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Morphological, biochemical and physiological characterization of indigenous rhizobia nodulating *Lablab purpureus*

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Abstract

Twenty-two rhizobial isolates of *Lablab purpureus* grown in soils from Babati district, Manyara region, Tanzania, were characterized for their morphological, biochemical and physiological characteristics. The isolates had the characteristics of slow and fast growers. Most of them had circular form and convex elevation. All showed a positive catalase activity. There were few isolates which formed poor growth in Peptone Glucose Agar, but all isolates grew in mannitol, maltose and sucrose sugars. Mannitol was the best source of carbon, while sucrose had much of weak growth (45%) than other sugars. The growth media pH at 4.5 was intolerable to most of isolates as 23% did not grow, while 55% had weak growth. All isolates tolerated alkalinity conditions, but growth was much reduced in salts at 3% NaCl (w/v). The optimum temperature for most isolates was 30˚C, while at 45˚C some few isolates managed to grow. The isolates BR3, BR18 and BR20 tolerated a wide range of environment ranging from acidic, saline and high temperature and hence were considered as stress tolerant. The dendrogram of observed characteristics formed two groups and had seven clusters at 70% similarity level. Five isolates did not form a cluster. Therefore, this preliminary study has identified lablab strains in Tanzania, which can further be characterized for their symbiotic performance and hence for bioprospecting for improved nitrogen fixation in *Lablab purpureus*.

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Introduction

Rhizobia are gram negative soil microorganisms which forms a symbiotic interaction with legumes. In symbiosis, legumes provide photosynthesis products to bacteria, and in return rhizobia fix atmospheric nitrogen to plant in readily available form (Suzaki *et al.*, 2015). Rhizobia have other important roles like promoting nutrients uptake in legumes (Ndakidemi *et al.*, 2011), synthesis of phytohormones and reduce toxic effects of metals (Koskey *et al.*, 2018). The rhizobia can either be inoculated to legumes as biofertilizer or be naturally available in soils. Rhizobia which are found occurring naturally in particular soil are termed as indigenous to that geographic area (Chakraborty & Ramkrishna, 2005). The characteristics of indigenous rhizobia vary widely and their diversity is affected by climate, land use systems and soil fertility status (Legesse & Assefa, 2014). Rhizobia have a defined host range for legumes which they can nodulate. It has been established that one legume species can be nodulated by different rhizobia, and one rhizobia species can nodulate different legume species (Gao *et al.*, 2004). Therefore, for better understanding of indigenous rhizobia, it is necessary to isolate them from legumes and characterize by different criterion so as to add knowledge of possible legume-rhizobia association.

Lablab purpureus is an African native legume and has been cultivated for so long in many parts of the world (Chang *et al.,* 2011). In Tanzania, the crop is mainly cultivated by small scale farmers in some parts. The crop can be grown as a single stand or intercropped with either, millet, sorghum or maize. Lablab is known for its drought tolerance characteristic when compared with other grown legumes such as common beans and cowpeas. With the current trends in climate changes, lablab has a great potential for the future of African agriculture (Maass *et al.*, 2010). The crop has important roles as source of protein in food and feeds which is obvious less expensive than soybeans, but also as green manures, as soil coverage to reduce erosion, as herbal medicines and in gardening or landscaping (Sheahan, 2012). Despite of the above mentioned roles, the crop is commonly mentioned as one of neglected and underutilized legume crop (Ewansiha *et al.*, 2016; Mabhaudhi *et al.*, 2017). This crop needs researchers' attention in order to promote its production and consumption as it has more beneficial roles than some other legumes which are widely cultivated.

Like all other legumes, lablab is nodulated by rhizobia and can fix nitrogen. Studies on the characterization of rhizobia which can infect lablab have been conducted in different parts of the world (Benselama *et al.*, 2018), and a very varied characteristics have been reported from area to area. This is caused by varied environment and soil factors but also the host-rhizobia relationship (Shutsrirung *et al.*, 2011). By considering rhizobia-host relationship and their adaptation to environments, characterization of local isolates becomes an important study so as to explore these microbial resources and optimize their use in legume cultivation. This study aims at isolating and screen for morphological, biochemical and physiological characteristics at different stress levels for the indigenous nitrogen fixing rhizobia of *Lablab purpureus* from soil samples which were collected in Babati district, Manyara region, Tanzania, one of the major lablab producing district in the country.

Materials and methods

Soil samples collection

The soil samples (4 kg each) were collected from 15 villages in Babati district in Manyara region in Northern Tanzania (4.2078° S, 35.7461° E). Field selection was based on farms where lablab was cultivated for at least three consecutive seasons. By using a spade and hand hoe, soils were taken at 0-20cm depth form five different locations in the field to make a composite sample, then it was and packed into plastic bags. Bags with soil samples were labelled and transferred to Nelson Mandela African Institution of Science and Technology (NM-AIST) for testing.

Rhizobia trapping

Each soil sample was divided into two pots to grow lablab in glasshouse for rhizobia trapping. Lablab variety Eldoret Black 2, acquired from Tanzania Agriculture Research Institution (TARI) - Selian, Arusha was used. At early flowering which was approximately 50 days after planting, the plants were

uprooted from pots and observed for active nodules (with reddish color) in their root. Active nodules were detached from roots and kept in small plastic bags for isolation and characterization of rhizobia.

Media preparation

Rhizobia needs aseptic condition and suitable media to support their growth in characterization study. A common and generally accepted media is Yeast Extract Mannitol Agar (YEMA). The composition of YEMA was 10 g mannitol, 0.5 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, 0.5 g yeast extract powder and 15 g agar in 1 L distilled water, at 6.8 ± 0.2 pH. (Somasegaran & Hoben, 1985). YEMA was autoclaved at 121˚C for 15 minutes and left to cool to 50˚C before it was poured in sterile petri dishes to solidify.

Isolation of rhizobia from root nodules

During isolation, the nodules from freezer were transferred in falcon tubes with sterile distilled water and kept overnight at 4˚C for imbibition. The nodules were surface sterilized by firstly immersing in 95% ethanol for 10 seconds, transferred to 3.5% Sodium hypochlorite for 2 mins and serial rinsed in 6 changed sterile distilled water. In sterile petri dish with a drop of sterile distilled water, 4-6 nodules were crushed by sterile forceps. The loopful of suspension was streaked by sterile inoculation loop into petri dishes containing YEMA. The cultures were incubated at room temperature for 4-7 days, with daily observation. Sub-culturing of a single, typical rhizobia colonies was done to get pure cultures (Woomer *et al.*, 2011).

Morphological characterization

The morphological characterization of the pure cultures of the colonies was done after 4-7 days of incubation. The visual observation made included: growth type, colony form, elevation, margins, colony color, opacity, surface appearance, texture and colony size. The shape of cells and their motility was observed under microscope as described by Howieson & Dilworth (2016).

Biochemical characterization

The pure cultures were also streaked in YEMA with congo red 25ppm and observed for congo red absorption. As most of rhizobia do not absorb congo red (CIAT, 1988). The Catalase activity test was done by spreading drops of hydrogen peroxide 3% into petri dishes with isolate colonies and observed for oxygen bubbles formation as an indicator for catalase enzyme presence (Hamza & Alebejo, 2017).

Physiological and stress tolerance characterization

The growth response of the isolates in 4 different media was tested. The media were YEMA, Peptone Glucose Agar (GPA), Sucrose and Maltose sugar that were used instead of mannitol in YEMA. Different pH levels 4.5, 5.5, 8.5 and 9.5 of YEMA were used to characterize isolates tolerance to pH. The pH was adjusted by 1M NaOH and concentrated HCl. Salt tolerance was tested in YEMA with NaCl 1%, 2%, 3% and 4% (w/v). Isolates' ability to grow under elevated temperature was tested at 30˚C, 35˚C, 40˚C and 45˚C. The growth responses were scored as "- (no growth), * (weak growth), ** (medium growth) and *** (Large growth) " (Datta *et al.*, 2015; Kucuk *et al.*, 2006).

Data Analysis

The collected data were used to plot a graph to show a percentage differences in their response to different condition. Data were binary coded (1 for presence and 0 for absence), for use in clustering of the isolates with respect to their characteristics. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was used for clustering in PAST software V 3.25, by Dice similarity coefficient (Hammer *et al.*, 2001).

Results

Twenty two out of 30 soil samples used to grow lablab were found to have roots with active nodules. The active nodules had pink-brown coloration, which was indicating presence of leghemoglobin (Delić *et al.*, 2010). The isolates from different villages were designated with numbers BR1-BR22, with BR as Babati Rhizobia.

Morphological characteristics

As shown in Table 1, there was variation in growth and colonies appearance. After 4-7 days of incubation, isolates showed different colony sizes. Some isolates (BR2, BR5 BR6, BR9, BR10 and

BR22) appeared to have small colonies even after 7 days and were regarded as slow growers. The rest of isolates forming large colonies after 4 days, were regarded as fast growers. The elevation of colonies was convex, flat and conical. The colonies formed regular and few irregular margins. The colonies were circular and oval in shape, with creamy, creamy-white and milky-white coloration. All colonies had smooth surfaces and dull or glistening appearance was observed. Mucus production for rhizobia isolates BR2, BR4, BR6, BR18 and BR22 was high, and was moderate to other isolates. Cells of the isolates appeared to have rod and coccus shapes, but all were motile.

Table 1. Morphological and Biochemical characteristics of rhizobia isolated from root nodules of lablab.

Village name	Isolates	Morphological Characteristics										Bioc hemical	
		Form	Elevation	Margin	Colour	Surface Appearance		Opacity	Texture	Diameter (mm)	Cell Shape	Motility	Congo Red	Catalase Activity
Maweni	BR1	Circular	Convex	Irregular	Creamy	Smooth	Glistening	Translucent	Mucoid	°4	Rod	Motile	Not absorbed	Positive
Maweni	BR2	Circular	Flat	Regular	Creamy-white	Smooth	Glistening	Translucent	Highly mucoid	°4	Rod	Motile	Not absorbed	Positive
Chemchemu	BR3	Circular	Convex	Regular	Creamy	Smooth	Glistening	Translucent	Mucoid	°4	Rod	Motile	Not absorbed	Positive
Chemchemu	BR4	Oval	Convex	Irregular	Creamy	Smooth	Dull	Opaque	Highly mucoid	°4	Rod	Motile	Not absorbed	Positive
Nakwa	BR5	Circular	Convex	Regular	Milky-white	Smooth	Dull	Translucent	Mucoid	°4	Coccus	Motile	Not absorbed	Positive
Moya	BR6	Circular	Convex	Regular	Milky-white	Smooth	Glistening	Opaque	Moist	°4	Rod	Motile	Not absorbed	Positive
Riroda	BR7	Oval	Flat	Regular	Creamy-white	Smooth	Dull	Opaque	Mucoid	°4	Rod	Motile	Not absorbed	Positive
Mwada	BR8	Circular	Convex	Regular	Creamy-white	Smooth	Glistening	Opaque	Mucoid	°4	Coccus	Motile	Not absorbed	Positive
Endakiso	BR9	Circular	Flat	Irregular	Creamy-white	Smooth	Glistening	Translucent	Mucoid	°4	Coccus	Motile	Not absorbed	Positive
Endakiso	BR10	Circular	Flat	Regular	Creamy-white	Smooth	Glistening	Opaque	Mucoid	°4	Rod	Motile	Not absorbed	Positive
Mwikantsi	BR11	Oval	Convex	Regular	Milky-white	Smooth	Glistening	Translucent	Mucoid	°4	Coccus	Motile	Not absorbed	Positive
Mwikantsi	BR12	Circular	Convex	Irregular	Creamy-white	Smooth	Dull	Translucent	Mucoid	°4	Coccus	Motile	Not absorbed	Positive
Mbuyuni	BR13	Circular	Convex	Regular	Creamy-white	Smooth	Dull	Translucent	Mucoid	°4	Rod	Motile	Not absorbed	Positive
Arri	BR14	Circular	Flat	Regular	Creamy-white	Smooth	Glistening	Translucent	Mucoid	°4	Rod	Motile	Not absorbed	Positive
Arri	BR15	Circular	Conical	Irregular	Creamy-white	Smooth	Dull	Translucent	Mucoid	°4	Coccus	Motile	Not absorbed	Positive
Manyara	BR16	Circular	Flat	Regular	Milky-white	Smooth	Glistening	Opaque	Highly mucoid	°4	Rod	Motile	Absorbed	Positive
Sarame	BR17	Circular	Convex	Regular	Creamy-white	Smooth	Glistening	Translucent	Mucoid	°4	Rod	Motile	Not absorbed	Positive
Singe	BR18	Circular	Convex	Regular	Milky-white	Smooth	Dull	Opaque	Highly mucoid	°4	Rod	Motile	Not absorbed	Positive
Singe	BR19	Oval	Convex	Regular	Creamy-white	Smooth	Dull	Opaque	Mucoid	°4	Rod	Motile	Not absorbed	Positive
Matufa	BR20	Circular	Convex	Regular	Milky-white	Smooth	Glistening	Translucent	Moist	°4	Rod	Motile	Not absorbed	Positive
Mawemario	BR21	Oval	Convex	Irregular	Creamy	Smooth	Glistening	Opaque	Mucoid	°4	Rod	Motile	Not absorbed	Positive
Mawemario	BR22	Circular	Convex	Regular	Milky-white	Smooth	Glistening	Opaque	Highly mucoid	°4	Coccus	Motile	Not absorbed	Positive

Biochemical characteristics

Only one isolate (BR16) absorbed the color and formed red colonies when grown in congo red and incubated in darkness, while the rest were white-pink. The bubbles were also formed in catalase test for all isolates, when the drops of 30% hydrogen peroxide (H_2O_2) were added to petri dishes with rhizobia culture (Fig. 1 A).

Physiological and stress tolerance characteristics

The results for physiological characteristics and tolerance to pH, temperature and salt are presented in Table 2. All isolates were able to grow well in mannitol, but there was reduced growth in maltose to 41% (large), 41% (medium), 18% (weak) and in sucrose to 14% (large), 41% (medium) and 45% (weak) (Fig. 3) when used as a sole source of carbon. Rhizobia responded with different patterns as weak and medium to large growth in different carbon sources. In GPA, poor growth of few isolates BR6, BR17, BR19 and BR22 was seen, while other isolates completely failed to grow.

Growth of the isolates in media with different pH levels was found to vary. There was a noted reduced growth of all isolates in acidic media (Fig. 1 B), while some failed to grow. Isolates BR6, BR12, BR15, BR19 and BR21 were highly susceptible in acidic media at 4.5 and 5.5 pH. In alkaline media the growth was normal for most of the isolates.

The salt concentration was found to affect the rhizobia growth in large extent. Growth was highly reduced at higher salt concentrations of 3 and 4% NaCl (w/v), as seen in Fig. 1 C where most of isolates failed to grow at that salt concentration. Some isolates like BR2, BR3, BR9, BR10, BR18 and BR20 were able to grow up to 4% NaCl. Temperature also had influence in rhizobia, the growth was decreased with increase in temperature. But there were some isolates that were able to grow up to 45˚C, while BR4, BR10, BR13, BR15 and BR21 were highly susceptible to high temperature.

Table 2. Growth responses of rhizobia isolated from root nodules of lablab to different physiological stress condition.

Village name	Isolates	Growth Media				Physiological Stress Condition											
						pH				NaCl Conc. (w/v)				Temperature °C			
		Mann	Malt	Sucr	GPA	4.5	5.5	8.5	9.5	1%	2%	3%	4%	30	35	40	45
Maweni	BR1	***	***	**	-	*	**	***	***	***	**	*	-	***	**	*	*
Maweni	BR2	**	***	***	-	**	***	***	***	***	**	**	*	**	***	**	**
Chemchemu	BR3	***	***	***	-	*	***	***	***	***	**	*	*	***	***	***	***
Chemchemu	BR4	***	**	*	-	*	*	**	**	***	*	-	-	***	*	*	-
Nakwa	BR5	**	**	**	-	*	*	**	***	***	*	-	-	**	**	**	*
Moya	BR6	**	**	*	*	-	*	**	***	***	*	-	-	**	***	*	**
Riroda	BR7	***	*	*	-	*	**	**	***	**	*	-	-	***	***	**	*
Mwada	BR8	***	**	**	-	*	*	**	***	**	*	-	-	***	***	**	*
Endakiso	BR9	**	*	**	-	*	**	**	***	***	***	*	*	**	***	***	**
Endakiso	BR10	**	***	**	-	*	*	***	***	***	**	**	*	**	**	*	-
Mwikantsi	BR11	***	**	*	-	**	**	***	***	**	*	-	-	***	**	*	*
Mwikantsi	BR12	***	**	*	-	-	*	***	***	***	*	-	-	***	***	**	**
Mbuyuni	BR13	***	**	*	-	**	*	***	**	***	*	-	-	***	**	*	-
Arri	BR14	***	***	*	-	*	*	**	***	***	*	-	-	***	***	**	*
Arri	BR15	***	*	*	-	-	*	***	**	***	*	-	-	***	***	*	-
Manyara	BR16	***	***	**	-	*	**	***	**	***	**	*	-	***	**	***	**
Sarame	BR17	***	*	**	*	*	**	**	**	***	*	-	-	***	***	**	*
Singe	BR18	***	**	**	-	**	**	**	**	***	**	*	*	***	***	**	***
Singe	BR19	***	***	*	*	-	**	**	*	***	*	-	-	***	***	**	*
Matufa	BR20	***	***	***	-	**	***	***	***	***	***	**	*	***	***	***	***
Mawemario	BR21	***	***	**	-	-	*	**	**	***	*	*	-	***	*	*	-
Mawemario	BR22	**	**	*	*	*	**	***	***	***	*	-	-	**	***	**	*

Note: Mann (Mannitol), Malt (Maltose), Sucr (Sucrose), GPA (Peptone Glucose Agar), - (No growth), * (Weak growth), **(Medium growth) and ***(Large growth)".

The combination of morphological, biochemical and physiological characteristics presented in Table 1 and table 2, were used to generated a dendrogram to show relationship between the isolates. The dendrogram formed 2 groups (A and B) at 55% similarity and there was a total of 7 clusters at 70% similarity level (Fig. 2). All groups composed slow and fasting growing isolates. Group A with total of 8 isolates formed Cluster I and II, also there were 4 unpaired isolates. Group B is composed of 16 isolates, with 5 clusters (III-VII) and one isolate (BR11) was independent. The cluster with higher number of isolates formed in dendrogram was cluster IV with 4 isolates (BR7, BR19, BR14 and BR 17), followed by cluster VII with 3 isolates, all other remaining clusters were having 2 isolates. For group A, the member isolates were all the salt and high temperature tolerant. All of them were capable to grow in 3% NaCl and 6 out of 8 isolates grew in 4% NaCl. Only one isolate BR21 of group B was able to grow in salt above 2% NaCl. Member isolates of cluster III, their growth was highly reduced at 2% NaCl and did not grow at higher salts.

Discussion

This study reports on morphological, biochemical and physiological characteristics of 22 rhizobia isolates of *Lablab purpureus* grown in soils from villages of Babati district, Manyara region, Tanzania. The studied isolates were showing the characteristics of fast (73%) and slow (27%) growing rhizobia. The result were similar to those reported by Benselama *et al.* (2013). The dominance of fast-growing over slow growing rhizobia was contrary to Jaiswal & Dakora (2019) who reported that slow growing rhizobia (*Bradyrhizobium*) were dominant in some African soils. Convex elevation and circular form of colonies were similar to those of lablab rhizobia characterized by Pervin *et al.* (2017). The variation in colony colors and opacity was also depicting diversity as also reported by Koskey *et al.* (2018) in *Phaseolus vulgaris* rhizobia. The variation in mucus production as high and moderate mucoid among the rhizobia isolated from the same plant species were like those observed by Teixeira *et al.* (2010). Mucus production has been an important feature in rhizobia as it helps in adapting to stress condition, a vital condition for enhancing the nodulation capacity (Ondieki *et al.*, 2017).

Its widely documented that rhizobia do not absorb congo red (Woomer *et al.,* 2011). Some few rhizobia can still absorb the dye, and it can be used as one of distinctive feature for those rhizobia as described by Somasegaran & Hoben (1994). In our study, only one isolate (BR16) absorbed congo red. Kneen & Larue (1983) identified some rhizobia which could absorb congo red like *R. trifolii* and *R. melilot*, so BR16 could be related to them. Further investigation is recommended on this isolate to identify its group. Rhizobia are known to have catalase enzyme that prevent harmful forms of oxygen as H_2O_2 (Elzanaty *et al.,* 2015). All 22 isolates formed bubbles when drops of 30% H_2O_2 was added and this showed the positive catalase activity as the enzyme hydrolyzes H_2O_2 to water and oxygen gas (Wadhwa *et al*., 2017; Elzanaty *et al*., 2015).

Mannitol that allowed 73% of isolates forming large growth, was the best among other sugars used. Mannitol as a sugar alcohol has been used widely as standard sugar for rhizobia culturing. It is a standard media that supports sufficient growth than other sugars, and it was also recommended by Vincent (1970) as the best source of carbon for rhizobia cultures. The insufficient growth of rhizobia in the maltose and sucrose (disaccharides) can be due to the inability of disaccharides uptake by some rhizobia (Dekak *et al*., 2018). Similar findings was reported by Naz *et al.* (2009); Patel & Dubey (2014).

Growth of the isolates was hampered in acidic medium, with 4.5 pH showing 0% of large growth, while alkaline medium at 8.5 and 9.5 pH did not affect rhizobia growth as most isolates formed large colonies. Acidic conditions have been reported to affect growth of rhizobia, and the optimum growth is found at pH 6.5-7 (Laurette *et al.,* 2015). Our findings mimic those reported by Dias *et al.* (2019) who found alkalinity was not a problem to rhizobia growth. Also, Howieson & Dilworth (2016) described that some rhizobia tolerates alkalinity up to pH level of 10. More studies are needed on isolates which were able to grow in low pH, as they can have high contribution to improving nitrogen fixation for legumes in low pH soils.

All rhizobia isolates grew at NaCl 1%, and 84% of isolates formed large growth, while at 2% reduced growth started to occur. The large growth in low salt concentration was caused by less salt effects in media and physiology of rhizobia. Maatallah *et al.* (2002) reported large growth of rhizobia at 1% salt, while Pervin *et al.* (2017) also observed reduced growth in 2%. More reduced growth was observed in 3% and 4% NaCl, as 59% and 73% of the isolates did not grow respectively (Fig. 3). Increased salt concentration in growth media could have affected rhizobia growth through a mechanism related to toxicity and osmotic stress (Zahran, 1999). Despite the toxicity in higher salt concentration, Datta *et al.* (2015); Kucuk *et al.* (2006) found higher growth of both *Rhizobium* and *Bradyrhizobium* at 4% NaCl. Our study also found a few slow growing (BR2, BR9 and BR10) and fast-growing (BR3, BR18 and BR20) isolates which tolerated and grew at 4% NaCl. These are considered as salt tolerant isolates. Laurette *et al.* (2015), described tolerance to high NaCl concentration as an important feature for rhizobia comptitiveness in high salt rhizosphere as it helps in the survival and nodulation ability in the host plant. Further studies are recommended on the identified isolates which were tolerant to higher salt concentration.

Temperature has effect on survival, growth and on physiological activities of rhizobia (Simon *et al*., 2014). In a current study, optimal temperature for the isolates' growth was observed to be 30˚C. The same optimum temperature was also reported by Benselama *et al.* (2013) in studies involving lablab rhizobia. Although, most of the isolates were temperature tolerant, however, few isolates (BR4, BR10, BR13, BR15 and BR21) did not grow at 45˚C. Panwar *et al.* (2012) in identifying temperature tolerance for *Trigonella foenum* rhizobia also found some isolates which could grow at higher temperature above 40˚C. Alike to our study, isolates BR3, BR18 and BR20 were more temperature tolerant than others. The results suggest that these isolates can perform better in high temperature soil environments where lablab is commonly grown. These results are encouraging because such isolates when used will support successive nodulation and nitrogen fixation, hence improving the final plant growth at high temperatures.

In a dendrogram all groups composed slow and fasting growing isolates. While, the largest cluster formed was cluster IV with 4 isolates, followed by cluster VII with 3 isolates, and all other remaining clusters were having 2 isolates. Being at different groups and clusters for the fast and slow growing isolates, it implies that they could show some similarities in morphological, biochemical and physiological traits. All isolates of group A were able to grow in acidic media with 4 pH level, and in high salt concentration up to 4% NaCl. While, group B isolates showed to be varying in their tolerance to most of stress conditions tested, and only one isolate BR21 was able to grow in salt above 2% NaCl. They had a mixed response to physiological changes that can suggest their grouping was much based on similarities of morphological characteristics. For example, cluster V had isolates of which one tolerated acidic condition at 4 pH level while the other failed to grow in the same condition. Another example of varied response within a cluster was for cluster VI, whereby one isolate tolerated acid condition and failed to grow in saline, but then the other isolate tolerated saline and failed to grow in acidic condition. This shows how rhizobia can have different responses but being similar to most of the morphological features, and it confirms that they highly differ in their adaptation to environmental conditions and hence symbiotic ability. The grouping and the differences between the two groups was in agreement to the study of Maatallah *et al.* (2002) who found physiological characteristics relating to adaptation such as in acidity and salinity can be used for grouping and as distinctive feature for rhizobia. There was no much differences in temperature tolerance between the isolates of the two groups, this could have been caused by warm climate for the most part of the year in the areas where soil samples were collected.

There was no relationship between the isolates clustering and their geographic original at 70% similarity level. Isolates from same field were placed in different clusters, also isolates from different villages clustered together. While at 55% similarity it occurred for some rhizobia to be in a same group, group A had BR1 and BR2 (Maweni) also BR9 and BR10 (Endakiso). While, group B had BR11 and BR12 (Mwikantsi), BR14 and BR15 (Arri) also BR21 and BR22 (Mawemairo). Several studies such as Rai *et al.* (2012) have found the similar diversity in rhizobia from same field of origin not clustered together. Also rhizobia in an geographic area presents a high diversity as it has been reported a possibility to find different rhizobia strains in the same root nodule (Denison & Kiers, 2011). Hence, the diversity of rhizobia that has been identified is an indicator that rhizobia are very diverse and more exploration is needed to identify the best candidate isolates which can suit our farming system and improve sustainability.

Conclusion

This as a first study for lablab rhizobia in Tanzania has identified much differences in their morphology, biochemical and physiological characteristics. The variation appears to be beneficial and important for inoculants bioprocessing as they could tolerate stress conditions such as acidic, high salt concentration and temperature. Clustering of isolates according to their studied features showed a wide diversity as a total of seven clusters were obtained while most of clusters were composed of two isolates and the isolates from same soil were even not clustered together. The tolerance to stress condition and diversity which was observed in this study is believed to have benefits to legume growers as these competitive strains can be used in harsh environments of similar characteristics in Tanzania and other parts. More studies are recommended on isolates such as BR3, BR 18 and BR 20, which tolerated wide range of environment ranging from acidic, saline and high temperature to check their genetic nature and potential of having rhizobia strains which can perform well in most of the poor African soils for sustainable legume production.

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