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RESEARCH ARTICLE

LENTIL- AND FABA BEAN- NODULATING RHIZOBIAL GENOSPECIES OBTAINED FROM CENTRAL AND SOUTHERN ETHIOPIA ARE DIVERSE IN THEIR PHENOTYPIC AND SYMBIOTIC PROPERTIES

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ABSTRACT: In our previous study, we isolated and genetically characterized various faba bean and lentil symbionts belonging to diverse Rhizobium genospecies from the soils of Ethiopia. Twenty lentil- and faba bean- nodulating rhizobial strains representing different isolation site, genospecies grouping, host plant and symbiotic gene groups were selected for this study. The aim of the study was to phenotypically characterize the test strains and to explore their nodulation and symbiotic properties to ultimately select those suited for inoculant purposes. Strain EAL 110, a commercial inoculant strain for faba bean, was used as a reference strain to compare the nodulation status (nodule number, nodule dry weight, shoot dry weight, total nitrogen and symbiotic efficiency scores) of the test strains. In vitro experiments were conducted to assess the effects of different eco-physiological stresses (pH, salinity, temperature, antibiotics and carbon and nitrogen substrates assimilation) on their growth. The greenhouse sand-pot experiment showed that the different strains displayed variations in their symbiotic performance on both legume hosts. The consequent results show strain EAL 110 (SE% = 85 with both lentil and faba bean) has still maintained its desired symbiotic property. However, 9 strains with lentil and 5 strains with faba bean from our collections have outperformed (≥85% SE) the reference control strain EAL110 ($p<0.001$). Of these strains, strain L33b, L53c, F32a and F42 have shown their phenotypic and symbiotic advantage over the remaining treatments under a controlled environment and should thus be selected for further tests to screen their ability of maintaining these traits in natural (field) settings for future bio-inoculant formulation.

Key words/phrases: Faba bean, Lentil, Nodulation, Rhizobia, Symbiotic property.

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INTRODUCTION

Nitrogen is an essential nutrient for plant growth and development. The inert nature of the element in its gaseous form as well as the inefficient use of its available (fixed) form (Beddington, 2010) has led to the decline in global agricultural produce (Folberth *et al*., 2013). Addressing yield gaps associated with nitrogen deficiency is thus vital to significantly contribute towards the efforts of increasing the global food supply. The use of leguminous crops in rotation with cereals is an efficient strategy that reduces dependence on nitrogenous fertilizers. Legumes provide a cleaner source of fixed nitrogen which results from their symbiosis with phylogenetically diverse soil bacteria collectively called rhizobia (Held *et al*., 2010).

Lentil and faba bean are important crops in Ethiopia with an annual production of 151 and 698 kilotons, respectively (CSA, 2020). They form an important dietary element, especially in the fasting culture of the country, and are accordingly prepared into different dishes (soups, stews, curries, roasted snacks, and so on). Both legumes are rich in proteins and contain high concentrations of essential amino acids, dietary fibers, vitamins and minerals (Rozan *et al*., 2001) making them good dietary sources for humans and animals (Lardy and Anderson, 2009).

Like most annual legumes, lentil and faba bean form symbiosis with Rhizobia to fulfill most of their nitrogen requirements, fixing up to 107 (lentil) and 120 (faba bean) kg of nitrogen per hectare when favourable conditions prevail (ICARDA, 2008; Rashid *et al*., 2012). Both lentil and faba bean require an effective and compatible rhizobium to fix atmospheric nitrogen. Traditionally, it was established that *Rhizobium leguminosarum* sv *viciae* nodulates legume hosts belonging to the *viciae* tribe that include lentil, faba bean and grass pea (Jordan, 1984). Recent studies however showed that other groups of rhizobia, such as *R. leguminosarum* complex (*R. leguminosarum*, *R. fabae*, *R. etli*, *R. pisi*, and *R. leguminosarum* sv. *trifolii*), *R. aegyptiacum*, and *R. bangladeshense* nodulate with lentil and faba bean (Sami *et al*., 2016; Siczek and Lipiec, 2016; Villadas *et al*., 2017). Besides their principal role in the nitrogen flow and uptake, rhizobia are able to directly induce plant growth through mechanisms that include Auxin (phytohormone) production and release of soil insoluble nutrients (eg. Iron and phosphorus), among others (Rashid *et al*., 2012). Several studies in Ethiopia (Amanuel Gorfu *et al*., 2000; Prakash *et al*., 2002; Kiros Habtegebrial and Singh, 2006; Solomon Legesse and Fassil Assefa, 2014; Dereje Tsegaye *et al*., 2015; Wondwosen Tena *et al*., 2017; Getahun Mitiku

et al., 2016) showed that Ethiopian soils harbour symbiotically competent strains that nodulate lentil and faba bean. However, most of the hitherto studies focused on a narrow range of taxonomic groups mainly strains of *R. leguminosarum* sv. *viceae*.

In our previous study, we isolated and genetically characterized 196 rhizobial strains from faba bean and lentil from some parts of Ethiopia and identified new genospecies dominated by *R. aethiopicum* and *R. aegyptiacum*) that had not been locally reported as symbionts for both legumes (Beimnet Asfaw *et al*., 2020). A fully functional symbiosis however requires the survival ability of rhizobia as they often are faced with various edaphic stresses that affect their growth as well as the initial steps in the process of nodulation and their effectiveness in nitrogen fixation (Wigley *et al*., 2016). The persistence and survival of strains can be determined by studying their ability to withstand various environmental stresses and compete with others in the rhizosphere (Graham, 2008). Thus, the continuous selection of new and more efficient rhizobial strains (inoculants) is thus vital to obtain strains with higher agronomic performance. The present work is thus focused on studying the phenotypic and symbiotic features of 20 lentil and faba bean nodulating rhizobial strains under controlled environment with the objective of identifying elite nitrogen-fixing strains with a potential to serve as an inoculant after validation study under field conditions.

MATERIALS AND METHODS

Bacterial origin

The selected twenty root nodule bacteria represent different isolation sites, isolation host plant, genospecies grouping as well as symbiotic gene groups and were collected from different parts of Ethiopia, genetically characterized and deposited in the culture collections of Addis Ababa University and the University of Helsinki. Details of the twenty strains are presented in Table 1 and in Beimnet Asfaw *et al*. (2020). The *Rhizobium* strain EAL110 was kindly supplied by the National Soil Testing Centre and used as a reference strain (positive inoculated control) for the symbiotic efficiency study. This study was carried out in the Microbiology Laboratory at Addis Ababa University and greenhouse of the National Soil Testing Centre, Ethiopian Institute of Agricultural Centre.

Table 1. Descriptions of the twenty test strains used in this study.

Strain code	Closest reference species	*recA* accession number	*nodC* accession number	Host and site of isolation*
L12y	*R. aethiopicum strain* HBR26T	MN386431	MN386485	Lentil, AG
L23a	*R. aegyptiacum strain* 1010T	MN386355	MN386553	Lentil, DB
L33b	*R. aegyptiacum strain* 1010T	MN386360	MN386575	Lentil, BU
L42a	*R. aegyptiacum strain* 1010T	MN386349	MN386549	Lentil, AK
L53c	*R. leg. sv. viciae* LMG14904T	MN386409	MN386530	Lentil, DZ
L62	*R. etli strain* Kim 5	MN386344	MN386667	Lentil, AL
L85b	*R. aethiopicum* strain HBR26T	MN386333	MN386558	Lentil, FI
L71	*R. aethiopicum* strain HBR26T	MN386377	MN386570	Lentil, CD
L84	*R. leg. sv. trifolii* strain CB782	MN386421	MN386577	Lentil, FI
L94d	*R. leg. sv. viciae* LMG14904T	MN386322	MN386572	Lentil, HO
F17a	*R. aethiopicum* strain HBR26T	MN386437	MN386519	Faba, AG
F22	*R. aegyptiacum* strain 1010T	MN386459	MN386475	Faba, DB
F32a	*R. etli strain* TAL 182	MN386347	MN386478	Faba, BU
F42	*R. etli strain* HBR5	MN386311	MN386568	Faba, AK
F56	*R. aegyptiacum* strain 1010T	MN386402	MN386532	Faba, DZ
F73	*R. leg. sv. trifolii* strain CB782	MN386384	MN386538	Faba, CD
F85	*R. leg. sv. viciae* LMG14904T	MN386327	MN386573	Faba, FI
F86	*R. etli* strain HBR5	MN386319	MN386569	Faba, FI
F95	*R. leg. sv. trifolii* strain CB782	MN386379	MN386482	Faba, HO
F102	*R. leg. sv. trifolii* strain CB782	MN386389	MN386559	Faba, HA

*AG-Alem-gena, DB-Debre-brehan, BU-Butajira, AK-Akaki, DZ- Debre-zeit, AL-Alagae, CD-Chefe-donsa, FI-Fiche, HO-Holetta, HA-Hawassa

In vitro physiological characterization tests

Experimental conditions

Each strain was grown on Yeast Extract Mannitol (YEM) broth (10 ml) tubes and incubated at 28°C ± 2 in a rotary shaker for 48 hours from which 1 ml of culture was used as inoculum for each experiment (Somasegaran and Hoben, 1994). The pipette culture was then inoculated on YEM solid medium in triplicates and incubated at 28°C ± 2 for 5–7 days for the various physiological tests exception being the temperature tolerance test which differed in the incubation temperature. The subsequent results, unless stated otherwise, were qualitatively scored in all cases as "+" for positive (growth) and "–" for negative (no growth) responses in reference to the control plates.

Determination of pH and salt tolerance

The ability of test bacteria to tolerate pH or salt on the growth of the test bacteria was evaluated following the procedures of Küçük *et al*. (2006). The test bacteria were inoculated on the medium adjusted to different pH levels (4, 4.8, 5.8, 6.8, 7.8, 8.8, 9.8 and 11) using 1M NaOH or HCl while the medium modified to contain different concentrations of NaCl (0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% and 10% w/v) was used for the salt

tolerance study. Plates adjusted to pH 6.8 (for the pH test) and those with 0% (no extra NaCl added) served as control treatments.

Temperature tolerance test

The ability of the study strains to grow at different temperatures was determined using YEM agar plates according to Lupwayi and Haque (1994). The inoculated plates were incubated at different temperatures (4°C, 10°C, 15°C, 20°C, 25°C, 28°C, 37°C, 44°C and 50°C) for the test.

Inherent antibiotic resistance test (IAR)

The ability of the strains to withstand different antibiotics was evaluated on YEM agar plates containing filter-sterilized (Millipore 0.2 µm) ampicillin, kanamycin, streptomycin, penicillin, tetracycline, erythromycin and chloramphenicol at concentrations 2.5, 5 and 10 mg l^{-1} (Küçük *et al*., 2006). Plates with no antibiotics (0% plates) were used as control.

Substrate utilization test

Subject strains were tested for their ability to utilize different carbon and nitrogen sources (*in vitro* heterotrophic competence (Amarger *et al*., 1997). They were inoculated on a basal medium containing galactose, maltose, starch, sorbitol, glycerol, arabinose, glucose, fructose, lactose, and sucrose. The basal medium contained; (g/L): K_2HPO_4, 1; KH_2PO_4, 1; $FeCl_36H_2O$, 0.01; $MgSO_4.7H_2O$, 0.02; CaCl2, 0.1; $(NH_4)_2SO_4$, 1; and 15 g of agar). They were also inoculated on the same basal medium containing nitrogen sources (amino acids); (L-tryptophan, L-alanine, L-arginine, L-glycine, L-glutamate, L-leucine, L-lysine, L-phenylalanine, L-tyrosine, and L-thymine) at a concentration of 0.5 g/l where ammonium sulfate was omitted and mannitol was added at a concentration of 1 g/l.

Symbiotic efficiency test

The nitrogen-fixing efficiency test of the 20 test strains was carried out under greenhouse conditions with lentil (Alemaya) and faba bean (Degaga) varieties kindly supplied by NSTC. Seeds were surface sterilized (1 min in 95% ethanol, 5 min in 3% sodium hypochlorite and rinsed 5 times with pre-sterilized distilled water) and germinated on sterile water agar plates according to Somasegaran and Hoben (1994). Five well-germinated seeds were transplanted into 70% ethanol sterilized 3 kg capacity plastic pots filled with acid washed (28.5% sulphuric acid for 24 h then water washed till neutral) river sand (Lupwayi and Haque, 1994). The inoculants were grown for 48 hours and adjusted to inoculum size of (about 10^9 cells ml^{-1})

and inoculated into each of the five seedlings after transplantation. The plants were thinned down to three seedlings per pot a week after planting. The pots were fertilized with quarter-strength Jenson's modified nitrogen-free nutrient solution every week and washed with water every two weeks to avoid salt accumulation (Somasegaran and Hoben, 1994).

A total of 138 pots (20 test strains + 3 controls X 2 host legumes in triplicate) were used for this test with the pots arranged in a Randomized Complete Block Design (RCB). Treatments used included 20 rhizobial strains, 2 positive controls where one was supplied with a nitrogen source (0.05% w/v KNO_3) on a weekly basis and the other inoculated with EAL 110 (a commercial inoculant strain for *Viciae* tribe legumes) as well as a negative control with no source of nitrogen (neither inoculated nor supplied with a nitrogen source).

The plants were carefully uprooted after 60 days of planting to collect and count the number of nodules from plant roots and determine nodules and shoot dry weight after oven dried at 70°C for 48 h (Lupwayi and Haque, 1994). The samples were then grounded to quantitatively determine the total nitrogen using the modified "Wet" Kjeldahl method (Sahlemedhin Sertsu and Taye Bekele, 2000). Lastly, the symbiotic efficiency percentage of the test strains was calculated by comparing the nitrogen content of inoculated plants with that of the nitrogen supplied control using the formula: (total nitrogen in inoculated plant/total nitrogen in N applied plant) x 100 with symbiotic efficiency classified as ineffective (<35%); lowly-effective (35–50%); effective (50–80%); and highly effective (>80%) (Ogutcu *et al*., 2008).

Data analysis

The greenhouse experimental data (nodule and shoot dry matter, nitrogen content) were tested with one-way variance analysis (one-way ANOVA) and the averages were compared by Duncan's mean range test ($p \leq 0.05$). All data were analyzed using the statistical package SPSS v 23.0.

RESULTS AND DISCUSSION

In vitro pH, salt and temperature tolerance

Almost all strains were tolerant to the pH range of 4.8–8.8 and 5% NaCl concentration. Most strains (55%) and few (25%) were also able to grow on YEMA medium measuring pH 9.8 and 6% NaCl, respectively while none grew at pH 4 and 11 (Table 2). The isolates also showed good growth at a temperature range from 10°C–37°C while 30% of the strains were tolerant

to 44°C. Generally, *R. aethiopicum* L12y and *R. aegyptiacum* L23a were the most tolerant isolates to the above mentioned eco-physiological stresses (Table 2). The observed adaptability to a wider pH, salt and temperature range amongst the test strains did not reflect or correspond to the edaphic conditions of the isolation soil/site which was in the neutral pH range of 6.5–7.7) and with an annual average temperature between 14 and 19.2°C (Beimnet Asfaw *et al*., 2020).

Jordan (1984) stated that fast growing rhizobia are more sensitive to lower pH than alkaline conditions. Our results however indicate that 80% of the strains grew at pH 4.8 while only 55% tolerated pH 9.8. Similarly, the strains showed higher thermo-tolerance as opposed to the one reported by Getahun Mitiku *et al.* (2016) and Wondwosen Tena *et al*. (2017) on lentil as well as by Zerihun Belay and Fassil Assefa (2011) on faba bean, whose isolates could only tolerate as much as 40°C.

Table 2. Eco-physiological characteristics of lentil- and faba bean- nodulating rhizobia grown on YEMA medium for 5–7 days.

Strain	**Corresponding**	**pH**		**Salt**			**Temperature**			**Positive**
Code	**reference species**	**4.8**	**9.8**	**4**	**5**	**6**	**10**	**37**	**44**	**Response**
L12y	*R. aethiopicum*	−	+	+	+	+	+	+	+	7
L23a	*R. aegyptiacum*	+	−	+	+	+	+	+	+	7
L33b	*R. aegyptiacum*	+	+	+	+	−	−	+	+	6
L42a	*R. aegyptiacum*	+	+	+	+	−	+	−	−	5
L53c	*R. leg. sv. Viciae*	−	+	+	+	−	+	+	−	5
L62	*R. etli*	+	−	+	−	−	+	+	+	5
L85b	*R. aethiopicum*	+	+	−	−	−	+	+	−	4
L71	*R. aethiopicum*	+	−	−	−	−	+	+	−	3
L84	*R. leg. sv. Trifolii*	+	−	+	+	+	+	+	−	6
L94d	*R. leg. sv. Viciae*	−	+	+	+	−	+	+	−	5
F17a	*R. aethiopicum*	−	+	+	−	−	+	−	−	3
F22	*R. aegyptiacum*	+	−	+	+	+	+	−	−	5
F32a	*R. etli*	+	+	+	+	−	−	+	+	6
F42	*R. etli*	+	−	+	−	−	+	−	−	3
F56	*R. aegyptiacum*	+	+	+	−	−	+	+	−	5
F73	*R. leg. sv. Trifolii*	+	−	+	+	−	+	+	−	5
F85	*R. leg. sv. Viciae*	+	−	+	+	+	−	+	+	6
F86	*R. etli*	+	−	−	−	−	+	+	−	3
F95	*R. leg. sv. Trifolii*	+	+	−	−	−	+	+	−	4
F102	*R. leg. sv. Trifolii*	+	+	+	+	−	−	+	+	6
Number of responding strains		16	11	16	12	5	16	15	6	-
Tolerant strains (%)		80%	55%	80%	60%	25%	80%	75%	30%	-

Solomon Legesse and Fassil Assefa (2014) reported that 26% of their faba bean isolates from northern Tigray were tolerant to 45°C which was similar to our study where more than 35% of the strains grew at 44°C. The pattern of osmo-tolerance of the strains to salt (5–6% NaCl) in the study was comparable to the faba bean isolates collected from northern Ethiopia

(Solomon Legesse and Fassil Assefa, 2014) and central Ethiopia (Getahun Mitiku and Abere Mnalku, 2019) that were tolerant to 6–7% NaCl while being slightly more than lentil isolates (5% NaCl) reported by Getahun Mitiku *et al*. (2016) and Wondwosen Tena *et al*. (2017). On the contrary, Amha Gebremariam and Fassil Assefa (2018) reported lentil and faba bean nodulating strains that could tolerate up to 10% NaCl.

Inherent antibiotic resistance test

Antibiotic resistance is a desirable trait for rhizobia intended for commercial/inoculant use as it increases the inoculant's chances of survival in the soil. The study showed that the strains displayed variation in their resistance to ampicillin, streptomycin, and chloramphenicol (Table 3). The test strains showed a high resistance pattern to kanamycin, penicillin, and tetracycline while also being mildly and strongly sensitive to erythromycin and tetracycline, respectively. It is interesting to note that the strains belonging to the *R. aegyptiacum* group were comparatively more resistant, with *R. aegyptiacum* L33b being the most resistant to many of the tested antibiotics (81%) followed by *R. aegyptiacum* F22 (76%) and *R. aegyptiacum* L23a (71%) (Table 3).

In general, the strains were more sensitive to higher concentrations of kanamycin, erythromycin and tetracycline. Similarly, Solomon Legesse and Fassil Assefa (2014) reported that erythromycin was toxic to faba bean isolates. However, 65% of our strains were resistant to higher concentrations of streptomycin and chloramphenicol.

Table 3. Intrinsic antibiotic resistance (IAR) of lentil- and faba bean- nodulating rhizobia grown on YEMA for 5–7 days.

Strain Code	Closest reference	Amp			Kana			Strep			Pen			Tet			Ery			Chl			A
		2.5	5	10	2.5	5	10	2.5	5	10	2.5	5	10	2.5	5	10	2.5	5	10	2.5	5	10	
L12y	*R. aethiopicum*	+	+	+	+	-	-	+	+	+	+	+	-	+	-	-	+	+	-	+	+	-	14
L23a	*R. aegyptiacum*	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+		-	+	+	+	15
L33b	*R. aegyptiacum*	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	17
L42a	*R. aegyptiacum*	+	+	+	+	+	-	+	+	-	+	+	-	-	-	-	+	-	-	+	+	+	13
L53c	*R. leg. sv. Viciae*	+	+	+	+	-	-	+	+	-	+	-	-	-	-	-	+	-	-	+	+	-	10
L62	*R. etli*	+	-	-	-	-	-	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	8
L85b	*R. aethiopicum*	+	+	+	+	-	-	+	+	-	+	-	-	+	-	-	+	-	-	+	+	+	12
L71	*R. aethiopicum*	+	+	+	+	-	-	+	+	+	+	+	-	+	-	-	+	-	-	+	+	+	14
L84	*R. leg. sv.trifolii*	+	+	+	+	-	-	+	+	+	+	-	-	+	-	-	+	-	-	+	+	+	13
L94d	*R. leg. sv. Viciae*	+	+	+	+	-	-	+	+	+	+	+	-	+	-	-	+	-	-	+	+	+	14
F17a	*R. aethiopicum*	+	-	-	-	-	-	+	+	-	+	-	-	-	-	-	+	-	-	+	+	-	7
F22	*R. aegyptiacum*	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+	-	+	+	+	16
F32a	*R. etli*	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-	+	+	-	+	+	+	14
F42	*R. etli*	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-	+	-	-	+	+	+	13
F56	*R. aegyptiacum*	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	+	-	-	+	+	+	14
F73	*R. leg. sv.trifolii*	+	+	+	+	-	-	+	+	+	+	-	-	+	-	-	+	+	-	+	+	+	14
F85	*R. leg. sv. Viciae*	+	+	+	+	-	-	+	+	-	+	+	-	+	-	-	+	+	-	+	+	-	13
F86	*R. etli*	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	-	-	+	+	-	11
F95	*R. leg. sv. Trifolii*	+	+	+	+	-	-	+	+	+	+	+	-	+	-	-	+	-	-	+	+	-	13
F102	*R. leg. sv.trifolii*	+	+	-	-	-	-	+	+	-	+	-	-	-	-	-	+	-	-	+	+	-	8
Positive response (%)		100	90	85	80	25	15	100	100	65	100	60	0	55	0	0	100	35	0	100	100	65	

*Amp-Ampicillin, Kana-Kanamycin, Strep-Streptomycin, Pen-Penicillin, Tet-Tetracycline, Ery-Erythromycin, Chl-Chloramphenicol, A-positive response count

Substrate utilization test

All test strains were able to catabolize galactose, maltose, glycerol, glucose, fructose, lactose and sucrose among the carbon sources; and alanine, arginine, glycine, glutamate, lysine, thymine and phenylalanine from the nitrogenous substrates (data not shown). However, only 75%, 70%, and 60% of the strains utilized sorbitol, arabinose and starch, respectively (Table 4). With regard to amino acid utilization, 80% of the strains were able to utilize tyrosine and tryptophan whereas 65% of the isolates grew on leucine.

Rhizobia can use a wide variety of substrates as they are equipped with several pathways for substrate catabolism. Though starch seemed recalcitrant relative to the other C sources, 60% of our strains were able to catabolize it. Similarly, our strains were also able to utilize 70–100% of the supplied N sources but only 65% of the strains catabolized leucine. The ability to utilize a broad range of substrates is important for rhizobia to thrive in the complex soil environment as well as intracellularly during symbiosis (Geddes and Oresnik, 2014) and to withstand pressure developing from other biota or environmental shock (Brencic and Winans, 2005; Nangul *et al*., 2013). In general, four strains in this study (*R. aethiopicum* L12y, *R. etli* L62, *R. leg. sv.viciae* L94d and *R. leg. sv. trifolii* F95) were found to be metabolically diverse, utilizing all 20 C and N-substrates (Table 4).

Symbiotic efficiency test

All isolates induced nodulation on both legume hosts, showing variation in their symbiotic efficiency amongst one another and in relation to the standard control isolate EAL 110 (Table 5). The one-way ANOVA revealed the presence of a significant (P=0.001) one-way effect with respect to the measured nodulation parameters (nodule number, nodule dry weight, shoot dry weight and total nitrogen) on both legume hosts (data not shown).

Table 4. Nutritional versatility pattern of lentil and faba bean rhizobia isolated from central and southern parts of Ethiopia.

Strain code	Closest reference species	Carbon source			Nitrogen source			(%)
		Starch	Sorbitol	Arabinose	Tryptophan	Leucine	Tyrosine	
L12y	*R. aethiopicum*	+	+	+	+	+	+	100%
L23a	*R. aegyptiacum*	+	+	+	+	-	+	95%
L33b	*R. aegyptiacum*	-	+	-	+	-	+	85%
L42a	*R. aegyptiacum*	-	+	-	+	+	-	85%
L53c	*R. leg. sv.viciae*	+	+	-	+	+	+	95%
L62	*R. etli*	+	+	+	+	+	+	100%

Strain code	Closest reference species	Carbon source			Nitrogen source			(%)
		Starch	Sorbitol	Arabinose	Tryptophan	Leucine	Tyrosine	
L85b	*R. aethiopicum*	+	+	+	+	-	+	95%
L71	*R. aethiopicum*	-	-	+	-	+	+	85%
L84	*R. leg. sv.trifolii*	+	-	+	-	+	+	85%
L94d	*R. leg. sv.viciae*	+	+	+	+	+	+	100%
F17a	*R. aethiopicum*	+	+	-	+	+	+	95%
F22	*R. aegyptiacum*	+	+	-	+	+	+	95%
F32a	*R. etli*	-	-	+	+	+	-	85%
F42	*R. etli*	-	+	+	-	-	-	80%
F56	*R. aegyptiacum*	-	+	+	+	-	-	85%
F73	*R. leg. sv.trifolii*	+	+	+	+	-	+	95%
F85	*R. leg. sv.viciae*	+	-	+	+	+	+	95%
F86	*R. etli*	-	-	+	+	+	+	90%
F95	*R. leg. sv.trifolii*	+	+	+	+	+	+	100%
F102	*R. leg. sv.trifolii*	-	+	-	-	-	+	80%
Response (%age)		**60%**	**75%**	**70%**	**80%**	**65%**	**80%**	-

The strains induced nodules ranging from 51–156 NN/plant on lentil and 85–149 NN/plant on faba bean (Table 5). Strain *R. leg. sv. viciae* L53c and *R. leg. sv. trifolii* L84 produced the highest number of nodules on lentil with more than 150 NN/plant while strain *R. etli* L62 and *R. aegyptiacum* F22 scored the highest on faba bean producing more than 130 NN/plant.

Similarly, the inoculated plants showed differences in nodule dry weight (NDW) in the range of 17 mg/P (F73) and 38 mg/P (L42a) on lentil and 74 mg/P (F85) to 121 mg/P (L62) on faba bean (Table 5). Strain *R. etli* L42a and *R. aegyptiacum* F56 with lentil as host and strain *R. etli* L62 and *R. leg. sv.viciae* L94d with faba bean produced the largest NDW, respectively (Table 5).

Shoot dry weight is the most reliable characteristic in the determination of symbiotic efficiency (Date *et al*., 1993). The isolates clearly showed a significant difference in the accumulation of shoot biomass from (0.184 g/P) (F73) to that of 0.45 g/P (L23a) on lentil as well as from 2.96 g/P (F22) to 5.4 g/P (F32a) on faba bean (Table 5). This indicates that the most effective isolates increased the shoot biomass 2.4 times (lentil) and 1.8 times (faba bean) more than the least effective isolates. The effective isolates also accumulated slightly higher biomass than the standard reference strain, EAL 110.

In general, nine isolates induced higher shoot biomass on faba bean compared with only four showing the same on lentil indicating that faba bean was more responsive to the isolates than lentil. *R. aegyptiacum* L33b resulted in the highest SDW of all rhizobial treatments including EAL110 that is currently being used as an inoculant for both host legumes in the country (Amare Tadesse *et al*., 2017). Results from the total nitrogen test in this study as well as the subsequently calculated symbiotic efficiency percentage also shows that strain EAL 110 (SE% = 85 with both hosts) has still maintained its desired symbiotic property.

Table 5. Nodulation and growth status of lentil and faba bean supplied with rhizobial treatments under greenhouse for 60 days.

Strain Code	Lentil					Faba bean				
	NN/Plant	NDW (mg/pl)	SDW (g/pl)	TN (%)	SE	NN/Plant	NDW (mg/pl)	SDW (g/pl)	TN (%)	SE
L12y	149^{f}	27.94^{b-g}	0.385^{b}	3.20^{b}	E	101^{bc}	102.69^{d-h}	2.85^{ab}	2.25^{b}	E
L23a	**102^{b-f}**	**34.78^{e-g}**	**0.447^{g}**	**3.26^{df}**	**HE**	**96^{bc}**	**91.21^{b-e}**	**3.34^{b-d}**	**3.44^{f-h}**	**HE**
L33b	**101^{b-f}**	**34.61^{e-g}**	**0.442^{h}**	**3.26^{i}**	**HE**	**114^{b-d}**	**99.12^{c-g}**	**5.38^{j}**	**2.98^{d-f}**	**HE**
L42a	57^{bc}	38.44^{g}	0.406^{e-g}	3.16^{bc}	E	128^{c-e}	113.11^{f-h}	4.71^{g-j}	3.34^{eg}	HE
L53c	**156^{f}**	**27.11^{b-g}**	**0.348^{g}**	**3.17^{hi}**	**HE**	**91^{b}**	**102.34^{d-h}**	**3.94^{d-g}**	**3.86^{gh}**	**HE**
L62	**65^{bcd}**	**36.11^{fg}**	**0.343^{fg}**	**2.98^{f-i}**	**HE**	**139^{de}**	**120.57^{h}**	**4.85^{h-j}**	**2.93^{d-f}**	**HE**
L85b	51^{b}	27.78^{b-g}	0.326^{d-g}	3.22^{df}	HE	97^{bc}	94.61^{c-e}	4.36^{e-i}	2.97^{d-f}	E
L71	**131^{f}**	**23.22^{b-e}**	**0.346^{g}**	**3.09^{gi}**	**HE**	**116^{b-d}**	**117.82^{h}**	**4.49^{e-j}**	**3.41^{f-h}**	**HE**
L84	**155^{f}**	**34.39^{d-g}**	**0.345^{fg}**	**2.95f-i**	**HE**	**85^{b}**	**95.84^{c-f}**	**3.76^{c-f}**	**3.93^{h}**	**HE**
L94d	132^{f}	25.83^{b-f}	0.193^{b}	2.51^{ce}	HE	127^{c-e}	120.43^{h}	3.07^{a-c}	2.78^{cd}	E
F17a	**108^{c-f}**	**26.61^{b-g}**	**0.263^{cd}**	**2.83^{eh}**	**HE**	**116^{b-d}**	**92.47^{c-e}**	**5.38^{j}**	**3.33^{eg}**	**HE**
F22	129^{ef}	24.33^{b-f}	0.327^{b}	3.29^{cd}	E	149^{e}	115.23^{gh}	2.96^{a-c}	2.34^{cd}	E
F32a	**121^{ef}**	**24.44^{b-f}**	**0.306^{d-g}**	**2.98^{f-i}**	**HE**	**101^{bc}**	**87.39^{b-d}**	**5.4^{j}**	**3.37^{eg}**	**HE**
F42	**148^{f}**	**26.28^{b-f}**	**0.313^{d-g}**	**3.11^{gi}**	**HE**	**86^{b}**	**97.5^{c-g}**	**5.18^{ij}**	**3.78^{gh}**	**HE**
F56	65^{b-d}	36.11^{fg}	0.343^{de}	2.98de	E	127^{c-e}	107.83^{e-h}	3.62^{b-e}	2.83^{ce}	E
F73	116^{d-f}	17.67^{b}	0.184^{b}	2.55^{de}	E	107^{b-d}	86.46^{b-d}	4.62^{f-j}	3.18^{d-f}	E
F85	109^{c-f}	21.11^{bc}	0.309^{d-g}	3.23^{i}	HE	101^{bc}	74.15^{b}	4.11^{d-h}	2.82^{ce}	E
F86	75^{b-e}	26.89^{b-g}	0.224^{bc}	2.36^{cd}	E	126^{c-e}	107.87^{e-h}	4.83^{g-j}	2.84^{ce}	E
F95	113^{d-f}	22.56^{b-d}	0.293^{d-f}	2.59^{de}	E	105^{bc}	86.35^{b-d}	3.97^{d-h}	3.36^{eg}	HE
F102	103^{b-f}	24.06^{b-e}	0.305^{d-g}	2.73^{df}	E	106^{b-d}	83.4^{bc}	4.55^{f-j}	2.72^{bd}	E
+ve	0^{a}	0^{a}	0.484^{h}	3.06^{gi}	HE	0^{a}	0^{a}	5.31^{j}	3.77^{gh}	HE
-ve	0^{a}	0^{a}	0.112a	0.69^{a}	-	0^{a}	0^{a}	2.25^{a}	1.33^{a}	-
EAL	104^{b-f}	30.81^{c-g}	0.302^{d-g}	2.79^{eg}	HE	105^{bc}	94.07^{c-e}	4.07^{d-h}	3.47^{f-h}	HE

*NN/plant = nodule number/plant, NDW (mg/plant) = nodule dry weight, SDW (g/plant) = shoot dry weight, TN (%) = total nitrogen, SE = symbiotic efficiency, HE= highly effective; E= effective, +ve = positive control, -ve = negative control, EAL=inoculant (control) strain.

However, most of the strains in our collection have outscored (≥85% SE) strain EAL 110 on both legume hosts (data not shown). Generally though, 10% of the strains proved to be highly efficient on lentil with 75% found to be effective nitrogen fixers. With faba bean as host, 45% of the strains proved to be highly effective with the remaining 55% categorized as

effective N-fixers. Wondwosen Tena *et al*. (2017) also reported lentil nodulating rhizobia isolated from parts of central Ethiopia that showed enhanced N-fixing potential.

Although EAL 110 is used as standard national reference commercial inoculum, the symbiotic test in this study showed the presence of equally competitive strains in our collections, some of which even outscored this inoculant strain in a controlled environment. Thus, the symbiotic result, coupled with that of the phenotypic result, might thus be inferred as supportive of the presence of diverse, viciae-tribe compatible, efficient N-fixing rhizobia across the soils of Ethiopia.

CONCLUSION AND RECOMMENDATIONS

Twenty lentil and faba bean nodulating rhizobial strains were subjected to phenotypic and symbiotic characterization study to ultimately select those suited for inoculant purposes. The biochemical tests proved the presence of versatile strains amongst our collections that are suited to withstand various edaphic stress levels while the greenhouse sand-pot experiment showed that the different strains displayed variations in their symbiotic performance on both legume hosts. Strains *R. aethiopicum* L12y, *R. aegyptiacum* L23a, *R. aegyptiacum* L33b and *R. aegyptiacum* F22 in general, showed their phenotypic and symbiotic advantage over the remaining treatments under a controlled environment. Further studies on the performance of these strains under a natural (farmers' field) setting are thus proposed to determine the likelihood of these strains to serve as a "super-sub inoculant" for EAL 110 and give the inoculant strain (EAL 110) its long-deserved break.

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