flavonoids, betaines and aldonic acids released by the host plant (Dakora, 1994). As a result of the genetic specificity dictated by bacterial Nod factors and the legumes's inducer signals, rhizobia from the six genera generally tend to nodulate specific groups of legumes, hence the so-called cross-nodulation groups (Fred et al., 1932).

Incidentally, members of each tribe within the Leguminosae seem to synthesise and release the same type of unique flavonoid molecules for nod-gene induction in symbiotic rhizobia. For example, members of the tribe Phaseoleae generally release similar root-based molecules (deidzein, genistein and coumestrol) that induce the expression of nod genes in Phaseoleae-nodulating rhizobia (Dakora, 2000). Whether symbiotic tribes within the Rhizobiaceae behave similarly, is yet to be properly established. Taxonomically, all the six genera of rhizobia with nodulation ability in legumes are α-proteobacteria, but some newly discovered strains include *Methylobacterium*, which nodulates *Lotononis bainesii* in South Africa (Jaftha et al., 2002) and *Crotolaria* in Senegal (Sy et al., 2001), as well as *Blastobacter denitrificans* and *Devosia* strains, which respectively nodulate the aquatic legumes *Aeschenomene* and *Neptunia* (van Berkum and Eardly, 2002; Rivas et al., 2002) in the tropical environments of Africa, Asia and South America.

Recently, however, a *Burkholderia* strain belonging to the β-proteobacteria was isolated from root nodules of *Aspalathus carnosa* in South Africa with nodulating ability on siratro (Moulin et al., 2001). *Ralstonia taiwanensis*, another β-proteobacteria was isolated from nodules of *Mimosa* species, and has been similarly confirmed to nodulate and fix N_2 in the homologous *Mimosa* host plants (Chen et al., 2003). Interestingly, the *Mimosa* is a pantropical genus that has been reported in earlier studies to nodulate with α-proteobacteria-type rhizobia in Phillipines, Mexico and Brazil (Oyainzu et al., 1993; Moreira et al., 1993; Wang et al., 1999), and now with strains of *Ralstonia taiwanensis* belonging to β-proteobacteria (Chen et al., 2003). Because these new nodulating *Ralstonia* and *Burkholderia* strains lack homologous sequences found in other rhizobial organisms, it is likely that they obtain their nodulation genes from "α-rhizobia" through lateral gene transfer.

Table 3. Estimates of fixed-N in symbiotic legumes in Africa.

Legume species	Country	Amount fixed kg N/ha	Reference
Food legumes			
Phaseolus vulgaris	Kenya	17-57	Ssali and Keya (1984)
Vigna unguiculata	Kenya	24-39	Ssali and Keya (1984)
	Ghana	201	Dakora et al. (1987)
	Nigeria	122	Eaglesham et al. (1981)
Arachis hypogea	Ghana	32-134	Dakora (1985b)
	Nigeria	11-63	Sanginga et al. (2003)
	Kenya	8	Guthambi et al. (2002)
Glycine max	Nigeria	15-125	Eaglesham (1982)
	Nigeria	94	Sanginga et al. (2001)
Cajanus cajan	Kenya	142	Guthambi et al. (2002)
	Zimbabwe	183	Mapfumo et al. (1999)
	Nigeria	86	Sanginga et al. (2001)
Vigna subterranea	Ghana	40-62	Dakora (1985b)
Tree/shrub legumes			
Leucaena leucocephala	Tanzania	110	Hogberg and Kvarnstrom (1982)
	Nigeria	448-548	Sanginga et al. (1985)
	Nigeria	304	Danso et al. (1992)
Sesbania sesban	Senegal	43-102	Ndoye and Dreyfus (1988)
	Kenya	52	Guthambi et al. (2002)
Lablab purpureus	Nigeria	215	Sanginga et al. (2001)
Acacia senegal	Nigeria	< 20	Sanginga et al. (2001)
	Senegal	5.25	Sprent and Parsons (2000)
Acacia seyal	Senegal	8.32	Sprent and Parsons (2000)
Acacia tortilis	Senegal	6.24	Sprent and Parsons (2000)
Acacia albida	Nigeria	< 20	Sanginga et al. (2001)
Albizia spp.	Nigeria	60-120	Kadiata et al. (1996)

Whatever the case, it is clear from these recent findings (Moulin et al., 2001; Vandamme et al., 2002; Chen et al., 2003) that many members of the Mimosaceae commonly found in Botswana and elsewhere in Africa are probably nodulated by non-rhizobial bacteria through gene transfer. It would therefore be interesting to know the proportion of nodule symbioses in the Mimosaceae of Botswana that are formed by "α-rhizobia" compared with "β-rhizobia" (Moulin et al., 2002).

Irrespective of whether symbiotic legumes are nodulated by "α-rhizobia" or "β-rhizobia" (Moulin et al., 2002), the benefit of these bacteria to the host plant is generally determined by the amount of N_2 fixed and released into the host-plant cells. The accumulated fixed-N in plant organs is easily quantified using ^{15}N isotope technology and/or the N difference method. As shown in Table 3, levels of N_2 fixation can vary markedly between species, as well as between sites or countries for the same species (Schulze et al., 1991) due to genetic and environmental factors (Dakora and Keya, 1997). For example, in Senegal, *Acacia senegal*, *A. seyal* and *A tortilis* have

been assessed to fix only 5.3, 8.3, and 6.2 kg N/ha, respectively (Sprent and Parson, 2000), levels probably enough to support plant growth, but too low to enhance the N economy of the ecosystem. Similar studies (Schulze et al., 1991) done in Namibia showed that members of the Mimosaceae exhibited varied δ ^{15}N values, ranging from about 0.15‰ for *A. hereroensis* to a high 7.5‰ for *A. tortilis*. Consequently, *A. tortilis* derived only 15 % of its N from symbiotic fixation of atmospheric N_2, and *A. hereroensis*, 50% (Schulze et al., 1991). Considering that Namibia (30-400 mm rainfall) and Botswana (150-650 mm rainfall) share similar dry environments, it would be interesting to know the levels of N_2 fixation in the Mimosaceae of Botswana. However, given the low symbiotic performance of the Mimosaceae in Senegal and Namibia, which have similar soil ecologies with Botswana, it is likely that symbiotic function would be similarly low due to the commonality of the prevailing abiotic factors. Many of the food grain legumes also showed a wide range of symbiotic perfor-mance in the same location (Table 3), thus indicating that these species are

not achieving their full potential in symbiotic N_2 fixation in those locales. Selecting legume genotypes for enhanced N_2 fixation and grain yield under the dry, low soil-nutrient conditions of Botswana is there-fore likely to promote adequate food production in that country and other parts of Africa.

DEVELOPING DROUGHT-TOLERANT LEGUMES FOR BOTSWANA

The level and range of N_2 fixation exhibited by the Leguminosae in Africa (Table 3) clearly indicate the need to select improved legume genotypes for increased N_2 fixation for food and fodder. Because of the generally low soil moisture as a consequence of low rainfall and high potential evapotranspiration in Botswana, any legume improvement programme must select genotypes or host/strain combinations with traits for drought resistance. Fortunately, a few recent reports have indicated the role of bacterial metabolites, including rhizobial molecules, in improving the water relations of plants (Dakora, 2003). Figueiredo et al. (1999) were probably the first to show that inoculating cowpea plants with *Bradyrhizobium* strains alleviated the effect of water stress in that species. So, even without knowing the mechanism involved in the plant's ability to save water with rhizobial inoculation, the use of different rhizobial inoculants on accessions or landraces of local legumes in Botswana could help identify genotypes and/or host/strain combinations that tolerate drought and provide increased grain yields.

In the study by Figueiredo et al. (1999), the leaves of cowpea plants showed decreased stomatal conductance, which then decreased transpiration and reduced water loss with rhizobial inoculation. In a similar study, decreased stomatal conductance and decreased transpiration were also observed when soybean, Bambara groundnut and maize plants were inoculated with infective strains of rhizobial bacteria (Matiru and Dakora, 2005a). Interestingly, applying the bacterium *Achromobacter piechaudi* ARV8 to tomato and pepper plants also conferred resistance to water stress (Mayak et al., 2004), presumably via a decrease in stomatal conductance and leaf transpiration. Clearly, these bacteria must release some chemical molecule that modulates stomatal function, and thus controls water loss.

Further studies involving the application of nanomolar concentrations of lumichrome (a rhizobial metabolite) to roots of legumes, showed that stomatal conductance and transpiration were significantly decreased in soybean and Bambara groundnut plants exposed to the bacterial metabolite (Matiru and Dakora, 2005b). Transpiration was also decreased in leaves of maize (cereal) with lumichrome application to roots (Matiru and Dakora, 2005b). These data suggest that the water-saving effect of rhizobial inoculation of cowpea (Figueiredo et al., 1999) and the resistance to water stress in tomato and pepper

inoculated with *Achromobacter piechaudi* (Mayak et al., 2004) were likely due to the effect of lumichrome released by these microbes. Thus, inoculating legumes in Botswana with high N_2-fixing rhizobial strains could assist in the identification of plant genotypes that can withstand drought and produce yields under dry conditions.

The fact that maize, a cereal, also responded to lumichrome application suggests that rhizobial infection of cereal species could enhance their water relations. In that regard, recent studies have demonstrated the ability of N_2-fixing rhizobia to colonise and infect roots of sorghum and millet (Matiru and Dakora, 2004), and in so doing, plant biomass and root length were increased via an improvement in P and K nutrition (Matiru et al., 2005). Taken together, the data suggest that field inoculation of legumes and cereals in cropping systems in Botswana could potentially result in the identifidation of genotypes with enhanced water relations and improved mineral nutrition for greater grain yields.

IDENTIFYING LEGUME GENOTYPES WITH TOLE-RANCE OF LOW SOIL NUTRIENTS FOR BOTSWANA

Because more than 70% of the soils in Botswana are sandy, nutrient-poor, and low in pH (Table 1), any attempt to increase food production would require the selection of crop plants with adaptation to low-nutrient conditions. Several studies have shown that plants can adapt to low-nutrient environments (Adu-Gyamfi, 2002) through the development of various nutrient acquisition strategies. For example, the presence of massive root hairs often developed in response to nutrient deficiency can substantially increase the root surface area available for nutrient uptake (Zhu et al., 2002), and therefore improve mineral nutrition in crop plants. Traits such as root architecture, number of fibrous roots and density of root hairs determine the legume's nutrient uptake efficiency and are apparently controlled by a multi-gene family (Caradus, 1995).

As discussed elsewhere (Dakora and Phillips, 2002), the exudation of organic acid anions, phenolics and phytosiderephores is a mechanism by which plants in low-nutrient environments adapt to nutritional deficiencies. So, screening legume genotypes for the profile and quality of root exudates should be a first step to identifying legumes adapted to low-nutrient conditions in Botswana. Interestingly, legumes such as pigeon pea release piscidic acid into root exudates as a mechanism for mobilizing unavailable P in low-nutrient soils (Ae et al., 1990). So quantifying the concentration of piscidic acid in root exudates of this legume could be a tool for selecting pigeon pea genotypes adapted to low-P conditions.

Additionally, with adequate technology, genes controlling the transport of mineral nutrients across root membranes could be identified and cloned. The genes for some primary ion pumps, co-transporters, and ion channels

have been cloned and their functions characterised (Dunlop and Phung, 2002). Molecular tools could produce legume genotypes that are better adapted to the low-nutrient soils of Botswana, and thus increase legume yields for food and fodder. Welch and Graham (2002) have however cautioned on the importance of maintaining adequate micronutrient concentrations in food legumes selected for low nutrient conditions. This is because micronutrient malnutrition (involving Fe, Zn and Vitamin A deficiency) currently affects over 40% of the world's population. Fortunately, it seems that micronutrient-rich seeds can increase crop yields and maintain adequate micronutrient levels when cultured in micronutrient-poor soils (Welch and Graham, 2002). However, selecting grain legumes for micronutrient enrichment traits in low-nutrient soils also has prospects for overcoming the health problems associated with crops from nutrient-poor soils.

FUTURE PROSPECTS FOR BIOLOGICAL NITROGEN FIXATION IN BOTSWANA

Because of the huge contribution symbiotic legumes make to the N economy of ecosystems, rhizobia and free living diazotrophs have a potential to reduce farmer's dependence on mineral fertilisers for increased crop yields. The economic incentiveS for exploring novel ways of increasing N_2-fixing efficiency in symbiotic legumes are therefore considerable. Although the legume-rhizobium symbiosis is currently well researched, most studies have concentrated on the genetic manipulation of the micro-symbiont for increased N_2 fixation (Tobar et al., 1996; Neves and Rumjanek, 1997; Zhang et al., 2003; Serraj and Sinclair, 1998; Wolde-Meskel et al., 2004; Jebara et al., 2005). Furthermore, studies on the host legume species have also tended to concentrate largely on the sub-family Papilionaceae (Kahindi et al., 1997) to the neglect of other sub-families. So, despite the extensive uses listed in Table 2 for the members of Mimosaceae and Caesalpiniaceae, little research has been conducted on the indigenous and endemic Mimosaceae and Caesalpiniaceae in Botswana. Similarly, fewer studies have focused on the rhizobia from Mimosaceae and Caesalpiniaceae, although there is the likelihood that new strains isolated from these legumes could be ideal candidates for making inoculants because of their ability to fix N_2 under the dry conditions of Botswana. A great potential therefore exists for the production of bio-fertilisers using local rhizobial isolates from Botswana. Furthermore, investing in microbial fertilisers for legumes and cereals would not only reduce the use of costly chemical fertilisers by farmers, but also decrease environmental pollution originating from the use of inorganic fertilisers.

It is therefore important to note that specific traits possessed by wild legumes and their associated rhizobia in the hot, dry, low-nutrient soils of Botswana could prove to be an asset for transfer to other crops to improve their tolerance of environmental stress. Rhizobia adapted to the harsh conditions of Botswana could possibly also possess specific genes that confer tolerance when expressed under such stress conditions. Future studies on rhizobia isolated from native Leguminosae in Botswana should focus on gene expression under conditions of abiotic stress such as high temperature, low nutrients and drought. That way, rhizobial strains could to be identified that tolerate environmental stress through gene expression.

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