academicJournals

Vol. 12(9), pp. 200-217, 7 March, 2018
DOI: 10.5897/AJMR2017.8756
Article Number: C53679356215
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Groundnut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata* L. Walp) growing in Ethiopia are nodulated by diverse rhizobia

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Received 2 November, 2017; Accepted 14 December, 2017

A total of eighty one (81) rhizobial isolates were recovered from root nodules of cowpea (*Vigna unguiculata* L. Walp.) and groundnut (*Arachis hypogaea* L.) grown in soils collected from eight different sites (Hawassa, Wondogenet, Chofa, Badawacho, Bodity, Gofa, Ziway, and Alemtena) in Ethiopia with no known history of inoculation. The test isolates together with seven reference strains belonging to five genera including *Rhizobium, Ensifer, Mesorhizobium, Bradyrhizobium* and *Azorhizobium* were characterized using ninety phenotypic traits. Thirty one isolates (38%) were found to be fast growers while fifty isolates (62%) were slow growers. The majority of the isolates showed an intrinsic resistance to antibiotics (µg/ml), Chloramphenicol (5 and 15), Lincomycin (100), Novobiocin (0.5 and 1.5), and Erythromycin (10 and 20) and to heavy metals manganese sulphate (500) and copper chloride (100). Most isolates did not tolerate NaCl concentration >3% (w/v) and high temperature (45°C). Dendrogram was constructed by applying the unweighted pair group method with arithmetic means (UPGMA) using NTSYSpc Version 2.1. They were grouped into seven clusters and eight unclustered positions, when 82% relative similarity was used as a cut point. Fifty eight percent of the test isolates were grouped with *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* superclades, thus indicating that rhizobia nodulating cowpea and groundnut are delineated within a branch that defines *Bradyrhizobium* genus. To elucidate the precise taxonomic positions of the isolates, further genetic studies are required using modern molecular biological methods.

Key words: Groundnut, cowpea, isolates, phenotypic traits, *Bradyrhizobium, Rhizobium*.

INTRODUCTION

Grain legumes such as groundnut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata* L. Walp.) are essential food sources in tropical and sub-tropical regions, including Ethiopia (Duke, 2012; Singh et al., 2003; Steele, 1985). Specifically, they sustain the nutritional balances of low income societies (Zhang et al., 2007). Grain legumes have a substantial dietary value for humans and animals due to their high contents in

proteins, vitamins and minerals (Ahenkora et al., 1998; Giller, 2001; Hallensleben et al., 2009; Singh et al., 2003). Besides their nutritional qualities, legumes with their biomass are known to improve soil fertility (Senaratne et al., 1995), a desirable feature in low input smallholder agriculture.

Generally, these benefits refer to the ability of legumes to establish, in their root zone, a symbiosis with rhizobia to initialize a process defined as biological nitrogen fixation (BNF) (Boogerd and van Rossum, 1997; Dakora, 2000; Dakora and Keya, 1997). In this respect, groundnuts and cowpea have been studied extensively with respect to their BNF capability and soil fertility benefits (Senaratne et al., 1995). For example, groundnut and cowpea revealed a net contribution of N to soil up to 100 kg N ha^{-1} (Toomsan et al., 1995) and 150 kg ha^{-1} (Dakora et al., 1987), respectively when they are associated with N-fixing soil bacteria generally known as rhizobia. For these reasons, subsistence farmers in sub-Saharan Africa usually intercrop their cowpea with maize, sorghum, millet, and cassava (Langyintuo et al., 2003). In Ethiopia, cowpea is mainly grown in the drier regions (Hararge, Konso) (Westphal, 1974), and it is getting importance from time to time. With respect to nutrition, over 90% of the world groundnut is produced in developing countries and roughly two-thirds of this is used for oil, making it the most important source of vegetable oil next to soybean (Giller, 2001). Groundnut is also the third most important vegetable protein, while cowpea is used for human food, as concentrate for farm animals, hay, silage, pasture, soil cover, and green manure (Westphal, 1974). In Ethiopia, groundnut is cultivated in eastern Hararghe (Babile area), which is the peanut belt of the country and western and southern part of the country (Pawe, Gojam, Illubabour, Gamo Gofa, Welega) are also potential groundnut producing areas (Wakjira, 1992).

N-fixing rhizobia are of polyphyletic origin that spread over various taxonomic groups within the different sub-classes of alpha-, beta- and gamma-proteobacteria. Notably, those rhizobia that require a match with individual legume counterparts to induce BNF are affiliated to alpha-proteobacteria. These include the genera *Allorhizobium (*de Lajudie et al., 1998), *Azorhizobium* (Dreyfus et al., 1988), *Rhizobium* (Jordan, 1984), *Mesorhizobium* (Jarvis et al., 1997), *Ensifer* (formerly *Sinorhizobium*) (de Lajudie et al., 1994) and *Bradyrhizobium* (Jordan, 1982).

The genus *Bradyrhizobium*, however, was created to circumscribe those N-fixing bacteria that establish a symbiosis with a variety of legumes including cowpea and groundnuts and distributed broadly over tropical and temperate regions. It is acknowledged that the BNF efficacy varies depending on the combination of the legume variety and N-fixing microsymbiont strain, climatic and edaphic conditions (Giller et al., 2013). In Ethiopia, there is limited information on indigenous rhizobia that nodulate cultivated legume crops including cowpea and groundnut in various locations in the country. Few studies available on Ethiopian collections, however, showed that Ethiopian soils harbor diverse populations of rhizobia with distinct genomic composition (Beyene et al., 2004; Degefu et al., 2013; Degefu et al., 2017; Wolde-Meskel et al., 2005). Furthermore, recently, we reported several phenotypic clusters of rhizobia from nodules of chickpea and pigeon pea growing in Ethiopia (Degefu et al., 2018; Negash et al., 2018). Hence, there is strong reason to believe that there is a large, uncovered biodiversity among rhizobial population nodulating different legumes including cowpea and groundnut in Ethiopia, an acknowledged geographic centre of many leguminous plants (Table S1) (Lie et al., 1987). Furthermore, to exploit the potential benefit from BNF and to improve the agricultural productivity, it is desirable to characterize the indigenous population of rhizobia compatible to cultivated crops and develop broad host range inoculants for use in various locations. This fact necessitates the need investigating the diversity of rhizobia isolated from cowpea and groundnut extensively cultivated in Ethiopia. Hence, eighty one rhizobial strains isolated from root nodules of the two target legumes species grown at diverse locations in Ethiopia were characterized using numerical taxonomic approach. The morphophysiological diversity of the test isolatesand their relative placement, on the dendogram, with respect to the seven reference strains included in our study were exploited by analyzing ninety phenotypic traits. Furthermore, preliminary symbiotic effectiveness test (based on leaf and nodule colors) were determined.

MATERIALS AND METHODS

Isolating rhizobia from nodules

Rhizobia were isolated from nodules collected from Hawassa, Chofa, Wondo Genet, Badawacho, Bodity, Gofa, Ziway, and Alem-Tena. The nodules were preserved *in silica* gel or from fresh nodules following the methods detailed elsewhere (Somasegaran and Hoben, 1994). Desiccated nodules were imbibed in sterile water overnight. Then the nodules were surface sterilized in 95% ethanol for 5 to 10 s and 3% solution of sodium hypochlorite (NaOCl) for 2 to 4 min, rinsed in five changes of sterile distilled water on sterilized Petri dishes using flame-sterilized blunt-tipped pair of forceps. The surface sterilized nodules were crushed in drops of sterile distilled water and a loopful of the crushed nodules sap was streaked on a Yeast Extract Mannitol Agar (YEMA) plate. The plate were incubated at 28±2°C and observed for growth daily. A single colony typical of rhizobia from primary isolation plates were

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Table 1. Leaf color, nodule color, nodule number and nodule dry weight per plant.

Leaf color		Nodule color	Nodule No./plant min-max/average	Nodule dry wt./plant min-max/average (mg)
Deep green	GN	28 deep red	10-135/60	10-95/44.7
	CP	22 pink	15-30/23	15-87/43.5
Green	GN	7 pink	5-90/35	10-50/25.7
	CP	4 pink	19-56/34	25-27/25.5
Pale green	GN	5 white	6-22/11	7.5-10/8.4
	CP	4 white	8-38/19	7.5-10/9.4
Yellow	GN	11 white	*	*

GN: Groundnut, CP: cowpea, *Many in number but very small in size.

re-isolated by streaking on YEMA containing (mannitol 10 g, K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.2 g, NaCl 0.1 g, yeast extract 0.5 g, and 15 g agar in 1 L distilled water) containing bromothymol blue (BTB) as pH reaction indicator, YEMA containing congo red (CR) and peptone glucose agar (PGA) (glucose 5 g, peptone 10 g, agar 15 g and 10 ml BCP stock solution per liter distilled water) containing bromocresol purple (BCP) and gram-stained as a presumptive test (Odee et al., 1997).

Authentication of the isolates as rhizobia

A rhizobial strain was tested for its ability to produce nodules on homologous host. Seedlings were grown in modified Leonard jar constructed from plastic pots which were filled with sterilized river sand. Surface sterilized and pregerminated seeds were transplanted into the pots. Each strain of rhizobia isolated was grown in yeast extract mannitol broth (YEMB) to logarithmic phase and the seedlings were inoculated with 1 ml of each isolates. The extra growth units which were not inoculated served as controls, negative control (non-inoculated and supplied with N-free nutrient solution). The plants were grown in triplicate under greenhouse condition. The pots were arranged in completely randomized design (CRD) and fertilized with quarter-strength modified Jensen's N-free medium ($CaHPO_4$ 1 g, K_2HPO_4 0.2 g, $MgSO_4.7H_2O$ 0.2 g, NaCl 0.2 g, trace elements stock solution 1 ml and $FeCl_3$ 0.1 g/L of distilled water). All seedlings were checked for nodulation after 45 days (Maâtallah et al., 2002; Somasegaran and Hoben, 1994). Nodule number per pot and internal color were scored. Nodules were dried at 80°C for 24 h and their dry weights were measured.

Characterization of the isolates

Morphological characteristics

After incubation of 3 to 13 days at 28°C, the colony morphology (Odee et al., 1997; Somasegaran and Hoben, 1994) and acid base production (Jordan, 1984) were examined following the procedures detailed in the respective references.

Biochemical and physiological characteristics

Sodium chloride (NaCl) tolerance (with concentration of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5 and 5.5% (w/v)) (Amarger et al., 1997), temperature (5, 20, 25, 30, 40 and 45°C), tolerance (Maâtallah et al., 2002), pH (4, 4.5, 5, 5.5, 6, 7, 8, 8.5, 9, 9.5, 10, and 10.5) (Amarger et al., 1997), utilization of different carbon sources including arabinose, rhamnose, xylose, galactose, mannose, maltose, trehalose, dextrin, inulin, raffinose, dulcitol, sorbitol, citric acid, malonic acid, xylitol, myo-inositol, and ribose (Somasegaran and Hoben, 1994), utilization of different nitrogen sources (L-aspargine, L-proline, L-leucine, L-alanine, L-theronine, L-arginine, L-phenylalanine, L-pyroglutamic acid, D-serine, L-histidine, Inosine, uredine, thymidine, glutamic acid, aspartic acid, and glycine) (Amarger et al., 1997), the resistance of the test isolates to different antibiotic (novobiocin (0.5, 1.5), streptomycin (10), spectinomycin (2.5, 5), kanamycin (5, 15), erythromycin (10, 20), chloramphenicol (5, 15), neomycin (5, 20), trimethoprim (200), and lincomycin (100), heavy metals ($MnSO_4.H_2O$, 500 µg/ml; Pb $(CH_3COOH).3H_2O$, 500 µg/ml; $ZnCl_2$, 100 µg/ml; CuCl, 100 µg/ml; $CoCl_2.6H_2O$, 500 µg/ml; and $AlCl_3.6H_2O$, 500 µg/ml) (Zhang et al., 1991) and phosphate solubilizing ability (Alikhani et al., 2006) were determined following the methods and procedures described in the respective references.

Numerical analysis

To investigate the phenotypic variability among the isolates, a dendrogram was constructed by using the average Unweighted Pair Group Method with Arithmetic Means (UPGMAM) using NTSYSpc version 2.1. Characters were coded as 1 for positive (growth) and 0 for negative (no growth).

RESULTS AND DISCUSSION

In total, 81 rhizobial isolates were isolated from root nodules of groundnut (51 isolates) and cowpea (30 isolates) (Table S1). Presumptive tests indicated, all the 81 test isolates were found to be Gram negative, formed white colonies on YEMA containing CR and showed no growth on PGA containing BCP, which are a characteristics feature of rhizobia (Somasegaran and Hoben, 1994). All isolates were able to nodulate their host species confirming that they are rhizobia. Preliminary symbiotic effectiveness test indicated that sixty one strains (35 from groundnuts and 26 from cowpea) formed effective nodule with deep green/green leaf color while twenty strains (16 from groundnut and 4 from cowpea) formed ineffective nodules with pale green/yellow leaf color (Table 1).

Table 2. Colony characteristics, acid and base production and date of first colony appearance.

Property		Cowpea	Groundnut	Total no. of isolates	%
Colony color	WT	21	40	61	75
	Milky	9	11	20	25
Colony size	<1 mm	25	25	50	62
	1-2 mm	4	18	22	27
	>2 mm	1	8	9	11
Shape	Convex	30	48	78	96
	Flat	-	3	3	4
Texture	Buttery	30	51	81	100
EPS production	-	-	10	10	12
Acid reaction	-	5	23	28	35
Alkaline r×n	-	25	28	53	65
Date 1st colony appeared	3-5 days	8	23	31	38
	5-10 days	22	28	50	62

WT: Watery translucent.

Colony characteristics

The test isolates varied in colony size; 62% of them formed small colonies with diameter of <1 mm in 5 to 10 days. This is in agreement with other similar work, where 67% of the strains formed colonies with diameter of ≤1 mm (Hungria et al., 2001). On the other hand, 27% formed colonies with diameter ranging between 1 and 2 mm when incubated for 4 to 5 days, and 11% of the test isolates formed larger colonies (2 to 4.2 mm in diameter) upon incubating them for 3 to 4 days. It has been reported that fast-growers form visible colonies on YEMA within 3 to 5 days and slow-growers, however, need more than 5 days to form colonies with diameter of 1 mm under the same conditions (Jordan, 1984). Accordingly, thirty one test strains (38%) were found to be fast growers while fifty test strains (62%) were slow growers. Convex elevation was detected in 96% of the isolates while only 4% (3 groundnut strains GZ014, GZ018 and GG060) were found to appear with colony shape as flat. The majority of the isolates (75%) expressed watery translucent colony color and the remaining 25% were milky. Only 12% of the isolates, all from groundnut, produced extracellular polysaccharide (EPS) (Table 2).

Reaction on YMA-BTB

Twenty eight isolates (35%) were acid producers and changed the media to yellow when incubated for 5 days on YEMA-BTB media. Fifty three isolates (65%) had alkaline reaction in which the media were changed to blue. Slow growing rhizobia produce alkaline reaction on YEMA-BTB while fast growing rhizobia produce acid (Jordan, 1984). However, unlike the fast-growing ones which commonly produce acid reaction, three isolates from cowpea (CC027, CC028 and CC029) were fast growers but alkaline producers. This indicates that reaction of rhizobia on YEMA-BTB media cannot be considered as diagnostic feature. Similar results have been reported in earlier undertakings of similar nature (Moreira et al., 1993; Wolde-Meskel et al., 2004a, b).

pH and temperature tolerance of the isolates

With the respect to pH profiles, all isolates grew at pH values ranging between 5.5 and 10.5 but 77% of cowpea and 69% of groundnut test strains grew at pH value as low as 4.0. On the other hand, 97% of cowpea and 90% of groundnut strains grew at pH 4.5 while 97% of the isolates from cowpea and 96% of the isolates from groundnut were able to grow at pH 5.0 (Table 3). In summary, all the tested isolates were found to be tolerant to high pHs. In earlier studies on rhizobia from cowpea and mungbean (Zhang et al., 2006) and chickpea (Nour et al., 1994), it was reported that rhizobial strains could grow at pH values ranging between 5.0 and 11.0 (for cowpea and mungbean isolates) and as high as 10.0 (for chickpea isolates). The isolates, which were found to have grown in a wider pH ranges, may have practical application with respect to selection of a wide-range pH tolerant strains that can perform well under acidic, neutral and alkaline soils.

Table 3. pH and temperature tolerance of the test isolates.

Site	No. of isolates	Proportion of tolerant isolates (%) at							
		pH levels				Temperature (°C)			
		4.0	4.5	5.0	5.5-10.5	5	20-30	40	45
Hawassa	CP 7	85	85	100	100	-	100	-	-
	GN 19	63	73	100	100	5	100	11	-
Ziway	CP 4	75	100	100	100	-	100	-	-
	GN 9	11	11	78	100	-	100	100	-
Bodity	CP 5	100	100	100	100	-	100	-	-
	GN 1	100	100	100	100	-	100	-	-
Gofa	GN 21	86	90	100	100	5	100	76	-
Badawacho	GN 1	100	100	100	100	-	100	100	-
Chofa	CP 10	40	60	90	100	-	100	10	-
Wondogenet	CP 2	100	100	100	100	-	100	50	50
Alemtena	CP 2	-	-	100	100	-	100	50	-

Regarding temperature requirements, all the rhizobial isolates in this study were able to grow at temperature values ranging between 20 and 30°C (Table 3), which to some extent was in agreement with others that reported the optimum growth temperature range for rhizobia varies between 25 and 31°C (Jordan, 1984; Somasegaran and Hoben, 1994). Only two isolates from groundnut (GG055 and GH101) were found to be tolerant to low temperature (5°C). About 7% of isolates from cowpea and 53% of isolates from groundnuts were able to grow at 40°C. One isolate (GH026) from groundnut tolerated a temperature of 45°C.

Similar to the present finding, the ability of rhizobial isolates to grow at high temperature were also reported by others (Zahran et al., 1994; Zhang et al., 1991). Practically, the existence of the isolates that could tolerate high temperature could potentially be helpful to develop inoculant that can perform better at high temperature, since cowpea and groundnuts grow in low lands where day temperature can be higher.

Salt tolerance

Tolerance of the test isolates to various salt (NaCl) concentrations is presented in Table 4. Accordingly, all the tested rhizobial isolates exhibited a wide range of variations in their tolerance to NaCl concentration. As the concentration of NaCl was increased the growth of the isolates were found to be inhibited. All the 81 rhizobial isolates grew in YEMA with 0.5 and 1% NaCl. But the proportion rapidly decreased as the concentration of NaCl increased. Thus, for cowpea, the tolerant isolates decreased from 83 to 3% when the NaCl concentration increased from 1.5 to 5.5%. For groundnut isolates, the proportion reduced from 80 to 2% when the NaCl concentration increased from 1.5 to 5.5%. Five rhizobial isolates, three from groundnut (GH026, GG055 and GH101) and two from cowpea (CC23 and CZ42) were found to be tolerant to a salt concentration of 4.5%. Only three isolates, two from groundnut (GH026, GH101) and one from cowpea (CC23) were found to be tolerant to a salt concentration of 5.5%.

It was reported that rhizobia can generally grow at salt concentration as high as 2% (Jordan, 1984). Another study on isolates from chickpea indicated that 3 out of 56 isolates were tolerant to 5% NaCl concentration (Maâtallah et al., 2002). The presence of such salt tolerant isolates in the collection can be regarded as a resource for applied research aiming to develop inoculant for the target legumes growing at localities where salinity is a problem.

Carbon and nitrogen source utilization

The ability of the test isolates to utilize different substrates as sole carbon and nitrogen source (Table S2) were presented. All of the tested isolates were able to grow on trehalose, raffinose, maltose, sorbitol, arabinose, xylitol, sucrose, mannitol, myo-inositol, D-glucose, inulin, galactose, ribose, mannose, starch, xylose, dextrin, rhaminose, and dulcitol. However, all the tested isolates failed to grow in the presence of fructose, malonic acid and citric acid. All the isolates grew on L-proline, L-arginine, L-alanine, L-leucine, L-phenylalanine, L-glutamic acid, L-histidine, aspartic acid, inosine, uredine, L-threonine, L-asparginine, thymidine and L-

Table 4. Salt tolerance of cowpea and groundnut isolates.

Site	No. of isolates	Proportion of tolerant isolates (%) at salt concentration % (w/v)									
		0.5-1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Hawassa	CP 7	100	100	100	100	100	86	-	-	-	-
	GN 19	100	68	68	68	68	16	16	11	11	5
Ziway	CP 4	100	100	50	50	50	25	25	25	-	-
	GN 9	100	78	78	78	78	78	-	-	-	-
Bodity	CP 5	100	80	20	20	20	-	-	-	-	-
	GN 1	100	100	100	100	100	-	-	-	-	-
Gofa	GN 21	100	90	86	81	71	5	5	5	-	-
Badawacho	GN 1	100	100	100	-	-	-	-	-	-	-
Chofa	CP 10	100	70	40	20	20	10	10	10	10	10
Wondogenet	CP 2	100	100	100	100	100	-	-	-	-	-
Alemtena	CP 2	100	50	50	50	50	-	-	-	-	-

phyroglutamic acid as nitrogen sources. However, the following nitrogen sources including glycine and D-Serine were found to selectively inhibit the growth of quite a number of our test isolates. Accordingly, glycine and D-serine were utilized only by 31 and 30% of the isolates, respectively. The results on carbon and nitrogen utilization by cowpea and groundnut isolates in the present study agrees with other works (Amarger et al., 1997; Gao et al., 1994; Lindström and Lehtomäki, 1988; Zhang et al., 1991). In general, the results imply that the test isolates, which were able to grow on diverse carbon and nitrogen sources, have selective advantage over those grown on restricted carbon and nitrogen sources.

Intrinsic antibiotic resistance (IAR)

The ability of the test isolates, when incubated on YEMA supplemented with different antibiotics, is presented in Table 5. Isolates generally displayed resistance to chloramphenicol, lincomycin, novobiocin, erythromycin and trimethoprim but were susceptible to 15 μgml^{-1} of kanamycin, 20 μgml^{-1} of neomycin and 10 μgml^{-1} of streptomycin. The lower levels of each of the antibiotics tolerated by the majority of the isolates includes 5 μgml^{-1} of neomycin (90%), 15 μgml^{-1} of kanamycin (88%), 2.5 μgml^{-1} of spectinomycin (86%) and higher level (5 μgml^{-1}) of spectinomycin (85%). It has already been indicated that fast growing strains are more sensitive to antibiotics than slow growing ones (Jordan, 1984), which agrees with the findings. The intrinsic antibiotic resistance of the test isolates is one of the survival strategies, thus to colonize the rhizosphere.

Intrinsic heavy metal resistance

Almost all the isolates were tolerant to the tested concentration of manganese and the majority showed an intrinsic resistance to copper (96%), lead (83%) and zinc (77%). Cobalt and aluminium seemed to be selective in that 24 and 47% of the isolates, respectively, tolerated a concentration of 500 μgml^{-1} of each (Table 6). In another study of similar nature, it was found out that 67% of the strains tolerated 0.5 mM of cobalt chloride and no isolate tolerated 2.5 mM lead acetate (Hungria et al., 2001). But, in this study, most isolates (83%) tolerated lead acetate (500 μgml^{-1}). The intrinsic heavy metal resistance of the test isolates observed in this study implies that the resistant isolates could be regarded as potential candidates to develop inoculant for environments polluted with heavy metals.

Phosphate solublizing ability

Out of the 81 tested isolates, 53 (65%) were found to solubilize phosphate as confirmed by the formation of clear zone around the colonies on agar plates (Table S2). This result agrees with other results generated in Iran The results indicated that the phosphate solubilizing isolates in our collection, in addition to their N_2-fixing ability, could avail plant phosphorus nutrition by mobilizing inorganic phosphate.

Numerical analysis of phenotypic data

The result of the cluster analysis performed on the 81 test isolates and 7 reference species for 90 phenotypic characteristics is as shown in Figure 1. The result grouped the strains into 7 clusters and 8 un-clustered positions (3 isolates and 5 reference strains) using 82% similarity level as a cut point. The majority of the isolates (58%) were clustered with *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii*, thus indicating that the slow

Table 5. Intrinsic antibiotic resistance of the isolates.

Antibiotic	Concentration (µg/ml)	% of resistant isolates		
		Cowpea	Groundnut	Total
Chloramphenicol	5	100	100	100
	15	100	100	100
Lincomycin	100	100	100	100
Novobiocin	0.5	100	100	100
	1.5	97	100	99
Erythromycin	10	97	100	99
	20	93	100	98
Trimethoprim	500	100	92	95
Neomycin	5	97	86	90
	20	90	69	77
Spectinomycin	2.5	83	88	86
	5	83	86	85
Kanamycin	5	90	86	88
	15	73	75	74
Streptomycin	10	83	75	7 8

Table 6. Intrinsic heavy metal resistance of the isolates.

Heavy metal	Concentration (µg/ml)	% of resistant isolates		
		Cowpea	Groundnut	Total
Mn	500	97	100	99
Cu	100	97	96	96
Pb	500	73	88	83
Zn	100	83	73	77
Al	500	20	63	47
Co	500	37	16	24

growing isolates nodulating cowpea and groundnut are most likely to comprise *B. japonicum, B. elkanii*, and other unidentified *Bradyrhizobium* species. The results, in some respects, were in agreement with other work on rhizobia from *A. hypogaea* L grown on Argentinean soils (Taurian et al., 2006).

Cluster I comprised three isolates (one from cowpea grown in Alem-Tena soil and two from groundnut grown in Gofaand Hawassa soil). They were slow growers. The isolates under this cluster were able to grow at 3% NaCl concentration, failed to utilize D-serine and glycine as sole nitrogen source, sensitive to antibiotics (spectinomycin, streptomycin and kanamycin) and heavy metals including cobalt and zinc.

Cluster II consists of 47 isolates (22 from cowpea and 25 from groundnut) from six study sites (Hawassa, Wondo Genet, Chofa, Bodity, Gofa and Ziway) and two reference strains *B. japonicum* (HAMBI 2314^{T}) and *B. elkanii* (LMG 6164). This cluster contained 58% of the isolates and it was very heterogeneous with two large sub-clusters at a similarity level of 83%. Sub-cluster IIA containing 29 isolates and sub-cluster IIB contained 18 isolates and two reference strains (*B. japonicum* and *B. elkanii*). It has been reported that very fast, fast,

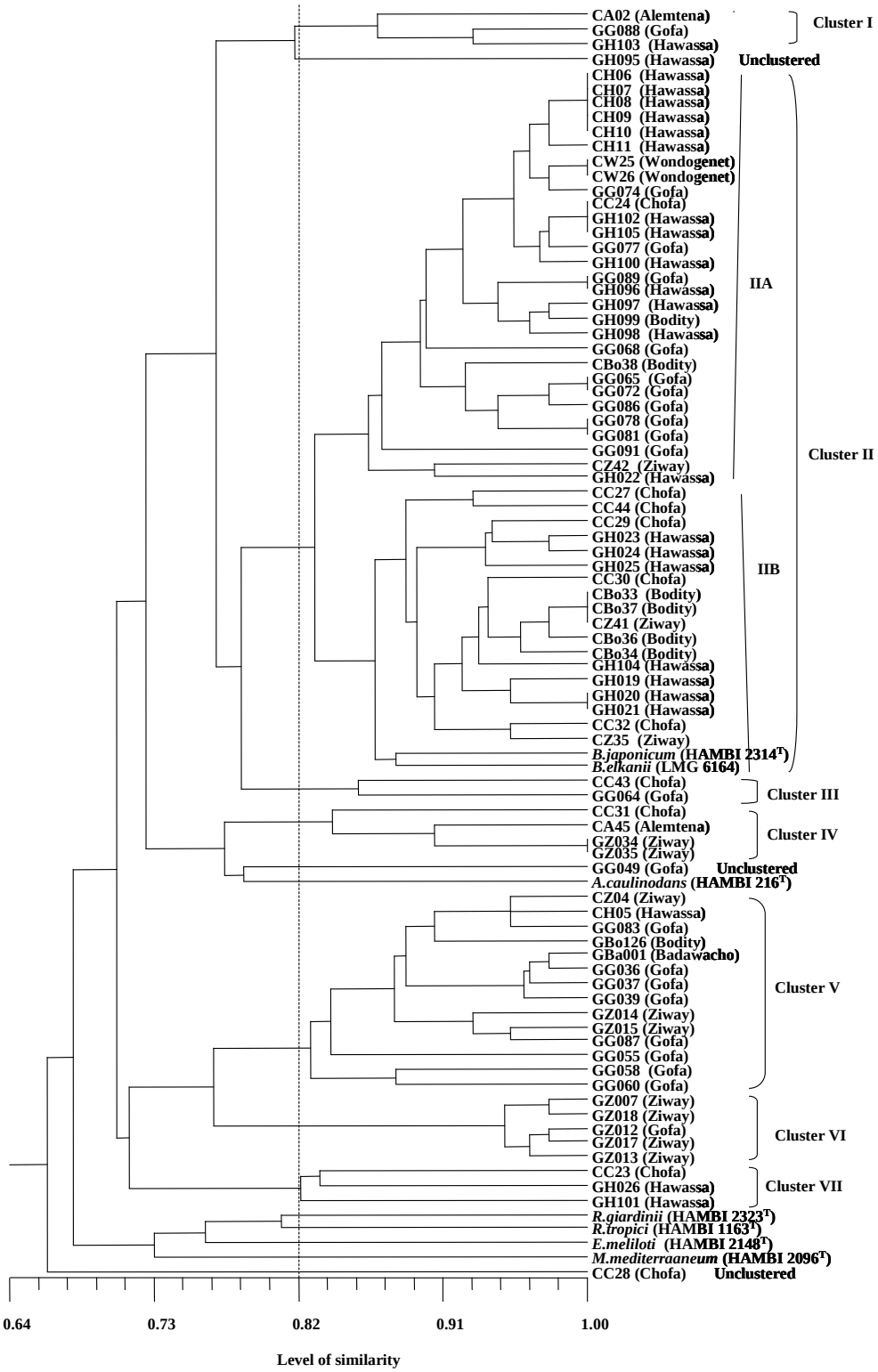

Figure 1. Dendrogram showing phenotypic similarity between test and reference strains.

intermediate and slow growing rhizobia are intermingled with one another and thus appeared as single cluster (Odee et al., 1997). Similarly, in this work slow growers and fast growers grouped together in clusters II and VII. The inclusion of *B. japonicum* and *B. elkanii* in this cluster is in agreement with other similar work, which reported

that rhizobia from cowpea grown in Senegal belong to the genus *Bradyrhizobium* (Krasova-Wade et al., 2003). On another study, rhizobia nodulating soybean and peanut growing in China were found to belong to the genus *Bradyrhizobium* (Yang and Zhou, 2008). The isolates under this cluster showed wide resistance to antibiotics, heavy metals, pH and a salt tolerance as high as 3%. They were found to utilize different substrates as their sole carbon and nitrogen sources.

Cluster III consisted of one cowpea isolate from Chofa (CC43) and one groundnut isolate from Gofa (GG064). They were fast growers. They were able to grow at pH values ranging between 4.0 and 10.5, NaCl concentration of 1%, sensitive to higher temperature, resistant to all tested antibiotics and sensitive to cobalt.

Cluster IV consisted of 4 isolates, two cowpea isolates (one from Chofa and one from Alem-Tena) and two groundnut isolates from Ziway. All the isolates under this cluster were slow growers. They were sensitive to pH 4.0, NaCl concentration of 1.5%, kanamycin, spectinomycin, streptomycin, lead, zinc, aluminum and cobalt.

Cluster V consisted of 14 isolates: two cowpea isolates (one from Ziway and one from Hawassa) and 12 groundnut isolates (eight from Gofa, two from Ziway, one from Bodity, and one from Badawacho). All the isolates were fast growers, sensitive to lower pH (4.0 and 4.5), tolerated NaCl concentration of 3%, temperature of 40°C and were resistant to heavy metals except cobalt.

Cluster VI consisted of 5 groundnut isolates from Ziway. All were fast growers, tolerated pH values ranging between 5.0 and 10.5, NaCl concentration of 3.5%, temperature of 40°C and were susceptible to kanamycin, neomycin, streptomycin, zinc and cobalt. All the five isolates in this cluster do not have phosphate solublizing ability.

Cluster VII consisted of three isolates, one cowpea isolate from Chofa and two groundnut isolates from Hawassa. The typical feature of this cluster was that they tolerated all salt concentrations tested (0.5 to 5.5%), continued to grow at a temperature of 45°C and all concentration levels of antibiotics tested.

Unclustered consisted of three isolates (GH095, GH049 and CC28) showed different test results, when compared with each other and to the other isolates in different clusters. GH095 and GH049 were isolated from groundnut grown in Hawassa soils and CC28 was isolated from cowpea grown in Chofa soil. All the three isolates tolerated pH 4.0 to 10.5, unable to utilize D-serine and glycine as nitrogen sources. But they showed wide variability in salt tolerance, IAR and intrinsic heavy metal resistance. Isolate GH095 tolerated NaCl concentration of 3% while CC28 tolerated 2% and GH049 1%. With respect to IAR, isolate CC28 was found to be susceptible to tested concentrations of kanamycin, erythromycin, spectinomycin, streptomycin and 20 μgml^{-1} of neomycin; isolate GH095 was sensitive to tested concentrations of spectinomycin, streptomycin, trimethoprim and 20 μgml^{-1} isolates of neomycin; GH049 was sensitive to tested concentrations of kanamycin, streptomycin neomycin and 5 μgml^{-1} of spectinomycin. Isolate GH049 was found to be sensitive to zinc and cobalt while GH095 was found to be sensitive to aluminium and CC28 to cobalt.

In earlier studies, based on numerical taxonomic approach, it was found that *B. japonicum* strains always clustered together with other *Bradyrhizobium* spp. at or above the 70% similarity level (Van Rossum et al., 1994). Furthermore, slowly growing rhizobia obtained from Hainan Province, China and three representative strains of *B. japonicum*, clustered together at similarity level of 81% (Gao et al., 1994). Similarly, majority of isolates (58%) under this study are clustered with *B. japonicum* and *B. elkanii* at similarity level of 82%, thus our slow growing strains might be closely related to these *Bradyrhizobium* reference strains. However, these remained to be established on further genetic analysis of the strains.

Conclusion

Based on the results generated from the authentication and preliminary symbiotic effectiveness test, it can be concluded that isolates with deep green and green leaf color and branching shoots and deep red and pink nodule color are effective in fixing atmospheric nitrogen. Morphophysiological characteristics and the clustering of the test isolates based on the 90 characters indicate that there is a wide diversity in rhizobial isolates nodulating cowpea and groundnut in the study sites. The rhizobial isolates that tolerated extreme environmental conditions may be potential candidates to develop them into inoculant for soils having such constraints. The ability of the test isolates to utilize a wide range of carbon and nitrogen sources imply that isolates with such ability can easily colonize the soil environment and compete with other microorganisms. To further elucidate the exact phylogenetic positions of the test isolates, genetic studies are required using modern molecular biological methods.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Table S1. Strain designations, geographic location and trap host species from which the test strains were isolated.

Location	Strain designation		No of isolates		
	Groundnut	Cowpea	Gn	Cp	T
Hawassa	GH019, GH020, GH021, GH022, GH023, GH024, GH025, GH026, GH095, GH096, GH097, GH098,GH099, GH100, GH101, GH102,GH103, GH104, GH105	CH05, CH06, CH07, CH08, CH09, CH10, CH11	19	7	26
Ziway	GZ007, GZ012, GZ013, GZ014, GZ015, GZ017, GZ018, GZ034, GZ035	CZ04, CZ35, CZ41, CZ42	9	4	13
Bodity	GBo126	CBo33, CBo34,CBo36, CBo37, CBo38	1	5	6
Gofa	GG036, GG037, GG039,GG049, GG055, GG058, GG060, GG064, GG065, GG068, GG072, GG074, GG077, GG078, GG081, GG083, GG086, GG087, GG088, GG089, GG091	-	21	-	21
Badawacho	GBa001	-	1	-	1
Chofa	-	CC23, CC24, CC27, CC28, CC29, CC30, CC31, CC32, CC43, CC44	-	10	10
Wondogenet	-	CW25,CW26	-	2	2
Alemtena	-	CA02, CA45	-	2	2
Total			**51**	**30**	**81**

Table S2a. Carbon source utilization of cowpea and groundnut isolates and reference strains. (*Bradyrhizobium elkanii* (LMG 6164), *Bradyrhizobium japonicum* (HAMBI 2314^T), *Rhizobium giardinii* (HAMBI 2323^T), *Rhizobium tropici* (HAMBI 1163^T), *Ensifer meliloti* (HAMBI 2148^T), *Mesorhizobium mediterraneum (*HAMBI 2096^T), *Azorhizobium caulinodans (*HAMBI 216^T)).

Strain	Cluster	Carbon source																					
		Trehalose	Dulcitol	Raffinose	Maltose	Sorbitol	Arabinose	Citric acid	Malonic acid	Xylitol	Myo-inositol	Sucrose	Fructose	Starch	Inulin	Galactose	Dextrin	Xylose	Mannose	Ribose	Rhaminose	Mannitol	D-glucose
CA02	I	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CZ04	V	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CH05	V	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CH06	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CH07	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CH08	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CH09	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CH10	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CH11	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CC23	VII	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CC24	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CW25	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CW26	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CC27	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CC28	Un	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CC29	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CC30	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CC31	IV	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CC32	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CBo33	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CBo34	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CZ35	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CBo36		+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CBo37	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CBo38	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CZ41	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CZ42	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CC43	III	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CC44	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CA45	IV	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GBa001	V	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GZ007	VI	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GZ012	VI	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GZ013	VI	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GZ014	V	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+

Table S2a. Contd.

Strain	Cluster	Carbon source																					
		Trehalose	Dulcitol	Raffinose	Maltose	Sorbitol	Arabinose	Citric acid	Malonic acid	Xylitol	Myo-inositol	Sucrose	Fructose	Starch	Inulin	Galactose	Dextrin	Xylose	Mannose	Ribose	Rhaminose	Mannitol	D-glucose
GZ015	V	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GZ017	VI	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GZ018	VI	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH019	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH020	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH021	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH022	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH023	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH024	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH025	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH026	VII	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GZ034	IV	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GZ035	IV	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG036	V	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG037	V	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG039	V	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG049	Un	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG055	V	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG058	V	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG060	V	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG064	III	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG065	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG068	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG072	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG074	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG077	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG078	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG081	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG083	V	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG086	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG087	V	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG088	I	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG089	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GG091	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH095	Un	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH096	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH097	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+

Table S2a. Contd.

Strain	Cluster	Carbon source																					
		Trehalose	Dulcitol	Raffinose	Maltose	Sorbitol	Arabinose	Citric acid	Malonic acid	Xylitol	Myo-inositol	Sucrose	Fructose	Starch	Inulin	Galactose	Dextrin	Xylose	Mannose	Ribose	Rhaminose	Mannitol	D-glucose
GH098	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH099	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH100	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH101	VII	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH102	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH103	I	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH104	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GH105	II	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
GBo126	V	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
HAMBI 216^{T}	Un	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
LMG 6164	II	+	-	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
HAMBI 2323^{T}	Un	+	-	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
HAMBI 1163^{T}	Un	+	-	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
HAMBI 2314^{T}	II	+	-	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
HAMBI 2148^{T}	Un	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
HAMBI 2096^{T}	Un	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+

Table S2b. Nitrogen source utilization, phosphate solubilizing ability (PSA) of the isolates and reference strains. (*Bradyrhizobium elkanii* (LMG 6164), *Bradyrhizobium japonicum* (HAMBI 2314^T), *Rhizobium giardinii* (HAMBI 2323^T), *Rhizobium tropici* (HAMBI 1163^T), *Ensifer meliloti* (HAMBI 2148^T), *Mesorhizobium mediterraneum (*HAMBI 2096^T), *Azorhizobium caulinodans (*HAMBI 216^T)).

		Nitrogen source																
Strain	**Cluster**	**L-aspargine**	**L-proline**	**L-leucine**	**L-alanine**	**Inosine**	**L-theronine**	**L-histidine**	**Uredine**	**L-phenylalanine**	**Thymidine**	**L-arginine**	**L-glutamic acid**	**L-pyroglutamic acid**	**Aspartic acid**	**D-serine**	**Glycine**	**PSA**
CA02	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CZ04	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CH05	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CH06	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CH07	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CH08	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CH09	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CH10	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CH11	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CC23	VII	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
CC24	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CW25	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
CW26	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
CC27	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
CC28	Un	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
CC29	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
CC30	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
CC31	IV	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
CC32	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CBo33	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CBo34	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CZ35	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CBo36	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CBo37	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CBo38	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
CZ41	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CZ42	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CC43	III	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-
CC44	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
CA45	IV	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GBa001	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
GZ007	VI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
GZ012	VI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

Table S2b. Contd.

Strain	Cluster	Nitrogen source																
		L-aspargine	L-proline	L-leucine	L-alanine	Inosine	L-theronine	L-histidine	Uredine	L-phenylalanine	Thymidine	L-arginine	L-glutamic acid	L-pyroglutamic acid	Aspartic acid	D-serine	Glycine	PSA
GZ013	VI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
GZ014	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
GZ015	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
GZ017	VI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
GZ018	VI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
GH019	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GH020	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GH021	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GH022	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GH023	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GH024	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GH025	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
GH026	VII	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GZ034	IV	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GZ035	IV	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GG036	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
GG037	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
GG039	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GG049	Un	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GG055	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GG058	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GG060	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GG064	III	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
GG065	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GG068	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GG072	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GG074	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GG077	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GG078	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GG081	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GG083	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GG086	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GG087	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
GG088	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
GG089	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-

Table S2b. Contd.

Strain	Cluster	Nitrogen source																
		L-aspargine	L-proline	L-leucine	L-alanine	Inosine	L-theronine	L-histidine	Uredine	L-phenylalanine	Thymidine	L-arginine	L-glutamic acid	L-pyroglutamic acid	Aspartic acid	D-serine	Glycine	PSA
GG091	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GH095	Un	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
GH096	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
GH097	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
GH098	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
GH099	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
GH100	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
GH101	VII	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
GH102	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GH103	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GH104	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GH105	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
GBo126	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HAMBI 216^{T}	Un	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LMG 6164	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HAMBI 2323^{T}	Un	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HAMBI 1163^{T}	Un	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HAMBI 2314^{T}	II	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
HAMBI 2148^{T}	Un	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
HAMBI 2096^{T}	Un	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-