Ethiop. J. Biol. Sci., 6(1): 77-95, 2007

ISSN: 1819-8678

GENETIC DIVERSITY OF RHIZOBIA IN ETHIOPIAN SOILS: THEIR POTENTIAL TO ENHANCE BIOLOGICAL NITROGEN FIXATION (BNF) AND SOIL FERTILITY FOR SUSTAINABLE AGRICULTURE

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ABSTRACT: Nitrogen is one of the most limiting nutrients to plant growth. It has to be fixed in the form of NH_4 through chemical (fertilizer production) and biological (bacterial) processes (BNF) in the soil. The endosymbiotic associations of root nodule bacteria (rhizobia) with leguminous plants fix 200-500kg N ha^{-1} yr^{-1}. Consequently, the legumes are integrated into different agro-ecosystems for plant production and soil protection. In view of the ever-increasing demand for food and feed for the burgeoning population in the country, the search for cheaper ways of enhancing soil fertility is very important. To that end, many research activities have been undertaken for the last 20 years to realize the full potential of the legume-rhizobia symbiosis in crop production and agro forestry systems. Although the pioneer research works were focused on the agronomic relationship of resident rhizobia with food legumes, recent studies encompassed rhizobial diversity and effectiveness on different pulse crops and other woody shrub and tree legumes. Some of the polyphasic studies on the rhizobia from Southern Ethiopia revealed that Ethiopian soils harbour diverse groups of rhizobia that are very distinct (more than 80%) from the hitherto known taxa of the Family Rhizobiaceae. This suggests that the country has enormous rhizobial resources for more phylogenetic studies and for the selection of elite strains to enhance effective *Rhizobium*-legume symbiosis in its agro ecosystems. In this review, the challenges and prospects associated with the exploitation of BNF in the country, in general, and the potential to develop and promote broad-host range inoculants to small-scale farmers, in particular, will be discussed.

Key words/phrases: Genetic diversity; Indigenous rhizobia; Legume-*Rhizobium* symbiosis; Nitrogen fixation.

INTRODUCTION

Since 1960, the world's population has doubled to 6 billion and is projected to increase to 8 – 9 billion people by the year 2030 – 2040. Despite the gloomy prediction of the 1960's that the food production would not keep pace with the growing population, advances in plant germplasm improvement, the unprecedented use of fertilizers, and expanded use of irrigation have increased food production to surpass the population of the developed countries (Vance, 2001). However, food production and

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insecurity is still a challenge in developing countries such as Africa and Southeast Asia due to breakdown of traditional practices, depletion of soil fertility, lack of purchasing power, and poor distribution (Nandwa and Bekunda, 1998; Sanchaz, 2002).

Over decades, small-scale farmers in developing countries have removed large quantities of nutrients from their soil without using sufficient quantities of manure or fertilizer to replenish it. This has resulted in a very high average annual depletion rate – 22 kg of nitrogen (N), 2.5 kg of phosphorus (P), and 15 kg of potassium (K) per hectare of cultivated land over the last 30 years in 37 African countries, an annual loss equivalent to U.S. $ 4 billion in fertilizer (Stoorvogel and Smaling, 1990; Sanchaz, 2002).

Ethiopia, with the current estimated population of 77 million which grows at a rate of 2.4%, has one of the world's highest incidences of malnutrition. Though agriculture is the most important source of livelihood employing 80% of the labor, the overall food production is far from self-sufficient. In 2005, humanitarian appeal was made for 8.6 million people, about 11 percent of the total population (http://www.wfp.org/country). Subsistence farming is the backbone of the economy and the meagre economic resources of Ethiopian farmers do not allow the use of a mechanized form of agriculture or to spend money on fertilizers. This, therefore, necessitates the intense use of rehabilitation and sustainable management system that can accommodate increased crop production and soil protection.

The legume-*Rhizobium* symbiosis plays a very important role in a productive and sustainable agriculture. While research has indicated many promising avenues for introduction of Nitrogen-fixing plants into cropping systems and for enhancement of the contributions from Nitrogen-fixation, to date few of these technologies have been adopted by farmers. An important note is that most of the technologies that are likely to lead to improvements of Nitrogen fixation in different cropping systems are well within the reach of research programs in developing countries. The technologies can deliver enormous benefits through judicious use of fertilizers, eg phosphorus and exploitation of the genetic diversity and symbiotic effectiveness of the hosts and their corresponding endosymbionts.

This review paper, by exploring the limited number of reports available on soil nutrient balances in Ethiopian soils, explores the genetic diversity and phylogeny of rhizobia indigenous to Ethiopian soils and their potential to enhance *Rhizobium*-legume symbiosis for replenishing soil fertility in the country.

Nitrogen, a limiting nutrient

Even though Nitrogen is one of the most abundant elements on Earth, it is the critical limiting factor for growth and biomass production of plants in terrestrial ecosystems as well as in high productive agricultural systems (Graham and Vance, 2000). It has to be fixed in available forms so as to be assimilated by plants. Plants acquire available Nitrogen from two principal sources: (1) the soil, through commercial fertilizer, manure, and/or mineralization of organic matter; and (2) the atmosphere through symbiotic Nitrogen fixation. In fact, the nitrogen needs of most crop plants are second only to their photosynthetic requirement. Production of high-quality, protein-rich food is extremely dependent upon the availability of sufficient Nitrogen. According to Vance (1998), in today's "Green Revolution", cereal yields of 6 – 9 tons of grain ha^{-1}, take up to 200 – 300 kg N ha^{-1} (Vance, 1998).

In Ethiopia, the nitrogen status of the different soils is found to be generally low. The most comprehensive study commissioned by FAO in 38 sub-Saharan African countries, including Ethiopia showed that there is a high nutrient depletion rates in N P K in different farming systems (Stoorvogel and Smaling, 1990; Stoorvogel *et al*., 1993). The same study revealed that Ethiopia is one of the countries that are characterized by the highest negative soil nutrient balance with national scale aggregated nutrient depletion estimated to be – 41 kg N, – 6 kg P and – 26 kg K ha^{-1} (Table 1).

Table 1 Average nutrient balance of N, P and K (kg ha^{-1} $year^{-1}$) of the arable land for some East and southern African countries over different years (after Stoorvogel *et al*., 1993).

Country	Nutrient balance (NPK) over years					
	N		P		K	
	1982 – 84	2000	1982 – 84	2000	1982 – 84	2000
Botswana	0	– 2	1	0	0	– 2
Ethiopia	– 41	– 47	– 6	– 7	– 26	– 32
Kenya	– 42	– 46	– 3	– 1	– 29	– 36
Malawi	– 68	– 67	– 10	– 10	– 44	– 48
Rwanda	– 54	– 60	– 9	– 11	– 47	– 61
Tanzania	– 27	– 32	– 4	–5	– 18	– 21
Zimbabwe	– 31	– 27	– 2	2	– 22	– 26

Eyasu Elias *et al*. (1998) also examined nutrient balances in the Kindo Koisha area of Southern Ethiopia and found that the main crop fields had strong negative Nitrogen balances in their soils. They also noted that the aggregated farm scale balances varied considerably across socio-economic groups and agro-ecological zones with severe Nitrogen depletion in the highland areas (Table 2). Later on, in a survey reported in the same area, Eyasu Elias and Scoones (1999) indicated that crop yields have been declining due to soil fertility. Generally, gradual depletion of soil nutrients (primarily Nitrogen) from these soils poses serious threat to food production.

Table 2 Nutrient flows (N and P, kg/field) for different field and socio-economic in Kido Koisha district, Wolaita (after Eyasu Elias *et al*., 1998).

Field and group	N		P	
	Highland	Lowland	Highland	Lowland
E1	+ 4	+1	+ 2.8	+ 6.6
E2	0	– 3	+ 1.9	+ 4.0
E3	– 1.2	–10	+ 0.6	– 0.4
E4	– 0.4	– 2	+ 0.2	+ 0.7
D1	– 0.6	– 0.6	+ 2.7	+ 0.8
D2	– 0.5	– 1.0	+ 1.6	+ 2.1
D3	– 0.8	– 2.8	+ 0.8	+ 0.2
D4	– 0.6	– 2.0	+ 0.7	– 0.8
T1	+ 1	–	+ 0.1	–
T2	+ 0.2	–	+ 0.3	–
T3	– 0.5	–	+ 0.2	–
T4	+ 0.4	–	+ 0.08	–
S1	– 51	– 47	+ 6.0	+ 23
S2	– 51	– 37	+ 0.7	+ 7.9
S3	– 17	– 42	+ 2.0	+ 4.0
S4	– 5	– 13	+ 0.1	– 1.5
F1	– 47	– 48	+ 11.7	+ 30.5
F2	– 51	– 41	+ 4.8	+ 17.3
F3	– 19	– 55	+ 3.6	+ 3.8
F4	– 6	– 20	+ 1.1	– 1.6

E – enset garden; D – darkoa; T – taro; S – shoka; F – farm.
1 – resource rich farmer; 2 – medium resource farmer; 3 – resource poor farmer; 4 – very poor farmer.
Shoka is the local name in “Wolaita language” designating one of the three farm plots where cereal crops (mainly, maize, teff, wheat or barley) are cultivated. This farm plot is one of the most important components in terms of contribution to total farm output.

Although increased use of Nitrogen from chemical fertilizers has resulted in significant increases in food production world-wide (Fink *et al*., 1999), Africa's consumption of mineral fertilizers ha^{-1} is the lowest in the world, ranging from 2.2 to 3.9% of global expenditure (FAO, 2001). This is mainly attributed to exorbitant prices of chemical fertilizers which the subsistence small-scale farmers in the region cannot afford. Since soil Nitrogen deficiency is common in the tropics and subtropics (Graham, 1981; Dakora and Keya, 1997; Sanginga, 2003), Nitrogen supply, Nitrogen management and Nitrogen-use efficiency will continue to be significant factors in crop production in the region.

What are Rhizobia?

The term rhizobia collectively refer to symbiotic bacteria capable of invading and eliciting root or stem nodules on leguminous plants, where they differentiate into N_2-fixing bacteroids. Based on 16S rDNA sequences and other parameters, these nodule endosymbionts are classified into 62 species in 12 genera, 10 of which belong to α-*Proteobacteria* and two members of β-*Proteobacteria* (Weir, 2006). These legume microsymbionts are phylogenetically intertwined with several non-symbiotic bacterial genera, comprising pathogenic, phototrophic and denitrifying strains. A remarkable feature of rhizobial ecology is their wide geographical distribution and ability to adapt their life style to the highly contrasting environments they can inhabit. They can be aquatic where they are able to infect and nodulate aquatic legumes such as *Aeschynomene* sp. and *Sesbania* sp. (Wang and Martinez-Romero, 2000); or they can be endophytic in addition to being soil bacteria. They can also be found as large populations of non-symbiotic rhizobia both in bulk soil and in the rhizosphere of legumes and other plants (Segovia *et al*., 1991; Sullivan *et al*., 1996). Some of these saprophytic or rhizospheric bacteria may become symbiotic by acquisition of symbiotic genes, which are required for nodulation of compatible legumes (Sullivan *et al*., 1995).

Symbiotic Biological Nitrogen fixation (SBNF)

The symbiotic association between legumes and rhizobia is the most important biocatalytic link for the flow of Nitrogen between the largest potentially available Nitrogen reservoir, the atmosphere, and the living world (Schlesinger, 1997). A widely cited estimate of global biological nitrogen fixation on land is 140×10^6 tons per year (Burns and Hardy, 1975), though this flux is not uniformly distributed among natural ecosystems. Out of this, the legume-*Rhizobium* symbiosis contributes to

80%; whereas the remaining is assumed to come from non-symbiotic fixation (Schlesinger, 1997). Fixed nitrogen eventually returns to the soil through Nitrogen-rich litter-fall, root and/or nodule turnover and increases soil fertility. Thus, when growing legumes, the results of symbiotic nitrogen fixation and plant growth are twofold: increase in yield and soil fertility. Vance and Graham (1995) indicated that 70% of Nitrogen input into global agriculture comes from symbiotic Nitrogen fixation. This biological process is certainly the cheapest and the most effective tool for maintaining sustainable yields in less developed countries such as Ethiopia where the consumption of Nitrogen fertilizer is limited for economic reasons. In 2003, Ethiopia imported Nitrogen fertilizer worth of 13.5 million USD (FAO, 2005). Introduction of judicious management of SBNF based cropping in traditional agricultural systems is one of the key components of sustainable agriculture, particularly for Africa (Dakora and Keya, 1997). Well planned intercropping and/or crop rotation using effectively inoculated legumes in alternating years, instead of chemical fertilizers, eventually benefits farmers. Thus, SBNF can contribute to the poverty reduction program, tackle environmental problems, and maintain the rare biodiversity resources.

The amount of Nitrogen gained from *Rhizobium*-legume symbioses vary considerably depending on the host species and locations due to differences in soil factors, legume genotypes, rhizobial strains and cropping patterns. Estimates of Nitrogen fixed by different grain legumes in the tropics ranges from few to well above 200 kg ha^{-1} $year^{-1}$ (Dakora and Keya, 1997; Giller, 2001). Though not widely practiced by African farmers, Nitrogen accumulation rates of over 300 kg ha^{-1} $year^{-1}$ have been reported from short season green manure fallow-cover species suited to the tropics (*Canavallia*, *Mucuna*, *Crotalaria*, *Tephrosia*) (Sanginga *et al*., 1996; Becker and Johnson, 1998). Perhaps more spectacular is that legume trees used as alley crops or shade plants have been reported to accumulate as much as 580 kg N ha^{-1} per annum (Nodye and Dreyfys, 1988; Kadiata *et al*., 1996). Estimation of fixed Nitrogen and Nitrogen flow in legume based cropping systems in Africa shows significant Nitrogen benefit (Dakora and Keya, 1997; Ståhl *et al*., 2002) and better whole-farm nutrient budget (Shepherd *et al*., 1995; Kho *et al*., 2001). The positive contribution of symbiotic legumes to associated crops is well acknowledged. However, these benefits could be fully realized only if the legumes are nodulated with effective rhizobia.

Ethiopia represents an important gene center for a few leguminous crop plants (Raven and Polhill, 1981; Hawkes, 1983; Cousin, 1997; Sing, 1997). An internet search on legumes of Ethiopia lists 739 species, out of which more than 80% are indicated to be native to the country (http://www.ildis.org/LegumeWeb/6.00/geo/g15.shtml).

Rhizobial research in Ethiopia

The rhizobial study and collection from indigenous legumes of Ethiopia has begun in the 1980's at Holetta Research Centre, Institute of Agricultural Reseaerch. The works focused on internationally funded projects on cool season legumes such as faba bean, field pea, etc. The major items revolved around the evaluation of symbiotic effectiveness of these legumes, and the data were published in subsequent annual progress reports (Desta Beyene and Angaw Tsigie, 1986). Quite recently, studies on other crops such as haricot bean, and woody legumes have been undertaken (Fassil Assefa and Kleiner, 1997; 1998; Desta Beyene *et al*., 2004; Endalkachew Wolde-meskel *et al*., 2004a). The study on Ethiopian collections (the ACA cultures) showed that Ethiopian soils harbour highly diverse rhizobial population (Endalkachew Wolde-meskel *et al*., 2004a, b, c; 2005).

The most recent works also emphasized the need to delve into the genetic diversity and phylogenetic relationships of rhizobia isolated from root nodules of 19 perennial (tree/shrub) and annual (crop) legume species from the hitherto unexplored part of the country. Morphological characterization using the Biolog system^{TM} (Biolog, Inc., Hayward, CA, USA), and whole genome AFLP (amplified fragment length polymorphism) revealed that the strains are phenotypically diverse and comprise several metabolically and genomically distinct groups where the bulk of the strains (80%) were not related to the known reference rhizobial species (Endalkachew Wolde-meskel *et al*., 2004a,b,c). Further PCR-RFLP (on all collections) and partial sequence analyses of the ribosomal (16S rRNA) genes for representative 67 strains indicated that the collection indeed comprised a large number of undescribed, unknown species of rhizobia (Endalkachew Wolde-meskel *et al*., 2005). By aligning these sequences and those of all the currently described root nodule bacteria of α-*Proteobacteria* subclass (retrieved from the Genbank/EMBL database) and by constructing a phylogenetic tree (Neighbor-Joining method in ClustalX), 46 distinct 16S rRNA sequence types representing six genera, *Agrobacterium*, *Bradyrhizobium*, *Mesorhizobium*, *Methylobacterium*, *Rhizobium* and *Sinorhizobium*, were found (Fig. 1). Twelve of the 46 sequence types showed 100% partial sequence similarity to one or more species of the first four genera while the other 34 were novel and related (with 94%-99% sequence similarity) to one or more members of all the six genera.

In addition to indicating the taxonomic diversity of the collection, the phylogenetic analyses by Endalkachew Wolde-meskel *et al*., (2004b, c; 2005) resulted in several interesting findings including:

- A number of possible new taxa for description; ¯at least four genotypes in the *Rhizobium* lineage (II, IV, VI and VIII) (Fig. 1a); one genotype in *Agrobacterium* (II); three in *Sinorhizobium* (II, III and V) (Fig. 1b); two in *Mesorhizobium* (I and II); several in *Bradyrhizobium* and one in the *Methylobacterium* branchs (Fig. 1c). The distinct nature and a wide range of metabolic and genomic diversity (not related to reference species) that most of the test strains showed (Endalkachew Wolde-meskel *et al*., 2004b,c; 2005) support these groupings.
- Several Ethiopian strains in the *Rhizobium* and *Sinorhizobium* lineages showed identical sequences to undescribed strains isolated from China in the database (Fig. 1a). While there is no known documented history of introduction of rhizobial inoculants from China to Ethiopia or *vice versa*, nor, in fact from any part of the world, it is intriguing to find that our strains are more similar to Chinese strains than the West African strains. A study of a core and accessory genome of these strains (the Ethiopian and Chinese) could reveal interesting insights about rhizobial evolution.
- Three of the four genotypes in the *Agrobacterium* lineages were novel sequences, and it is the first time a strain related to *A. albertimagni*, an arsenite-oxidizing bacterium isolated from a hot creek in California in the USA (Salmassi *et al*., 2002), is isolated from root nodules in an African soil.
- *S. terangae*, *S. arboris* and *S. kostiense* were described from isolates of *A. senegal* and *P. chilensis* growing in East African soils (Kenya and Sudan). Despite extensive sampling, which covered a wide range of ecoclimatic and altitudinal zones in Ethiopia, and use of the same or related trap host species of similar rhizobial affinities, it is surprising that no strains closely related to these species were found.
- Irrespective of host species and site of isolation, the partial 16S rDNA sequences of all but one strain in the *Mesorhizobium* branch

were closely related to *M. plurifarium* (99% similarity). However, these sequences were so heterogeneous that they represented seven different genotypes. Earlier, the strains showed a wide range of differences in their metabolic and genomic characteristics (Endalkachew Wolde-meskel *et al*., 2004b, c). The intergenic spacer gene (ITS) sizes were also very variable (500 – 1200bp).

- In the *Methylobacterium* lineage, one strain (AC72a), which was isolated from root nodules of *P. vulgaris*, was related (90% similarity) to the recently described methylotrophic bacteria, *Mt. nodulans*, and the only symbiotic organism in this genus. In view of the low level of sequence similarity, it is highly likely that AC72a will represent a second species in this genus. In contrast to the very specific nature of *Mt. nodulans*, which is effective only in *Crotalaria* sp., AC72a was able to nodulate at least three host species (*V. unguiculata*, *S. sesban* and *F. albida*).
- The discovery of several strains of rhizobia less related to the described species further strengthens the view that exploration for new nodule forming bacteria should focus on legume species (wild or domesticated) growing in previously unexplored bio-geographical regions, such as Ethiopia.

Desta Beyene *et al*. (2004), the only phylogenetic study available in the literature (other than the work by Endalkachew Wolde-meskel and co-workers), found out that rhizobia they isolated from common bean (*Phaseolus vulgaris*) grown in central Ethiopian harbour natural populations with distinct genomic composition. The same study also indicated that there was no evidence for introduction of rhizobial inoculants to Ethiopian soils. These findings (Endalkachew Wolde-meskel *et al*., 2004 b,c; 2005; Desta Beyene *et al*., 2004) strongly support the view of Odee *et al*. (2002) that this sub-Saharan region might be a center for rhizobial biodiversity. While this warrants further explorations and studies to unearth as yet unidentified rhizobia and to clarify how the distinct population of Ethiopian soils acquired their symbiotic characteristics, the evaluation of the symbiotic efficiency of the Ethiopian strains has great significance to realize their potential for improving the livelihood of the subsistence farmer in the region.

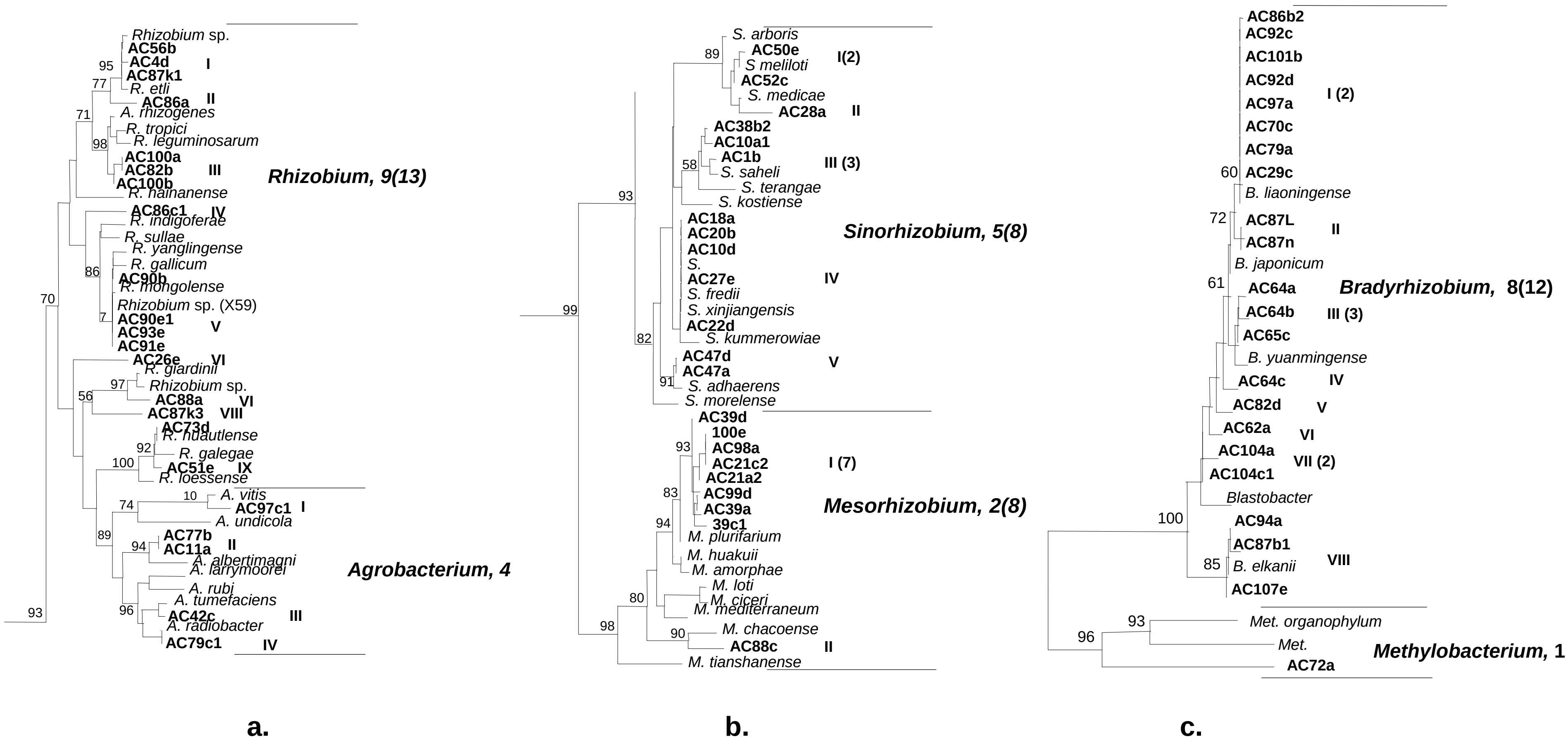

Fig. 1. Phylogenetic tree of Ethiopian rhizobial strains (accessions in bold typeface) within the class '*Alphaproteobacteria*'. **a**. *Agrobacterium* and *Rhizobium* clade; **b**. *Mesorhizobium* and *Sinorhizobium* clade; **c**. *Bradyrhozibium* and *Methylobactrium* clade. The tree was constructed by neighbour – joining method from partial 16S rDNA sequences. Bootstrap probability greater than 50% are indicated at the branch points. Roman numerals indicate groups of different test strains (unnamed) in the respective genus while numbers in parenthesis represent the different 16S rDNA sequence types in the group (after Endalkachew Wolde-meskel *et al*., 2005).

Legumes: the door-way to tap benefits from rhizobial biodiversity

Although crop legumes are good sources of protein and short-term Nitrogen inputs through biological nitrogen fixation, the integration of tree/shrub legumes in cropping systems are becoming all the more important to replenish soil fertility in sustainable, low-input land use systems. The integration of crop and tree legumes is particularly important in the enset land-use systems in Southern Ethiopia where grain legumes form essential complement to the predominantly carbon rich staples (enset, maize, root and tuber crops). It is also well understood that planned crop rotations and/or intercropping with legumes provide considerable potential in sustainable agriculture and thus maintain soil fertility and reduce the need for nitrogen fertilizers. In Ethiopia, pulse crops rank second after cereals both in terms of area coverage (occupying about 17.7% of the total cultivated area) and volume of production (about 12% of the total production) (Asfaw Hailemariam and Angaw Tsigie, 2006). The major food legumes in Ethiopia include field pea, haricot bean, fenugreek, faba bean, chickpea, groundnut, cowpea, grass pea, and lentil.

The crop legumes vary in amount of Nitrogen that they fix in symbiosis with compatible rhizobia (Table 3). In the literature, one can easily find out a striking variation in estimated total Nitrogen accumulation of a given crop legume species, between years and sites as well. Such estimates may indeed have little relevance to local growing condition. To accommodate such variations, Unkovich and Pate (2000) and Giller (2001) compiled, from the literature, ranges in N_2 fixation (rather than average figures) for the different legume crops (Table 3). While the incorporation of legumes in cropping systems results in net accumulation of Nitrogen in general, the amount varies depending on the legume crop species, environmental conditions and availability of rhizobial strain compatible to the target legume species *in situ*.

When planning integration of legumes in cropping systems, it is necessary to consider the differences in Nitrogen fixation capacity and their potential for Nitrogen contribution. For instance, compared to faba bean, field pea, chickpea or common bean (pulses most commonly grown in Ethiopia), soybean is the best in terms of largest amounts of Nitrogen accumulation and Nitrogen fixation (Table 3). The latter is a recent introduction to Ethiopia (Asfaw Hailemariam and Angaw Tsigie, 2006). However, the Nitrogen fixing potentials of this crop (also the other pulses) and occurrence of effective rhizobia compatible to these crops in local soils at different agro ecological zone are not known. Recently, *Rhizobium* inoculation in field experiment on soybean in Awassa resulted in improved crop yield and soil Nitrogen accumulation, reflecting lack of compatible strains in indigenous rhizobial population and hence the need for inoculation (Dereje Asaminew, 2007). Soybean forms a determinate type of nodule (Allen and Allen, 1981). Such type of nodule is characteristically short-lived and several nodule turnovers occur in one growing season, thus significantly improving the soil Nitrogen status. Also, it is interesting to note differences between pulse crops in shoot:root Nitrogen ratio (Table 3). The latter determines the amount of Nitrogen left over in the soil after crop harvest, and thus the net Nitrogen input to the soil. A higher shoot:root Nitrogen ratio contributes less fixed Nitrogen to the soil system, especially if the crop residue is completely removed for animal feed or other purposes which are a common management practice carried out by small holder farmers in Ethiopia. These inherent differences between legume crops in amount of Nitrogen fixation and root Nitrogen turnover potential (shoot:root ratio) represent important research subjects to realize the potentials of these pulse crops to be integrated into different agroecological zones in Ethiopia.

Table 3 Range in N_2 fixation observed for principal annual crop legumes (after Unkovich and Pate[a], 2000; Giller[b], 2001).

Crop	Estimated N_2 fixation (Kg ha^{-1})	Shoot::root Nitrogen ratio
Soybean (*Glycine max*)	0 – 450 [a]	2.4
Common bean (*Phaseolus vulgaris*)	0 – 165 [a]	2.8
Groundnut (*Arachis hypogaea*)	32 – 206 [a]	2.0
Chickpea (*Cicer arietinum*)	0 – 141 [a]	1.4
Field pea (*Pisum sativum*)	4 – 244 [a]	3.0
Lentil (*Lens culinaris*)	5 – 191[a]	1.8
Faba bean (*Vicia faba*)	12 – 330 [a]	1.5
Lupin (*Lupinus angustifolius*)	19 – 327 [a]	2.5
Pigeonpea (*Cajanus cajan*)	0 – 166[b]	–
Mung bean (*Vigna radiata*)	0 – 107 [b]	–
Black gram (*Vigna mungo*)	0 – 140 [b]	–
Cowpea (*Vigna unguiculata*)	9 – 201[b]	–

Grain legumes, similar to their tree/shrub counterparts, show genetic differences in the amount of nodulation and nitrogen fixation between species and varieties (Endalkachew Wolde-meskel *et al*., 2004a; Herridge and Rose, 2000). Also, this represents another untapped biological resource to improve both yield and Nitrogen fixation in legumes through selection of microsymbionts and/or breeding programs (to maximize Nitrogen fixation in the host species). The biodiversity of rhizobial resource resident in

Ethiopian soils (as discussed above) provide ample opportunity to select efficient Nitrogen fixing strains that are compatible to different legume genotypes to be grown in targeted agroecological zones.

Potentials and perspectives

Coupled with the natural conditions like topography and high intensity rainfall, the poor management of the soil accelerates the rate of soil erosion. Studies indicate that soil erosion in the country ranges from 16 to 300 tons $ha^{-1}year^{-1}$ depending mainly on the slope, land cover, and rainfall intensities (Tamire Hawando, 2000). Associated with the soil, the loss of nitrogen ranged from 0.39–5.07 million tons per year and that of phosphorus ranged from 1.17–11.7 million tons per year (Tamire Hawando, 2000) from 78 million ha of cultivated and grazing lands. This is equivalent to 0.85 - 11.02 million tons and 5.8 - 58 million tons of Urea and TSP, respectively. Use of inoculant's technology together with introduction of appropriate soil management/conservation methods is a viable alternative for resource poor farmers in Ethiopia.

Rhizobial strains from Ethiopia have not been previously characterized using modern molecular biological techniques; hence, their diversity was not known. Rhizobial diversity studies contribute to the existing global body of knowledge on the subject, while information on biodiversity of rhizobia in local soils is also an essential prerequisite for selection and promotion of effective symbiosis. However, despite great need and much available potential, there has been very little effort to promote rhizobial inoculant's technologies in agriculture or forestry in Ethiopia. Despite the limited number of samples studied, rhizobia indigenous to Ethiopian soils show high genetic diversity. This provides ample opportunity for improving Nitrogen fixation in legumes, and thus benefit farming. The following points indicate the scope for further work in this area that can be considered in the future.

Assessment of occurrences of compatible rhizobia in target agroecological zones

The benefit of symbiotic Nitrogen fixation is achieved only if the host is nodulated with effective *Rhizobium* strain. There seems geographical differences in nodulation among nitrogen fixing legumes in Ethiopia, thus demonstrating differences in the occurrence of compatible rhizobia resident in soils at different locations, and so the need for inoculation (Endalkachew Wolde-meskel *et al*., 2004a, Asfaw Hailemariam and Angaw Tsigie, 2006;

Dereje Asaminew, 2007). This calls for assessment of nodulation status of legumes (crops or woody species) at various agro ecological zones in the country to establish occurrence of compatible rhizobia and the need for inoculation. This is the first task when planning inoculant technology. Absence of compatible rhizobia, or low population size or presence of less effective strain justifies for inoculation of rhizobia.

Evaluation on growth promoting effect and efficiency of *Rhizobium* strains

Despite a bounty of rhizobial resources resident in Ethiopian soils, the symbiotic efficiency of the strains is not known and remains to be an important impediment for realization of their potentials. The present rhizobial culture collection that the country possesses (at HARC, NSRC, ACA, AAU, HU) represents an already large investment of time, money and effort (among other things, sampling of nodules from roots and the work to obtaining pure isolates in the laboratory). It is, therefore, necessary to evaluate the growth promoting effect and efficiency of these strains in order to develop effective broad-range inoculants for use in agriculture and forestry at various locations. Inoculation of strains to field condition introduces them to dynamic environment where genetic recombination occurs from time to time. Therefore, inoculant development and monitoring of their performance is a continuous activity by its very nature.

Promotion of inoculant technology for improved growth, yield and Nitrogen fixation in legumes

Often the specificity of both symbiotic partners is tight and thus legumes may require bacterial inoculation to realize their yield and Nitrogen fixation potentials. For example, lack of compatible rhizobial strain in the soil has been a problem for production of beans in Wolaita area and soybean in Jima and Sidama zones (Endalkachew wolde Meskel, unpublished data), as well as for production of faba beans in Semen Shewa (Aynabeba Adamu *et al*., 2001). Compared to neighbouring African countries (for instance Kenya and Uganda) in the region where commercial inoculants (BIOFIX) are used by small holder farmers (Odame, 1997), inoculant technology as plant growth promoting factor is very little known in Ethiopia. This calls for testing the efficiency and persistence of rhizobial inoculants in laboratory and field condition at various agro ecological zones for use as inoculants.

Exploration of genetic differences in legume species for improvement of Nitrogen fixation

Cultivars/varieties/provenances in legume species show considerable genetic differences in maturity, in plant Nitrogen derived from the atmosphere (Ndfa) or for Nitrogen fixation and related traits (nodule traits and scores, ureide content of xylem sap, etc). The variability of cultivars of pulse crops such as soybean (Ravuri and Hume, 1993), pigeon pea (Kumar Rao *et al*., 1995; 1996), common bean (Harderson *et al*., 1993) has been demonstrated under various growth environments. Investigation of local and recommended cultivars (including CIAT genotypes) of crop legumes help to select superior accessions for increased amount of Nitrogen fixation at target agro ecological locations.

Development of improved cropping practices and management techniques

Lack of improved crop management practices and low soil Nitrogen fertility are among the main constraints in Ethiopia (Eyasu Elias *et al*., 1998; Eyasu Elias and Scoones, 1999; Asfaw Hailemariam and Angaw Tsigie, 2006). Often, most farmers' spare fertile soils for the production of cereal crops while pulses are grown in marginal soils, usually as a rotation crop, thus rendering them lower yielding (Nitrogen fixing) than their genetic potentials.

Agronomic investigation on several combinations of alternative cropping practices (crop rotation, relay cropping and intercropping) using pulses and their specific micro-symbionts, should be carried out in target agro-ecological zones to realize the yield and soil fertility replenishment effects that a planned incorporation of legumes in cropping systems offers. This should also include formulation of fertilizer (especially P) application and crop residue management strategies

Development of rhizobial inoculant production

Pure and effective strains identified through research will make a difference if only they are produced in large-scale and delivered for use by farmers. To promote this, it is necessary to initiate a small-scale inoculant production and plan an outreach mechanism through agricultural extension services. Inoculant production is not a quick profit making enterprise, and the initial production has to be carried out by a government organization. Inocula-carrier production and alternate materials for rhizobia mass production has been identified in Ethiopia (Asfaw Hailemariam, 2002).

Further exploration and phylogenetic analysis of rhizobia indigenous to Ethiopian soils

Endalkachew Wolde-meskel *et al*. (2004a, b, c; 2005) and Desta Beyene *et al*. (2004) pioneered interesting insights into the biodiversity of rhizobia in Ethiopia. However, the limited indigenous rhizobial isolates covered in their works could not warrant the revelation of the true picture of rhizobial diversity of the Ethiopian soils within the established species of the Family Rhizobiaceae. Consequently, there is a need for a collection of more rhizobial isolates to undertake further phylogenetic analyses in order to explore, conserve, and utilize the rhizobial resources in Ethiopia.

ACKNOWLEDGEMENTS

I am thankful to Ato Teramaje Tesfaye and Dr. Andargachew Gedebo for their support during the preparation of this manuscript to its final stage.

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