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Morphophysiological diversity of rhizobia nodulating pigeon pea (*Cajanus cajan* L. Millsp.) growing in Ethiopia

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Pigeon pea (*Cajanus cajan* (L.) Millsp.) is an important protein source grown in several tropical and sub-tropical countries, and is considered a multi-purpose plant that is resistant to the conditions where drought and salinity is a common phenomenon. The aim of this study was to evaluate the diversity of rhizobial isolates obtained from root nodules of pigeon pea plants grown in central and southern Ethiopia. A total of 116 nitrogen-fixing rhizobial strains were isolated. The bacterial isolates were characterized by 91 phenotypic traits including cultural characteristics, intrinsic antibiotic and heavy metal resistance, salt, pH and incubation temperature tolerance, and carbon and nitrogen sources utilization ability. Preliminary symbiotic properties of the isolates were also evaluated. The isolates were compared with seven reference strains of rhizobia by application of the unweighted pair group method with arithmetic means (UPGMA) using NTSYSpc Version 2.1 software program. The dendrogram constructed from cluster analysis of 91 phenotypic traits, grouped them into six clusters and eight un-clustered positions at 80% relative similarity. Cluster I contained 83% of the test isolates that were grouped together with the reference strains *Bradyrhizobium japonicum* (HAMBI 2314^T) and *Bradyrhizobium elkanii* (LMG 6164), suggesting that pigeon pea is commonly nodulated by bradyrhizobia. Results from symbiotic effectiveness test revealed that majority of the isolates were found to be effective. Generally, this investigation demonstrated that rhizobial population nodulating *C. cajan* on the study area were phenotypically diverse and symbiotically effective. Furthermore, the result indicates the existence of strains in the collection, which can tolerate environmental stresses, thus can be developed into inoculant for pigeon pea inoculation and production in Ethiopia and beyond.

Key words: *Bradyrhizobium*, *Cajanus cajan,* phenotypic characteristics, *rhizobium*.

INTRODUCTION

Pigeon pea (*Cajanus cajan* L Millsp.) is the only crop member of the Cajaninae tribe (Hancock, 2012), which grows vigorously in soils with low fertility, mainly in marginal lands (Beltrame and Rodrigues, 2007). Indeed,

Table 1. Sites and associated environmental variables of the locations from where root nodules of pegion pea was obtained.

Site of nodule	Annual T°C ranges	Annual rainfall ranges (mm)	Elevation in m.a.s.l.	Latitude °'N	Longitude °'E
Humbo	17.6-25	801-1600	1001-2500	6.51-6.79	37.59-38.05
Bodity	17.6-22.5	100-1400	1501-2500	6.88-7.12	37.75-38.00
Gofa	17-29	1401-1600	501-3000	6.12-6.72	36.68-37.20
Hawassa	12-27	801-1600	1501-3000	6.11-6.52	37.58-37.98
Badwacho	17.6-22.5	801-1400	1501-2500	7.07-7.30	37.73-38.10
Ziway	16-27	450-850	1640	7.56	38.43

Source: EARO (1998); AARC (2004).

pigeon pea is able to associate with a large diversity of indigenous rhizobia in soil, reaching more than 150 kg of fixed N per hectare per year (Peoples et al., 1995). To exploit the biological nitrogen fixation (BNF) potential of this crop, the selection and evaluation of new rhizobial strains from different areas where pigeon pea is cropped must be carried out.

The slow and fast growing pigeon pea rhizobia present great genetic and metabolic diversity and are likely to have new species among the culture collections worldwide (Fernandes et al., 2012; Ramsubhag et al., 2002). In addition to being efficient in fixing nitrogen in field conditions, pigeon pea rhizobia also present other biotechnological applications, such as biopolymer production and enzymatic activity (Fernandes et al., 2012; Júnior et al., 2011). Nevertheless, it should be noted that for Ethiopia, information is scarce about indigenous rhizobia that nodulate grain legumes such as pigeon pea. Few studies on Ethiopian collections of rhizobia from different legume species showed, however, that Ethiopian soils harbour diverse populations of rhizobia with distinct genomic composition which also differ in symbiotic effectiveness (Beyene et al., 2004; Degefu et al., 2013; Tena et al., 2017a, b; Wolde-Meskel et al., 2005). Hence, it could be logical to hypothesize that there is a large, undiscovered genetic diversity of rhizobia nodulating pigeon pea growing in Ethiopia, an acknowledged geographic centre of many leguminous plants (Lie et al., 1987). Furthermore, to improve productivity of the target crop (that is, pigeon pea), it is relevant to characterize the indigenous population of rhizobia compatible with cultivated crops and develop inoculants for use in legume production in various locations in the country and beyond. This fact necessitates investigation of rhizobia nodulating pigeon pea growing in various locations in the country.

As a first step to such approach, (either from field standing nodule or by trapping techniques) rhizobia nodulating pigeon pea growing on soils of six locations in Ethiopia was systematically recovered. Accordingly, the investigations were primarily based on considering an array of morphophysiological features including cultural characterization, intrinsic antibiotic and heavy metal resistance, salt, pH and incubation temperature tolerance, carbon and nitrogen sources utilization ability of rhizobial test isolates recovered from surface-sterilized root nodules of pigeon pea grown at diverse locations in central and southern Ethiopia. Cluster analysis of the test isolates were carried out using numerical taxonomic approach where 91 features were examined. Furthermore, preliminary symbiotic potential of the test isolates were studies using nodule and leaf color.

MATERIALS AND METHODS

Rhizobial source and isolation procedure

The rhizobial strains were isolated from desiccated and fresh nodules obtained from pigeon pea grown at the different locations in central and southern Ethiopia (Table 1) following procedures detailed elsewhere (Somasegaran and Hoben, 1994). All isolates were stored in 20% glycerol stock at -21°C (Coutinho et al., 1999). Presumptive tests were also carried out based on the methods described earlier (Somasegaran and Hoben, 1994). Plates were incubated at 28°C, observed daily for colony appearance with their characteristics color (colorless on congo red, yellow for fast growers and blue for slow growers) (Jordan, 1982; Vincent, 1970).

Authentication of the test isolates

The ability of isolates to form nodules was determined by re-inoculating them into homologous host (pigeon pea). Authentication was carried out in modified Leonard jars constructed from plastic cups, which was sterilized after filling it with washed river sand. Surface-sterilized pre-germinated seedlings were asceptically transplanted into each Leonard jar. Pure single colony of each isolates was inoculated on Yeast Extract Manitol Broth (YMB) and from the logarithmic growth phase the seedlings were inoculated with 1 ml of each isolate (Somasegaran and Hoben, 1994). Positive control (none inoculated and supplied with N-solution where 0.1% KNO_3 was added to N-free solution) and negative control (none inoculated and supplied with N-free nutrient solution) were included during authentication. The plants were grown in triplicate under

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Table 2. Geographical origins, strain designations (alphabets along with numbers) and number of isolates obtained from each sampling sites.

Location	Isolate designation	Total No. of isolates
Ziway	PZ11, PZ81, PZ82	3
Badawacho	PBa32, PBa33, PBa92, PBa93	4
Bodity	PB86, PB87, PB88, PB89, PB90, PB91, PB94, PB96, PB97, PB98, PB99	11
Hawassa	PH104, PH105, PH106, PH107, PH127, PH128, PH147, PH148, PH149, PH150, PH151, PH152, PH153	13
Gofa	PG13, PG17, PG27, PG28, PG29, PG31, PG35, PG59, PG61, PG63, PG64, PG67, PG68, PG69, PG70, PG71, PG72, PG 73, PG74, PG75, PG76a, PG76b, PG79, PG80, PG84	25
Humbo	PHu34, PHu36, PHu49, PHu50, PHu100, PHu101, PHu108, PHu109, PHu110, PHu111, PHu112, PHu113, PHu114, PHu116, PHu117, PHu119, PHu120, PHu121, PHu122, PHu123, PHu124, PHu125, PHu126, PHu130, PHu131, PHu132, PHu133, PHu134, PHu135, PHu136, PHu137, PHu138, PHu139, PHu140, PHu141, PHu143, PHu144, PHu145, PHu146, PHu154, PHu156, PHu157, PHu159, PHu161, PHu162, PHu163, PHu164, PHu165, PHu166, PHu169, PHu170, PHu172, PHu173, PHu174, PHu175, PHu176, PHu177, PHu178, PHu179, PHu181	60

*PZ: Pigeon pea Ziway, PBa: Pigeon pea Badawacho, PB: Pigeon pea Bodity, PH: Pigeon pea Hawassa, PG: Pigeon pea Gofa, PHu: Pigeon pea Humbo

greenhouse condition. The modified Leonard jars were arranged in complete randomized design (CRD) and fertilized with quarter strength Jensen's N-free medium. Seedlings were checked for nodules after 45 days and roots from the plants were gently washed and nodules were carefully removed. Nodule number per plant and internal color (pink, white and green) were scored.

Characterization of the isolates

Morphological characteristics

The colony morphology was observed on Yeast extract mannitol agar (YMA) after incubation for 2 to 13 days at 28°C. Colony appearance was characterized based on their color, shape, size, texture and ability to produce extracellular polysaccharide (Somasegaran and Hoben, 1994).

Salinity tolerance

Isolates were tested for salt tolerance in YMA supplemented with NaCl (Bouhmouch et al., 2001), temperature (Chen et al., 2002) and pH tolerance (Kishinevsky et al., 2003). The results were recorded as (+) for growth and (-) for no growth.

Carbon sources utilization

Carbon source (Somasegaran and Hoben, 1994) and amino acid utilization (Amarger et al., 1997), intrinsic heavy metal resistance (IHR) (Zhang et al., 1991), intrinsic antibiotic resistance (IAR) (Lindström and Lehtomäki, 1988), phosphate solubilizing ability (PSA) (Alikhani et al., 2006) for the entire test isolates were carried out following the methods described in their respective references.

Numerical analysis

Characters were coded 1 for positive and 0 for negative. Cluster analysis for the final matrix containing 123 strains and 91 features was carried out using similarity coefficient and a phenotypic dendrogram was constructed by the unweighted pair group method with arithmetic means average (UPGMA) clustering method using Numerical Taxonomic Analysis System NTSYSpc version 2.

RESULTS AND DISCUSSION

In a systematic collection of rhizobia nodulating pigeon pea growing in central and southern Ethiopia, a total of 116 rhizobial isolates were recovered from root nodules of *C. cajan* grown in Ziway (3 strains), Badawacho (4 strains), Bodity (11 strains), Hawassa (13 strains), Gofa (25 strains) and Humbo (60 strains) (Table 2). In order to investigate their diversity and relationships with the reference strains, they were characterized based on various morphophysiological features. From the total isolates, 20% were found to be fast grower, as the first colony was found to appear between 3 and 5 days, while 80% of the isolates were slow growers (colony appeared between 5 and 10 days). This indicated that both fast and slow growing rhizobia are the natural endophytes nodulating pigeon pea in the study sites. The results from this study was in agreement with results generated elsewhere, which reported that fast-growing root nodule bacteria form visible colonies on YMA within 3 to 5 days, and slow growers needs 5 to 7 days when incubated under 28°C (Jordan, 1984). In another study of similar nature, it was clearly shown that pigeon pea was found to be nodulated by both fast and slow growers (Anand and Dogra, 1991), indicating that pigeon pea is non-selective to make symbiotic association with rhizobia for biological nitrogen fixation.

With the respect to presumptive test, it has been well documented that rhizobia showed little or no congo red (CR) absorption when incubated in the dark, and they showed no growth or poor growth on PGA-BCP (Jordan, 1984; Somasegaran and Hoben, 1994). In agreement

Table 3. Summary table on the overall symbiotic effectiveness of the isolates.

No. of Isolates	Leaf color	Nodule color	Remark
51	Deep green	R/P	Effective
52	Green	P	Effective
13	Pale green	P/W	Ineffective

*R: Red, P: pink, W: white.

Table 4. Colony characteristics, acid/base reaction, of the test isolates.

property		Ziway n=3	Badawacho n=4	Bodity n=11	Hawassa n=13	Gofa n=25	Humbo n=60	Total No. of isolate	% of isolates
Colony size mm	<1.0	3	3	7	6	20	37	76	66
	1-1.5	-	-	4	7	5	22	38	32
	>1.5	-	1	-	-	-	1	2	2
Colony color	WT	-	3	5	12	16	55	91	78
	M	3	-	6	1	9	3	22	19
	WO	-	1	-	-	-	-	1	1
	Y	-	-	-	-	-	2	2	1
Colony shape	C	3	3	11	13	25	59	114	98
	F	-	1	-	-	-	1	2	2
Colony texture	B	3	3	11	13	24	59	144	98
	E	-	1	-	-	1	1	2	2
EPS		-	1	-	-	1	1	3	3
Acid/Base reaction	Al	2	3	10	12	59	59	107	92
	A	1	1	1	1	1	1	9	8
Date first colony appeared	3-5 days	-	2	11	-	6	6	23	20
	5-10 days	3	2	-	13	54	54	93	80

*n: Number of isolates, M: milky, WT: watery translucent, WO: white opaque, Y: yellow, B: buttery, E: elastic, C: convex, F: flat, Al: alkaline, A: acid, EPS: exopolysaccharide production.

with this, the present study clearly showed that, all isolates did not absorb CR, and they did not grow on PGA-BCP, confirming that the test isolates were all rhizobia. Furthermore, Gram reaction of the isolates was detected, and all were found to be Gram negative.

Based on the authentication test, 116 isolates were found to form nodule with the host plant. The preliminary symbiotic effectiveness test (based on nodule and leaf color) indicated that more than 44% of the test isolates were found to produce leaf (withgreen or deep green color) and red/pink nodule, when inoculated to pigeon pea, whereas the rest produced leaf (with pale green color) (Table 3). It can be concluded that, the isolates with deep green and green leaf color as well as with red/pink nodule color were effective strains, whereas, the isolates with pale-green leaf color can be categorized as being ineffective.

Colony morphology

As shown in Table 4, the isolates were found to vary in morphology; 66% formed small colonies (<1.0 mm), whereas 32% had diameter ranging between 1 and 1.5 mm, and only strains designated as PHu110 (from Humbo) and PBa93 (from Badawacho) had diameters of 4.9 and 5.5 mm, respectively. PHu110 and PBa93 had flat shape and produced copious EPS, but the rest of the isolates had convex or domed shape and did not produce EPS. Previous report indicated that fast growing rhizobia produce excessive EPS within 3 to 5 days, while slow growing isolates produce less or no mucus and do not grow more than 1 mm size within ten days of incubation (Jordan, 1984). The majority of the isolates (78%) have expressed watery translucent, while 19% have shown milky appearance on YMA. In agreement with the

Table 5. Salt tolerance of the isolates NaCl% (W/V).

Isolate origin	NaCl%										
	0.5	1.0	1.5	2	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Ziway (n=3)	+	+	+	+	1	1	-	-	-	-	-
Badawacho (n=4)	+	+	+	3	2	2	-	-	-	-	-
Bodity (n=11)	+	+	+	+	7	7	1	1	-	-	-
Hawassa (n=13)	+	+	+	+	2	1	-	-	-	-	-
Gofa (n=25)	+	23	23	18	11	6	3	3	2	2	2
Humbo (n=60)	+	52	51	44	17	3	1	1	1	1	1

*n: Number of isolates, '+'=growth, '-'=no growth. Figures in the table indicate the number of test isolates, which were able to grow on the indicated salt concentration.

findings, all isolates recovered from Kenyan soils fell into watery, milky translucent and curdle milk color type (Odee et al., 1997). PBa93 has produced white opaque colonies while, PHu110 and PHu114 (isolated from Humbo) were found to appear as yellow colonies. Taken together, the color of the colonies investigated in the study is a characteristic feature of rhizobia (Somasegaran and Hoben, 1994).

Acid/Base reaction of the isolates on YMA-BTB

Acid and alkaline production has been used as a tool to elucidate the general characteristics of rhizobia. Generally, slow-growing rhizobia produce alkaline, while fast-growing rhizobia produce acid (Jordan, 1984). However, in another study of similar nature, it was reported that slow growth and alkali production were not mutually inclusive characteristics (Kennedy and Greenwood, 1982). In the present study, only 8% of the isolates were acid producing strains when inoculated and incubated on YMA containing BTB, while the others were alkali-producing strains (Table 4), which is in agreement with other studies conducted elsewhere (Hernandez and Focht, 1984; Moreira et al., 1993; Wolde-meskel et al., 2004), and that our test isolates represent both fast and slow growing groups.

Salinity tolerance

The characteristic salt tolerance profile of the test isolates is presented in Table 5. Accordingly, 80% of the test isolates were able to grow in YMA with 2% NaCl, while 41% at 2.5% NaCl and only 17% of the test isolates were able to grow at salt concentration of 3%. Isolates PG73 (from Gofa) and PB87 (from Bodity) continued to grow at 4% NaCl, while PG68 and PG80 (from Gofa), and PHu162 (from Humbo) tolerated salt concentration of 5.5%. In previous undertakings, rhizobia were reported to grow at salt concentration of 2% (Jordan, 1984), 3% (Odee et al., 1997), 4% (Swelim et al., 1997) and 5% (Surange et al., 1997). Successful rhizobium-legume symbiosis under salt stress require isolates that could resist higher NaCl concentration, thus the presence of high salt concentration tolerant isolates in the collection could potentially offer a possibility for developing inoculant for legume production in sites where salinity is a problem.

pH and temperature tolerance of the isolates

The pH and temperature tolerance of the test isolates is presented in Table 6. The isolates were shown to have wide diversity with respect to their pH and temperature requirements. With the exception of the following isolates including PG27, PG29 and PG74 (which failed to grow at acidic and/or alkaline pH), most of the tested isolates were shown to have wider range of pH profile for their growth. This was to some extent in agreement with other study (Kalita and Małek, 2004). Soil acidity is a significant problem for agricultural production in many areas of the world and limits legume productivity (Bordeleau and Prévost, 1994). Isolates with high adaptability to acidic and alkaline pH can be used as inoculant for legume production where acidity and alkalinity is a problem.

With respect to the temperature tolerance of the test isolates, the optimum growth temperature was found to range between 20 and 30°C, which is in line with results reported elsewhere (Jordan, 1984). The exceptions to this finding was that, the following test isolates including PG59, PG 68, PB87, and PBa93 were able to grow at extreme temperatures (at 5, 40, and 45°C). In previous studies, rhizobia were reported to grow at temperature values of 44°C (Zhang et al., 1991) and 50°C (Surange et al., 1997). It has been reported that high temperatures decrease rhizobial survival and establishment in tropical soils (Hungria and Vargas, 2000). Nevertheless, the high temperature tolerant rhizobial isolates investigated in this study might be regarded as good opportunity to develop efficient inoculum for legumes production tropical soils, where high temperature is a common phenomenon. Furthermore, isolates that tolerated a wide range of temperature can be used for production of effective inoculum for legumes grown in different eco-climatic

Table 6. pH and temperature tolerance of the isolates.

Isolate	pH					Temperature (°C)			
	4.0	4.5	5.0-9.5	10.0	10.5	5	20-30	40	45
PZ (n=3)	-	1	+	+	+	-	+	-	-
PBa (n=4)	2	2	+	+	+	1	+	1	1
PB (n=11)	8	9	+	+	+	1	+	1	1
PH (n=13)	8	11	+	+	+	-	+	-	-
PG (n=25)	9	14	+	23	22	2	+	2	2
PHu (n=60)	30	45	+	+	+	7	+	-	-

*n: Number of isolates, '+'=growth, '-'=no growth. The figures in the table show the number of test isolates which tolerated the tested parameter.

regions.

Carbon and amino acids sources utilization pattern of the isolates

With respect to carbon source utilization (Table S1), it was observed that there is only a slight difference among the isolates. All isolates were able to grow on the 17 carbon sources provided, while 88% of the isolates utilized arabinose. Several studies reported that most carbon sources were utilized by rhizobia (Amarger et al., 1997; Workalemahu and Assefa, 2007; Zhang et al., 1991). Sixty four percent of the isolates utilized dulcitol and only 15% were able to metabolize fructose. Strain selection to produce inoculum requires effective isolates with high resistance to various environmental constraints and competence for resources. Since the isolates in this study have utilized a wide range of carbon sources, they will have a selective advantage over those with limited carbon source utilization ability, thus paramount importance for production of inoculum that can be useful in soil with different carbon sources.

Regarding the amino acids source utilization (Table S2), most of the amino acids were utilized by about 95% of the isolates. However, the most selective amino acids were Glycine and D-serine. Of the total 116 isolates tested, only 13 strains, namely, PG27, PHu50, PG59, PG68, PG72, PG76b, PG79, PG80, PG84, PB87, PBa93, PHu114, and PHu117 were able to utilize Glycine. On the other hand, isolates designated as PG 27, PG68, PG72, PG79, PG80, PG84, PB87, PHu114, PHu179, and PHu181 have utilized D-serine as N sources. The overall results presented in S2 have shown that the isolates of *C. cajan* in this study had an ability to utilize a wider range of nitrogen sources. It can thus be concluded that the test isolates in this study were not fastidious in their amino acids requirement; rather they were able to grow on wide range of amino acids. The ability of the test isolates to utilize a wide range of amino acid sources would be selected for a system thus can survive and establish themselves in an environment where nitrogen sources are a limiting factor for growth.

IAR, IHMR, and PSA of the test isolates

The intrinsic antibiotic resistance (IAR; Table S3), intrinsic heavy metal resistance (IHMR; Table S4) and phosphate solubilizing ability (PSA) clearly indicated the existence of variations among the test isolates. Accordingly, isolates of *C. cajan* showed high degree of resistance to the tested concentration of antibiotics, and this is in agreement with results of other several investigations (Eaglesham, 1987; Elkan, 1992; Jordan, 1984; Zhang et al., 1991). Similarly, the tested isolates resisted different concentration of heavy metals including Pb, Zn, Cu, and Al (Table S4), which is in agreement with other study of similar nature (Zhang et al., 1991). In the present study, cobalt seems to be selective in that only 43% of the isolates have resisted $CoCl_2.6H_2O$ 100 µg/ml. It is known that heavy metals are regarded as persistent in soils. Therefore, isolates with high intrinsic heavy metal resistance, is very useful for production of inoculum, which can be applicable in polluted soils with heavy metals released from industries.

Phosphorus is one of the limiting factors to crop production in many tropical soils (Singleton et al., 1985). In the present study, 68% of the isolates had solublized phosphate in medium containing tricalcium phosphate (TCP). Therefore, it can be concluded that, in addition to their beneficial nitrogen fixing activity with legumes, the tested rhizobial strain can potentially improve plant P nutrition by mobilizing inorganic and organic P. Phosphate solubilizing ability is a agood attributes for the strain to be used in soil with limited phosphorus source.

Numerical analysis

Cluster analysis of the 116 isolates and 7 reference strains was performed based on 91 phenotypic characteristics. The phenogram shows six phena and eight unclustered positions that were separated at a similarity cut point of 80% (Figure 1). Phenon I contained majority of the isolates (83%) representing fast and slow growing rhizobia, and grouped with *Bradyrhizobium japonicum* (HAMBI 2314^T) and *Bradyrhizobium elkanii*

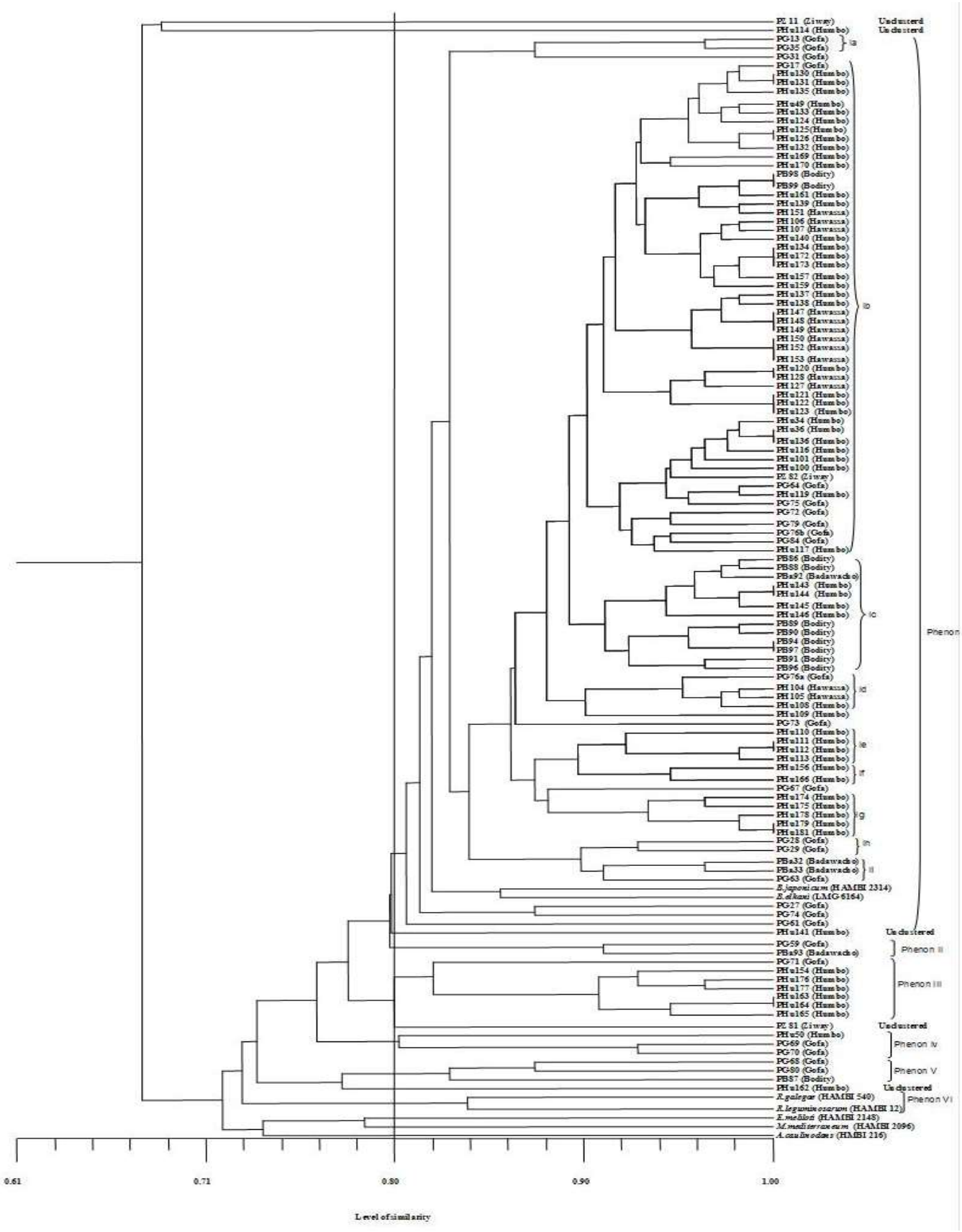

Figure 1. Phenogram showing similarity between the tested isolates and the reference species.

(LMG 6164). This phenon was very heterogeneous with 9 subphena at a similarity of 90%. It was reported that a significant number of slow-growing isolates of *C. cajan* are phylogenetically related to *B. elkanii* and *B. japonicum* strains (Ramsubhag et al., 2002). In addition, the existence of fast as well as slow-growing strains of rhizobia infecting *Cajanus* species were reported (Anand and Dogra, 1991). From this study, it was observed that pigeon pea strains isolated from the study area were diverse, and thus still there are untapped rhizobial resources in Ethiopian soils.

In conclusion, this preliminary study based on morphophysiological features has provided well characterized and preserved collection of rhizobial strains from nodules pigeon pea, and has explored the presence of indigenous rhizobia nodulating pigeon pea in the study areas. This study recommends to characterize the test isolates further using modern molecular techniques in order to elucidate the proper identity of the strains. Moreover, symbiotic effectiveness under greenhouse and field conditions, as well as evaluation of the competitiveness against indigenous strains should be studied to exploit the benefits these test isolates could offer for pigeon pea production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Table S1. Carbon sources utilization of pigeon pea isolates.

Carbon source	PZ (n=3)	PBa (n=4)	PB (n=11)	PH (n=13)	PG (n=25)	PHu (n=60)	% of utilizing isolates
Maltose, Sorbitol, Trehalose, Raffinose, Xylitol, Myo-inositol , Sucrose, Glucose, Mannitol, Starch, Galactose, Mannose, Ribose , Rhamnose , Dextrin, Inulin, Ribose, and Xylose	+	+	+	+	+	+	100
Arabinose	-	2	+	+	17	+	88
Dulcitol	+	-	3	2	23	56	64
Fructose	1	-	-	-	12	4	15
Citric and malonic acid	-	-	-	-	-	-	0

*n: Number of isolates, '+'=growth, '-'=no growth. Figures in the table show the number of the test isolates which were able to grow on the tested parameter.

Table S2. Amino acid utilization pattern of the isolates.

Characteristic	PZ (n=3)	PBa (n=4)	PB (n=11)	PH (n=13)	PG (n=25)	PHu (n=60)
L-Phenylalanine	+	+	+	+	+	+
L-Histidine	+	+	+	+	24	+
L-Pyroglutamic acid	2	+	+	+	+	59
L-Theronine	2	+	+	+	+	+
L-Alanine	+	+	+	+	+	+
L-Aspartic acid	2	+	+	+	24	59
L-Arginine	+	+	+	+	+	+
L-Proline	2	+	+	+	+	59
L-Asparagine	+	+	+	+	24	+
L-Gutamic acid	+	+	+	+	+	+
Inosine	+	+	+	+	+	+
Leucine	+	+	+	+	+	59
Thymidine	+	+	+	+	+	+
Uridine	+	+	+	+	+	+
Glycine	-	1	1	-	8	3
D-serine	-	-	1	-	6	3

*n: Number of isolates, '+'=growth, '-'=no growth. Figures in the table show the number of the test isolates which were able to grow on the tested parameter.

Table S3. Intrinsic antibiotic resistance of the isolates (IAR).

Antibiotic	Concentration (µg/ml)	Resistant isolates (%)
Lincomoycin	100	100
Trimtoprin	500	100
Streptomycin	10	83
Erythromycin	10	95
	20	95
Novobiocin	0.5	100
	1.5	100
Spectinomycin	2.5	94
	5	94
Kanamycin	5	91
	15	74
Neomycin	5	100
	20	98
Chloramphenicol	5	98
	15	98

Table S4. Intrinsic heavy metal resistance of the isolates.

Heavy metal	Concentration (µg/ml)	Resistant isolates (%)
$Pb(CH_3COO)_2$	500	83
$ZnCl_2$	100	95
$CoCl_2.6H_2O$	100	43
CuCl	100	96
$MnSO_4.H_2O$	500	99
$AlCl_3.6H_2O$	500	79