

1 **Genetically diverse lentil- and faba bean- nodulating rhizobia are present in soils across Central**  
2 **and Southern Ethiopia**

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19 **ABSTRACT**

20 In total 196 bacterial isolates were obtained from root nodules of lentil (*Lens culinaris*) and faba bean (*Vicia*  
21 *faba*) grown on soil samples collected from ten different sites in central and southern parts of Ethiopia. All  
22 isolates were identified as members of the genus *Rhizobium* by using *recA* gene sequence analysis. In the *recA*  
23 phylogenetic tree 195 rhizobial strains were classified into nine genospecies. The phylogeny of symbiotic  
24 genes *nodC* and *nifH* revealed five and six distinct groups respectively, largely dominated by symbiovar *viciae*.  
25 A multivariate analysis showed that environmental variables of the sampling sites considered in this study had  
26 more effect on the distribution and composition of the genospecies than the host legumes of the strains. Twenty  
27 representative strains selected based on their isolation site, host plant and *nodC* group were able to nodulate  
28 all lentil, faba bean, field pea (*Pisum abyssinicum*) and grass pea (*Lathyrus sativus*) plants in a greenhouse test  
29 in axenic conditions. The majority of the rhizobial strains were effective nitrogen-fixing symbionts for all  
30 tested legumes, indicates their potential to serve as broad-host-range inoculants in agriculture. The present  
31 work suggests the presence of taxonomically and symbiotically diverse rhizobial species for legumes in the  
32 *Viciae* tribe in Ethiopia.

33  
34 **Keywords:** gene center; genus *Rhizobium*; rhizobial genospecies; symbiotic genes; host range

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36 **INTRODUCTION**

37 Symbiotic nitrogen fixation in legumes by root- nodulating bacteria, rhizobia, plays a crucial role in  
38 ecologically sustainable agricultural systems. Symbiotic nitrogen fixation is especially important in  
39 areas where legumes form the staple food and where access to synthetic or organic nitrogen fertilizer  
40 is limited.

41           The grain legumes faba bean (*Vicia faba L.*) and lentil (*Lens culinaris M.*) have the  
42 ability to grow over a wide range of soil and climatic conditions, making them suitable candidates for  
43 sustainable agriculture particularly in marginalized areas (Nadal *et al.* 2003; Brink and Belay 2006).  
44 Both species are major pulse crops commonly grown in Ethiopia with an annual production of 698  
45 and 151 kilotons respectively (CSA 2013). They are grown as field crops throughout the highlands  
46 and are most common between altitudes of 1700 m and 3000 m above sea level (Agegnehu *et al.*  
47 2006). Ethiopia is a major global producer of faba bean (Mussa and Gemechu 2006) with the crop  
48 occupying over 31% of the farmed pulse fields and accounting for 34% of the total annual pulse  
49 production in the country (CSA 2013). The country also ranks first in terms of area coverage of lentil  
50 within the African continent but is placed second to Egypt in terms of yield (FAO 2015). Both  
51 legumes play significant roles in improving the productivity of soil when used as a break crop in a  
52 cereal-based rotation scheme (Reiter *et al.* 2002). In favorable conditions, faba bean and lentil can fix  
53 up to 120 and 107 kg nitrogen per hectare annually, respectively (ICARDA 2008; Rashid *et al.* 2012).

54           Faba bean and lentil form symbiotic associations with several species of rhizobia in the  
55 genus *Rhizobium*. *Rhizobium leguminosarum* (Allen 1981), *R. fabae* (Tian *et al.* 2008) and *R.*  
56 *laguerreae* (Saidi *et al.* 2014) have been reported symbiotic with faba bean and *R. leguminosarum*  
57 (Tena *et al.* 2017), *R. binae* (Rashid *et al.* 2015), *R. lentis* (Rashid *et al.* 2015), *R. pisi* (Ramirez-  
58 Bahena *et al.* 2008) and *R. bangladeshense* (Rashid *et al.* 2015) were reported as lentil symbionts.  
59 However, the lentil or faba bean rhizobia were reported to promiscuously form also symbiotic  
60 associations with both host plants (Laguerre *et al.* 2003; Tian *et al.* 2008; Tena *et al.* 2017).

61           The 16S rRNA gene has low resolution power for classification of rhizobia below the  
62 genus level (Gevers *et al.* 2005; Martiny *et al.* 2006; Li *et al.* 2012), whereas several housekeeping  
63 protein-coding genes have been used as powerful tools for taxonomic studies at the species level.  
64 Recombinase A (*recA*) gene has been demonstrated as a very good marker for identification,  
65 taxonomic and phylogenetic studies of rhizobia by many authors (e.g. Aserse *et al.* 2012, 2013;  
66 Mousavi *et al.* 15; Tena *et al.* 2017).

67           Symbiotic genes are involved in symbiotic interactions with the host plants and their  
68 phylogenies, especially those of *nod* genes, are often congruent with host plant phylogenies,  
69 indicating co-evolution of rhizobia with their hosts (e.g. Suominen *et al.* 2001; Lindström *et al.* 2015).  
70 Co-evolution of rhizobia and their host plants was also demonstrated for the species *Galega orientalis*  
71 and *G. officinalis*, with symbiovars *orientalis* and *officinalis* of *Neorhizobium galegae*. Further, the  
72 diversity of the microsymbiont *N. galegae* sv. *orientalis* was greatest in the gene center of the host  
73 plant in the Caucasus (Österman *et al.* 2011).

74           Ethiopia is considered as a secondary gene center of faba bean (Raven and Polhill 1981;  
75 Khazaei *et al.* 2016), thus we hypothesized that the soils of Ethiopia harbored diverse faba bean-  
76 nodulating microsymbionts. This study aimed to explore the presence and diversity of faba bean- and  
77 lentil- nodulating rhizobia in soil samples collected in central and southern Ethiopia. We used  
78 the *recA* gene as a phylogenetic marker to determine the taxonomic diversity of rhizobia. The  
79 phylogeny of symbiotic genes involved in nodulation (*nodC*) and nitrogen fixation (*nifH*) was studied,  
80 and the nodulation capacity of the rhizobia was also tested on lentil, faba bean, field pea and grass  
81 pea plants in greenhouse conditions. We looked for environmental factors of the sampling sites to see  
82 if they have effects on the distribution and composition of the genospecies.

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## 85 **MATERIALS AND METHODS**

### 86 **Soil sample collection and description of sampling sites**

87 Ten soil samples were collected from farmers' fields across the major lentil and faba bean growing  
88 region in central Ethiopia as well as from sites in the southern part of the country where these legumes  
89 are less dominant (Fig.1). The GPS location, elevation, soil and climatic data of the sampling sites  
90 are presented in Table 1. The annual average temperature and annual rainfall data were provided by  
91 the National Meteorological Agency of Ethiopia. For each site, soils taken from three soil pits having  
92 an area of 30 cm x 30 cm and with a depth of 20 cm were packed in pre-sterilized plastic bags. The  
93 collected soils were then mixed properly to form composite samples and air-dried in the greenhouse  
94 for further use. Soil parameters such as particle size distribution, pH (H<sub>2</sub>O), electrical conductivity,  
95 total organic carbon, total nitrogen, exchangeable aluminum and basic cations, and potential cation  
96 exchange capacity at pH 7.0 were analyzed following the method described in Aserse *et al.* (2019)  
97 and the results are presented in Table 1. The soils were fine-textured, eight of them having a texture  
98 of clay and two being silty clays. The pH was close to neutral (6.5-7.7) except in one acidic (5.4) soil.  
99 The soils were not sodic or saline, even though in the Hawassa soil the electrical conductivity was  
100 comparatively higher than the rest. According to the Olsen soil test, available phosphorus ranged  
101 from a relatively low (0.4 mg kg<sup>-1</sup>) to a very high (162 mg kg<sup>-1</sup>) score.

### 102 **Greenhouse trapping experiment, nodule collection and isolation of bacteria**

103 Faba bean and lentil were used to trap and recover rhizobia present in the soil samples. The seeds  
104 were surface sterilized (1 min in 95% ethanol, 5 min in 3% sodium hypochlorite and 5 times rinsing  
105 with sterile water) and germinated on sterile water agar plates according to Somasegaran and Hoben  
106 (1994). The germinated seeds were then aseptically transferred to pre-sterilized (70% ethanol) plastic  
107 pots containing about 3 kg of the sampled soil. A total of 40 pots (10 soil samples with 2 capture  
108 hosts, both in duplicate) were used. The pots were laid out in quadruplets with five seeds initially



109 transplanted in each pot, and later thinned to 3 plants per pot. The plants were adequately given sterile  
110 water every other day and were uprooted after 8 weeks of growth. The uprooted roots were washed  
111 with running tap water and healthy and intact nodules were carefully collected from the root crown.  
112 Nodules were then surface sterilized with 95% ethanol for 5 min, followed by 3% sodium  
113 hypochlorite for 5 min and 5 rinses with sterile water (Vincent 1970). Sterilized individual nodules  
114 were then crushed, suspended in a drop of saline solution (0.85% NaCl), and streaked onto individual  
115 Yeast Extract Mannitol (YEM) agar plates containing 25 mg/L Congo red (CR) (Lindström *et al.*  
116 1985). The plates were incubated for 3 – 5 days at 28°C after which single colonies were picked from  
117 the plates and re-streaked to obtain pure cultures. Purified isolates grown in YEM broth were  
118 transferred to Eppendorf tubes and stock culture preserved in 20% glycerol–YEM broth at –70 °C.  
119 Intact nodules were in parallel stored in preservation vials containing silica gel.

## 120 **DNA extraction**

121 Genomic DNA was extracted from bacterial cultures grown in YEM broth for 3 – 5 days at 28°C  
122 after pelleting. DNA was isolated from the cell pellets through the phenol-chloroform protocol  
123 (Aserse *et al.* 2012; Li *et al.* 2012) and using the ultraclean™ microbial DNA isolation kit (Mo Bio  
124 Laboratories, Solana Beach, Calif.) according to the manufacturer’s instructions. The quality and  
125 quantity of the DNA samples were checked by gel electrophoresis (Aserse *et al.* 2012) and stored at  
126 –20°C.

## 127 **Gene amplification, sequencing and data analysis**

128 PCR amplification and sequencing of the housekeeping gene *recA* and symbiotic genes *nodC* and  
129 *nifH* were according to Aserse *et al.* (2012) and Li *et al.* (2012). Primers used for *recA*, *nodC* and  
130 *nifH* gene amplifications were obtained from literature (Gaunt *et al.* 2001; Sarita *et al.* 2005; Rivas *et*  
131 *al.* 2002). The primes are listed in supplementary material Table S1.

132 The PCR protocols used for the amplifications of the genes were according to Aserse *et al.* (2012).  
133 The quality of the PCR products was checked by gel images from 1.5% (w/v) agarose electrophoresis  
134 gels, and the size of the products was determined with GENE RULER™ 100bp plus as a standard.  
135 The sequencing of the PCR products was made via the Sanger method at the Institute of  
136 Biotechnology, University of Helsinki. The quality of all the sequences was checked and edited using  
137 Gap v4.11.2-r program imbedded in the Staden-package 1.7.0 (Staden *et al.* 1998). The *recA*, *nodC*  
138 and *nifH* sequences obtained in this study have been deposited in the GenBank database under the  
139 accession numbers of MN386279-MN386474, MN386475 - MN386577 and MN386578 -  
140 MN386684, respectively.

141 Sequences were compared against reference sequences using a nucleotide database  
142 (NCBI) BLAST program (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>; Altschul *et al.* 1990). Bacterial  
143 strains were identified based on their *recA* sequence similarities to reference sequences in  
144 the GenBank database. Multiple nucleotide sequence alignment was by using CLUSTALW  
145 (Thompson *et al.* 1994) of MEGA version 6.0 (Tamura *et al.* 2013). Similar approaches were used  
146 for *nodC* and *nifH* gene sequence analyses. Phylogenetic trees were constructed with the maximum  
147 likelihood method with Tamura 3-parameter model and discreet Gamma distribution using the  
148 MEGA version 6.0 program. Bootstrap analysis with 1000 replications were used to calculate the  
149 robustness of the tree topology.

## 150 **Host range test**

151 Twenty representative strains were selected based on their site of origin, host of isolation and *nodC*  
152 phylogeny to test their nodulation ability on faba bean, lentil, grass pea and field pea. Seeds of the  
153 four host plants were surface sterilized and germinated as described above. Three seedlings were then  
154 transplanted aseptically into sterile, correspondingly labeled, 3 Kg capacity pots filled with  
155 autoclaved and acid washed river sand. The seedlings were inoculated with 1 ml of test bacterial  
156 cultures grown to late logarithmic phase in YEM broth. A total of 176 pots (20 test strains + 2 controls

157 x 4 host plants in duplicates) were randomly laid in the greenhouse. The pots were supplied with  
158 sterile, quarter-strength Jensen's modified nitrogen-free nutrient solution and sterile distilled water  
159 on a weekly and bi-weekly basis respectively (Somasegaran and Hoben 1994). Un-inoculated plants  
160 served as controls. Plants were grown for 45 days until the flowering stage, after which the nodulation  
161 status and nitrogen-fixing efficiency of the isolates were visually assessed. Nodulation was recorded  
162 as positive when nodules were found and negative if absent. The test bacteria were considered  
163 effective nitrogen-fixers if the internal color of excised nodules was reddish to pink in color and the  
164 host plants appeared healthy and green. Nitrogen fixation was considered ineffective when nodules  
165 were whitish and plants appeared pale and yellowish.

## 166 **Statistical analysis**

167 Community structure and diversity of the rhizobial strains were estimated based on the total number  
168 of individuals ( $N$ ) and number of genospecies ( $S$ ) identified per site. The diversity regarding both  
169 genospecies richness (number of species) and evenness (species distribution) of the different  
170 sampling sites was assessed using mathematical biodiversity indices Shannon–Wiener diversity ( $H'$ )  
171 and Simpson ( $D$ ) indexes respectively. Simpsons' index gives more weight to dominant species and  
172 assumes that a few rare species (those with only a few representations) will not affect diversity.  
173 Pielou's evenness ( $J'$ ) index quantifies how equally the species are distributed within the  
174 community/site while Margalef's species richness ( $d$ ) index estimates the number of different species  
175 (species richness) (Magurran 2004). The biodiversity indices were calculated using the program  
176 PRIMER v7.0.11 and their statistical significance were determined with the SPSS v23 program.

177 One-way Analysis of Variance (ANOVA) was performed with SPSS v.23 to determine  
178 the significance of the differences among the sampling sites in terms of their soil and environmental  
179 parameters. Distance-based linear models (*DISTLM*), a routine for analyzing and modeling the  
180 relationship between a multivariate data cloud (species abundance data along with their respective  
181 environmental variables), was used to investigate the possible relationships between the

182 environmental ecological variables (soil properties and climatic factors) and the different genospecies  
183 of faba bean and lentil of the sampling sites. The abundance data was square root transformed and  
184 Jaccard's similarity matrix (transformed to dissimilarity) was obtained for the analysis. The predictor  
185 variables were transformed and normalized to overcome issues of differing measurement scales  
186 (Clarke and Gorley 2001). Monte Carlo permutations (9999) with specified selection procedure and  
187  $R^2$  selection criteria was then used to test the null hypothesis of no relations between the ecological  
188 variables and the different genospecies. The fitted model was visualized using the distance-based  
189 redundancy analysis (*dbRDA*) routine (Anderson *et al.* 2008) after removing highly correlated  
190 variables to clarify the resulting plot.

## 191 **RESULTS**

### 192 **Isolation of root nodule bacteria**

193 Nodule samples were successfully recovered from faba bean and lentil plants grown in the greenhouse  
194 in soil samples collected from the ten sampling sites, except from lentil in soil sampled from the  
195 Hawassa site. In total, 196 bacterial strains were isolated from the root-nodules of the two plants,  
196 108 from faba bean and 88 from lentil. The distribution of the isolates with respect to the sampling  
197 sites are shown in Table S3.

### 198 **Identification, genetic diversity and distribution of the isolates**

199 All of the isolates were identified as belonging to genus *Rhizobium* by using *recA* (516 bp) sequence  
200 data. The phylogeny of 195 test strains with appropriate *recA* reference sequences was determined  
201 (Fig. 2). One of the strains (strain F37c, with *recA* accession number MN386280), although belonging  
202 to *Rhizobium* (showing 83% similarity with *Rhizobium aethiopicum* species), upon blasting, contained  
203 an extra set of nucleotides and was not suitable for making alignment and in *recA* phylogenetic tree  
204 reconstruction. Therefore, this strain was not included in the phylogenetic tree (Fig. 2). The  
205 phylogenetic groups distinguished were assigned to genospecies and numerically indexed from 1 to  
206 9. Five genospecies (1, 2, 4, 7 and 8) were found to be the most abundant and widely distributed,

207 occurring in almost all of the ten sampling sites. Genospecies 1 belonged to *Rhizobium*  
208 *leguminosarum* species (97 – 100 sequence similarity) with the majority of the strains isolated from  
209 the host lentil and from the site Debre-zeit, Alem-gena and Fiche. Genospecies 2 included strains  
210 spreading across all ten sampling sites and both host plants, and had an identical or nearly identical  
211 *recA* sequence to reference named as *R. leguminosarum* CB782. The strains classified under  
212 genospecies 4 had identical or 96 – 97% sequence identity with *R. etli* HBR5, which was isolated  
213 from nodules of common bean in Ethiopia. The genospecies 7 and 8 each contained 39 strains and  
214 were closely related to *R. aegyptiacum* and *R. aethiopicum* respectively (Fig. 2 and Table S3).  
215 Genospecies 7 was further divided into subgroups either in terms of isolation host or site of origin  
216 and genospecies 8 was spread across five sites.

217 Genospecies 3, 5, 6 and 9 were minor phylo-groups, consisting of 3 to 12 strains each,  
218 with limited spread that spanned from two to six sampling sites (Fig.2 and Table 2). Genospecies 3  
219 consisted of four strains isolated solely from the Alagae soil sample with lentil as the capture host.  
220 These strains were tightly grouped (100 % identity) with the Mexican bean-nodulating *Rhizobium* sp.  
221 Kim5, corresponding to the *phaseoli-etli-leguminosarum* lineage 1 (PEL1) (Ribeiro *et al.* 2013) and  
222 were also found to be distantly related to the Bangladeshi lentil nodulating *R. binae* BLR235 (94%  
223 sequence identity). Genospecies 5 consisted of three test strains strictly limited to the host lentil and  
224 site Chefe-donsa, for which the closest reference (99% identity) was another lentil symbiont *R.*  
225 *bangladeshense* obtained from Bangladesh (Rashid *et al.* 2015). Genospecies 6 comprised three  
226 unique strains obtained from nodules of both lentil and faba bean with no close references.  
227 Genospecies 9 was formed by strains isolated from both hosts and with the closest reference (96-97%  
228 similarity) *R. mesosinicum* CCBAU 25217, obtained from nodules of *Kummerowia stipulacea*  
229 growing in China (Lin *et al.* 2009) (Fig. 2 and Table S3).

230 The distribution of the genospecies across the sampling sites and the diversity index  
231 results are presented in Table 2. The highest genospecies richness (Margalef) value was obtained for

232 Butajira (1.97), followed by Holetta (1.94) and Chefe-donsa (1.91) sites, and seven genospecies were  
233 obtained from each of these sites. The lowest genospecies richness value was recorded from Hawassa  
234 (1.30), with four genospecies found at this site. The Shannon-Weiner (H') diversity index results  
235 showed positive correlation with the species richness values ( $r = 0.86$ ), with the highest Shannon-  
236 Weiner was for Holetta (1.77) followed by Chefe-donsa (1.73), Fiche (1.69), Alagae (1.69) and  
237 Butajira (1.66) and the lowest value was for the Hawassa (1.31) site. The values of Simpson's index  
238 (D) varied 0.84 – 0.76 and were mostly consistent with Shannon-Weiner (H') diversity index results,  
239 with the highest value recorded for Holetta, Alagae and Fiche sites. The score of the evenness measure  
240 (Pielou, J) ranged from 0.95 for Hawassa to 0.85 for the Butajira site. Generally, the diversity index  
241 results corroborated the presence of diverse genospecies with varied compositions of the faba bean-  
242 and lentil-nodulating rhizobial communities at our sampling sites.

#### 243 **Phylogeny of the symbiotic genes**

244 The genes involved in nodulation (*nodC*, 521 bp) and nitrogen fixation (*nifH*, 293 bp) were  
245 successfully amplified and sequenced partially for 104 and 107 strains respectively. As shown in the  
246 phylogenetic trees (Fig.3 and 4), five *nodC* and six *nifH* distinct groups were identified. The *nodC*  
247 and *nifH* groups of the strains and their corresponding genospecies, host plants and isolation sites are  
248 presented in Table S3.

249                 Four of the *nodC* groups showed closely similarity to different members of symbiovar  
250 *viciae* rhizobia of faba bean, field pea and lentil identified in different geographic regions of the globe.  
251 The *nodC* group 1 contained 47 strains that had identical or similar *nodC* sequences (95 – 97%  
252 identity) with the type strains *R. leguminosarum* sv. *viciae* USDA2370<sup>T</sup> and *R. pisi* sv. *viciae*  
253 DSM30132<sup>T</sup> and with *R. leguminosarum* sv. *viciae* Vaf-09 and Vaf 10 isolated from root nodules of  
254 *Vavilovia formosa* in Russia (Safronova *et al.* 2014). Strains (26) in the *nodC* group 2 showed close  
255 similarity (95-99% *nodC* sequence identity) to field pea-nodulating *R. leguminosarum* sv. *viciae*  
256 BIHB1107 obtained in India (Rahi *et al.* 2012), and *R. anhuiense* sv. *viciae* CCBAU 33508 isolated

257 from faba bean in China (Zhang *et al.* 2015). The *nodC* group 3 was composed of 18 strains that had  
258 identical or 95-99% *nodC* sequence similarity with faba bean- and lentil-nodulating rhizobial species;  
259 type strains *R. fabae* sv. *viciae* CCBAU33202<sup>T</sup>, *R. laguerreae* sv *viciae* FB206<sup>T</sup>, and *R. binae* sv  
260 *viciae* BLR195<sup>T</sup>. The *nodC* group 4 included strains mainly representing genospecies 4 (7/10 strains).  
261 Strains in this group had identical or nearly identical *nodC* sequences to lentil-nodulating *R.*  
262 *leguminosarum* sv. *viciae*, *R. bangladeshense* sv. *viciae* and *R. lentis* sv. *viciae* (Rashidi *et al.* 2012;  
263 Sbabou *et al.* 2016) (Fig.3).

264 The *nifH* phylogenetic tree showed similar topology with the *nodC* tree, although the  
265 two trees had some differences in their branches. The *nifH* groups I and II contained strains that were  
266 mainly classified as *nodC* group I and also strains found in *nodC* groups III and IV. The *nifH* group  
267 III and V contained most strains classified as *nodC* group II. In *nifH* group I, 42 strains had identical  
268 or very similar *nifH* gene sequences to the reference type strains *R. leguminosarum* sv. *viciae*  
269 USDA2370<sup>T</sup> and *R. pisi* sv. *viciae* DSM30132<sup>T</sup> and other rhizobial species belong to symbiovar  
270 *viciae*. The majority of the strains (9 of 11) forming *nifH* group II were restricted to the host lentil,  
271 genospecies 1, and *nodC* group II. These strains had about 97% *nifH* sequence similarity with *R.*  
272 *leguminosarum* sv. *viciae* Vaf-09 and Vaf 10 and *R. leguminosarum* sv. *viciae* BIHB1107. The *nifH*  
273 group III included 34 strains that showed about 97% *nifH* similarity with *R. leguminosarum* R45964  
274 obtained from nodules of cow vetch (*Vicia cracca*) in Belgium (De Meyer and Willems 2011). Fifteen  
275 strains formed a unique *nifH* group V without close references. Phylogenetic groups *nifH* VI (four  
276 strains) and *nodC* 5 (two strains) contained strains isolated solely from nodules of lentil. Unlike the  
277 above mentioned symbiotic groups that belong to symbiovar *viciae*, strains in these groups had  
278 identical or highly similar *nodC* and *nifH* gene sequences to the corresponding genes of common  
279 bean-nodulating rhizobial species, such as *R. aethiopicum* sv. *phaseoli* HBR26<sup>T</sup> (Figs. 3 and 4).

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281

## 282 **Host range test**

283 All four test plants (faba bean, lentil, grass pea and field pea) were successfully nodulated by all 20  
284 rhizobial test strains, resulting in numerous nodules on the plant roots. All test strains belonging to  
285 *nodC* groups 4 and 5 formed an effective symbiosis with all four host plants. The test strains from  
286 *nodC* groups 1, 2 and 3 showed mixed results, forming an effective symbiosis with either one, two or  
287 all of the test plants. For example, strains F56, L71, and F73 from *nodC* groups 1, 2, and 3  
288 respectively, were able to form an effective symbiosis with only their original host plant faba bean or  
289 lentil. A few other strains representing *nodC* groups 1 (F85) and 3 (L12y, F22) formed less effective  
290 symbioses with all of the test strains (Table 3).

## 291 **Effect of environmental variables of the study sites on the distribution of identified genospecies**

292 The ANOVA results revealed that the sampling sites were significantly different ( $p \leq 0.001$  data not  
293 shown) in their soil and environmental parameters and hence were appropriate for the downstream  
294 *DistLM* analysis. According to the *DistLM* analysis result, all of the measured ecological variables  
295 were important parameters ( $P = 0.001$ , Table S2) in defining the distribution rhizobial genospecies.  
296 The dbRDA ordination plot was constructed with the main contributing selected variables (Ca, K, Al,  
297 P, annual rainfall) in order to view the relative contribution of these variables in shaping the  
298 genospecies and variation between the genospecies (Fig. 5). In the plot the first two axes explained  
299 38% of the fitted and 15% of the whole (total) variation present between the genospecies. The soil  
300 exchangeable Al and P and annual rainfall had more influence and positive correlation with the  
301 distribution of four genospecies (4, 5, 6, and 8) than other variables. However, these variables showed  
302 opposite or negative correlation with the distribution of genospecies 1 and 3. Soil exchangeable K  
303 and elevation showed positive influence on the distribution of genospecies 2. Genospecies 7 tend to  
304 correlate with soil exchangeable Ca. Genospecies 9 held an intermediate position in the plot,  
305 indicating that the contribution of all the environmental variables was small on the distribution of this  
306 genospecies (Fig.5).



307 **DISCUSSION**

308 In the present study, 196 bacterial strains were isolated from nodules of faba bean and lentil plants  
309 grown in the greenhouse on 10 soil samples collected from central and southern Ethiopia. Based on  
310 the *recA* gene sequence analysis results, all the bacterial strains were identified as belonging to the  
311 genus *Rhizobium*, but with a high diversity within the genus, forming 9 distinct taxonomic  
312 genospecies. Symbiotic and nodulation genetic characteristics of faba bean- and lentil-nodulating  
313 rhizobia were investigated. The rhizobial strains were also diverse in their symbiotic genes, with five  
314 and six distinct *nodC* and *nifH* groups, respectively identified among the test strains.

315 Ethiopia is endowed with enormous biodiversity, known as a center of origin of diverse  
316 fauna and flora, including legumes (Raven and Polhill 1981; FAO 1996). Previous studies also  
317 revealed the presence of diverse and unique N<sub>2</sub>-fixing rhizobial species nodulating native legumes of  
318 Ethiopia (Aserse *et al.* 2012, 2017; Wolde-meskel *et al.* 2004, 2005). The country is considered one  
319 of the secondary gene centers of the cool season food legumes, such as faba bean, pea, lentil and  
320 chickpea (Raven and Polhill 1981; Keneni *et al.* 2007; Khazaei *et al.* 2016). The diversity of N<sub>2</sub>-  
321 fixing rhizobial symbionts is expected to be higher in the gene center of the host plant than places  
322 where the host is introduced (Österman *et al.* 2011). The observed high diversity of rhizobia  
323 nodulating faba bean and lentil in our study was thus to be expected. The high diversity could be a  
324 result of prolonged interaction of the hosts and their symbionts, as cultivation of lentil and faba bean  
325 in Ethiopia dates back to antiquity (Dawit *et al.* 1994).

326 According to previous studies, faba bean rhizobia were taxonomically classified as *Rhizobium*  
327 *leguminosarum* (Allen 1981), *R. fabae* (Tian *et al.* 2008), *R. anhuiense* (Zhang *et al.* 2015; Chen *et*  
328 *al.* 2018) and *R. laguerreae* (Saidi *et al.* 2014). *R. leguminosarum* species was also commonly  
329 identified as symbionts of lentil and field pea (Tegegn 2006; Rashid *et al.* 2012; Zou *et al.* 2016). The  
330 population composition of the rhizobial species might be different depending on geographic regions;  
331 in China, *R. anhuiense* was found to be the main faba bean symbiont among rhizobial species

332 identified in the Panxi region (Chen *et al.* 2018). Lentil-nodulating *R. binae*, *R. lentis* and *R.*  
333 *bangladeshense* (Rashid *et al.* 2015) were dominant in Bangladesh. In this study, based on *recA* gene  
334 sequence analysis, the different genospecies (1, 3, 4, 7, 8, 9,) showed close similarity to *R.*  
335 *leguminosarum*, *R. bangladeshense*, *R. etli*, *R. aegyptiacum*, *R. aethiopicum*, and *R. mesosinicum* or  
336 stand as unique genospecies (Fig. 2). This result suggests the presence of taxonomically diverse and  
337 distinct rhizobial species for faba bean and lentil in Ethiopia. Although most of the genospecies were  
338 related to *Rhizobium* species reported as faba bean and lentil rhizobia from other regions, to our  
339 knowledge this is the first time to find *R. etli*, *R. aegyptiacum*, *R. aethiopicum*, and *R. mesosinicum*  
340 related faba bean or lentil rhizobial species in Ethiopia. *R. etli* and *R. aethiopicum* were among the  
341 main common bean-nodulating rhizobial species identified in Ethiopia (Aserse *et al.* 2012, 2017).  
342 However, *R. aegyptiacum* was isolated in Egypt from nodules of *Trifolium alexandrinum* L  
343 (Shamseldin *et al.* 2016), and *R. mesosinicum* species was isolated from nodules of *Kummerowia*  
344 *stipulacea*, annual herb native to China (Lin *et al.* 2009). Generally, further studies with more gene  
345 sequence analyses would clarify the taxonomic positions of those strains related to *R. aegyptiacum*,  
346 *R. aethiopicum*, and *R. mesosinicum* and the novel genospecies groups.

347         The rhizobial symbiotic genes, which code for nodulation and nitrogen fixation, are important  
348 determinants for the success of the legume-rhizobium symbiosis. Phylogenies of rhizobial *nod* genes  
349 often follow the phylogenies of the host legumes, and thus indicate the host ranges and co-evolution  
350 of rhizobia with their hosts (Perret *et al.* 2000; Suominen *et al.* 2001; Österman *et al.* 2011). The  
351 *nodA*, *B*, and *C* genes are essential for the synthesis of the lipo-oligosaccharide core structure known  
352 commonly as the Nod factor backbone, an important signal molecule in the rhizobial host infection  
353 process (Roche *et al.* 1996). The *nifH* gene encodes a nitrogenase Fe protein, an enzyme involved in  
354 nitrogen fixation (Hu *et al.* 2006). Principally, the symbiotic genes of faba bean, lentil and field pea  
355 symbionts were reportedly closely related to corresponding genes of *R. leguminosarum* sv. *viciae*  
356 (Laguerre *et al.* 2003; Santillana *et al.* 2008; Tian *et al.* 2010; Rahi *et al.* 2012). According to Rashid

357 *et al.* (2012), taxonomically diverse lentil symbionts isolated in Bangladesh shared identical  
358 nodulation gene (*nodA*, *nodC*, and *nodD*) sequences, most showing a close relationship to *R.*  
359 *leguminosarum* sv. *viciae*. On the other hand, diverse *nodC* and *nifH* types showing close relationship  
360 with the corresponding genes in *R. leguminosarum*, *R. fabae* CCBAU 33202 or *R. laguerreae* were  
361 reported from faba bean rhizobia in China (Chen *et al.* 2018). The four *nodC* and *nifH* major groups  
362 identified in this study had close similarity to one or more reference rhizobia belong to *R.*  
363 *leguminosarum*, *R. pisi*, *R. anhuiense*, *R. laguerreae*, *R. bangladeshense*, and *R. lentis* isolated from  
364 nodules of legumes in *viciae* plant tribe from different geographic locations (Rahi *et al.* 2012; Rashidi  
365 *et al.* 2012; Saidi *et al.* 2014; Zhang *et al.* 2015; Sbabou *et al.* 2016) (Fig. 3 and 4). This suggests that  
366 the rhizobia in Ethiopia are of high diversity in their symbiotic genes within symbiovar *viciae*. The  
367 finding of group V without known close relatives in the *nifH* phylogenetic tree (Fig. 4), probably  
368 indicate the presence of a unique variant of *nifH* gene for faba bean and lentil rhizobia in Ethiopia.  
369 The detection of *nodC* (Fig.3) and *nifH* (Fig. 4) groups that had close similarity to common bean  
370 rhizobial species (symbiovar *phaseoli*) in strains isolated from nodules of lentil, reflects that Ethiopian  
371 rhizobia are more diverse in their symbiotic genes than expected. These strains also formed an  
372 effective nitrogen-fixing symbiosis with all faba bean, lentil, field pea and grass pea plants tested in  
373 the greenhouse. Common bean is traditionally grown in Ethiopia and the soil harbors a unique  
374 common bean symbiont *R. aethiopicum* sv. *phaseoli* HBR26<sup>T</sup> (Aserse *et al.* 2017). Thus, those  
375 symbiotic genes related to common bean rhizobia in our collections, suggests the occurrence of  
376 horizontal gene transfer of symbiotic genes (Sullivan *et al.* 1995).

377           The genospecies based on *recA* phylogeny (Fig. 2) and the symbiotic gene (*nodC* and  
378 *nifH*) groups (Figs. 3 and 4) were generally incongruent, suggesting an independent evolutionary  
379 history between the housekeeping chromosomal genes and plasmid born-symbiotic genes (Laguerre  
380 *et al.* 1996; Rashid *et al.* 2012). The *nodC* and *nifH* phylogenies, tend to follow rhizobia host of  
381 isolation, in which *nodC* groups (1, 4) and *nifH* groups I, were largely dominated by strains isolated

382 from faba bean while the *nodC* groups (2, 5) and *nifH* group VI contained mostly or completely  
383 rhizobia isolated from lentil. However, the *nifH* and *nodC* groups were not perfectly congruent,  
384 suggesting that the *nodC* and *nifH* genes of the rhizobial strains probably evolved separately.

385           The legumes in the Viciae tribe; faba bean, lentil, field pea and grass pea are  
386 phylogenetically very close to each other among the cool season legumes (Zhu *et al.* 2005). These  
387 legumes, generally also share rhizobia and make promiscuous symbiotic associations with  
388 taxonomically different rhizobial species (Laguerre *et al.* 2003; Tian *et al.* 2008). In agreement with  
389 the previous reports, the test rhizobial strains obtained from nodules of faba bean and lentil which  
390 belong to different genospecies and *nodC* groups, were able to cross nodulate all faba bean, lentil,  
391 field pea and grass pea in our study.

392           Soil properties were found to be important determinants in shaping the community  
393 structure and distribution of rhizobia (Jiao *et al.* 2015; Zhang *et al.* 2011). Although acidic soils are  
394 believed to be less favorable for bacterial growth, higher bacterial diversity and richness were  
395 reported from acidic soils (pH of 5.2) than those found at neutral pH (Cho *et al.* 2016). In our study,  
396 one of the sites (Butajira) with acidic pH (5.4) of the soil and with high exchangeable aluminum  
397 scores showed the highest species richness diversity index (Table 2), and was represented by seven  
398 of the genospecies. Generally, the variation in composition of the rhizobial genospecies between the  
399 sampling sites, and the differences in relative abundance of genospecies and diversity indexes (Table  
400 2), could be explained by the differences in soil and environmental variables of the sites. According  
401 to Zhang *et al.* (2017), the distribution of the soybean rhizobia was influenced by the collective effects  
402 of the ecological variables. In our study, all variables included in the test had also a significant  
403 contribution to the variations between the genospecies and their compositions (P=001, Table S2). The  
404 soil parameters (exchangeable Al, P, K, and Ca), annual rainfall, or elevation were among the main  
405 factors correlating to the distribution of the majority of the genospecies (Fig. 5).

406

407 **CONCLUSIONS**

408 The genospecies identified from the nodules of faba bean and lentil in Ethiopia were diverse both in  
409 their taxonomic composition and symbiotic genes phylogenies. Such a result was expected from the  
410 gene center of the hosts in Ethiopia and long history of the legume cultivations in the country (Dawit  
411 *et al.* 1994), since the extended interaction between the hosts and their symbionts, would result in  
412 high rhizobial diversity. The ecological variables of the sampling sites were found determinant for  
413 the distribution and composition of the genospecies. The phylogeny based on *recA* gene and  
414 symbiotic gene groups of the strains were not generally consistent, which strengthen the previous  
415 findings indicating an independent evolutionary history between the housekeeping chromosomal  
416 genes and plasmid born-symbiotic genes (e.g. Laguerre *et al.* 1996; Rashid *et al.* 2012). The rhizobia  
417 belonging to different genospecies and distinct symbiotic groups were able to form an effective  
418 nitrogen-fixing symbiosis with all tested hosts, i.e. faba bean, lentil, field pea and grass pea, which  
419 revealed the promiscuous nature of the legumes and broad symbiotic promiscuity of the rhizobial  
420 strains. Thus, the result shows the potential of the rhizobial strains to serve as broad host range  
421 inoculant in agriculture.

422 **SUPPLEMENTARY DATA**

423 Supplementary files are included

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429

430 **DISCLOSURE STATEMENT**

431 No conflict of interest.

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Table 1. Edaphic and climatic data of the ten soil sampling sites used in the study.

Site	pH H <sub>2</sub> O	EC ( $\mu$ S cm <sup>-1</sup> )	Clay (%)	P Olsen (mg kg <sup>-1</sup> )	N total (%)	C total (%)	Ca ex	K ex	Mg ex	Na ex	Al ex 1M KCl	CEC potential (cmol kg <sup>-1</sup> )	BS (%)	Alt. (m)	Avg. Temp (°C)	A. Rain (mm)	Latitude	Longitude
							(mg kg <sup>-1</sup> )											
AG	7.69	155	79	3.6	0.11	1.2	12620	703	872	29	0.7	72.3	100	2100	14.8	994	8.554N	38.3919E
Ho	6.56	129	68	46	0.24	2.1	4440	2090	916	14	1.7	38.3	92	2389	15.9	1134	9.0452N	38.3017E
Fi	7.50	48	77	1.4	0.10	1.1	10390	337	1390	57	0.5	65.4	99	2800	14.0	1008	9.4643N	38.4324E
DB	6.45	27	66	18	0.12	1.4	9360	221	1740	49	1.6	65.1	95	2800	14.4	964	9.4021N	39.3357E
Ak	7.60	69	52	15	0.10	0.9	7310	466	895	45	0.7	45.8	99	2180	18.7	907	8.5248N	38.5006E
DZ	7.65	108	46	18	0.11	1.0	6360	528	902	131	0.7	41.5	99	1860	18.7	892	8.4128N	39.0218E
CD	7.64	85	78	0.4	0.10	1.2	10560	494	1150	14	0.1	64.0	100	2309	16.8	986	8.5759N	39.0851E
Ala	7.69	77	73	1.2	0.09	1.1	10810	493	1260	16	9.4	66.4	99	1810	16.3	957	7.364N	38.2744E
Bu	5.39	53	72	6.0	0.18	1.5	5070	226	1100	47	72.4	44.0	80	2975	17.4	1055	8.0747N	38.2158E
Ha	6.64	324	67	162	0.30	3.0	6480	2120	1269	143	0.8	51.9	95	1810	19.2	1007	7.5036N	38.3019E

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AG, Alem-gena; Ho, Holetta; Fi, Fiche; DB, Debre-brehan; Ak, Akaki; CD, Chefe-donsa; DZ, Debre-zeit; Ala, Alagae; Bu, Butajira; Ha, Hawassa.

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EC, Electrical conductivity of soil extract (1:2.5); (Ca, K, Mg, Na, Al) ex.; exchangeable (Ca, K, Mg, Na, Al); BS, base saturation;

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CEC potential, cation exchange capacity potential at pH 7.00; Alt., altitude in meters asl; Avg.Temp, annual average temperature; A. Rain, annual rainfall.

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690 Table 2. Distribution of the genospecies according to sampling sites and

Sampling site	Genospecies									Diversity index*					
	1	2	3	4	5	6	7	8	9	S	N	<i>d</i>	<i>J'</i>	<i>H'</i>	<i>D</i>
Alem-gena	5	4	0	1	0	0	5	6	1	6	22	1.62	0.90	1.62	0.82
Holetta	1	7	0	3	0	1	3	4	3	7	22	1.94	0.91	1.77	0.84
Fiche	5	4	0	2	0	0	6	2	2	6	21	1.64	0.94	1.69	0.84
Debre-brehan	2	3	0	0	0	0	4	1	4	5	14	1.52	0.94	1.51	0.82
Akaki	2	1	0	4	1	0	8	4	0	6	20	1.67	0.86	1.54	0.78
Debre-zeit	9	4	0	0	0	0	4	3	1	5	21	1.31	0.88	1.42	0.76
Chefe-donsa	0	6	1	6	2	1	2	5	0	7	23	1.91	0.89	1.73	0.83
Alagae	2	4	2	7	0	0	3	4	0	6	22	1.62	0.94	1.69	0.84
Butajira	0	2	1	6	0	1	3	7	1	7	21	1.97	0.85	1.66	0.81
Hawassa	0	3	0	3	0	0	1	3	0	4	10	1.30	0.95	1.31	0.80
Total count	26	38	4	32	3	3	39	39	12	=196					

691 \***S**, number of genomic species in each sampling site; **N**, Total number of strains per site; **d**, Species richness (Margalef);  
692 **J'**, Pielou's evenness index; **H'**, Shannon-Weiner's index (loge); **D**, Simpson's index (1-Lamda).

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Table 3. Nodulation status of faba bean (*Vicia faba*), lentil (*Lens culinaris*) grass pea (*Lathyrus sativus*) and field pea (*Pisum abyssinicum*) inoculated with representative rhizobial strains in the pot experiment in the greenhouse.

Strain Code	nodC Group	Host (test) plant			
		faba bean	lentil	grass pea	field pea
<b>F17a</b>	Group 1	E	E	E	E
<b>F56</b>	Group 1	E	LE	LE	LE
<b>F85</b>	Group 1	LE	LE	LE	LE
<b>L53c</b>	Group 1	E	E	E	E
<b>F102</b>	Group 2	E	E	E	E
<b>L23a</b>	Group 2	E	E	E	LE
<b>L42a</b>	Group 2	E	E	LE	LE
<b>L71</b>	Group 2	LE	E	LE	LE
<b>F22</b>	Group 3	LE	LE	LE	LE
<b>F32a</b>	Group 3	E	E	E	E
<b>F73</b>	Group 3	E	LE	LE	LE
<b>F95</b>	Group 3	E	E	E	E
<b>L12y</b>	Group 3	LE	LE	LE	LE
<b>L85b</b>	Group 3	E	E	E	E
<b>F42</b>	Group 4	E	E	E	E
<b>F86</b>	Group 4	E	E	E	E
<b>L84</b>	Group 4	E	E	E	E
<b>L94d</b>	Group 4	E	E	E	E
<b>L33b</b>	Group 5	E	E	E	E
<b>L62</b>	Group 5	E	E	E	E

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E, Effective (positive nodulation and nitrogen fixation); LE, less effective (nodulation positive but with weak nitrogen-fixing efficiency). Nitrogen fixing efficiency was visually assessed by comparing plant and internal nodule color with the negative control.

713 **Figure legends**

714  
715 Figure 1. Geographical locations of the soil sampling sites in Ethiopia.

716 Figure 2. Maximum likelihood phylogenetic tree based on *recA* gene (516 bp) sequences of faba bean  
717 (F) and lentil (L) rhizobial strains, showing the taxonomic positions of nine genospecies. The tree  
718 was constructed using Tamura 3-parameter model plus discrete gamma distribution. Bootstrap values  
719  $\geq 50\%$  are presented at the branch nodes. The scale bar, 0.02 indicates estimated nucleotide  
720 substitution rates. Gene sequence accession numbers of the references are in parenthesis, and type  
721 strains are indicated with superscript "T". *B.*, *Bradyrhizobium*.

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723 Figure 3. Phylogenetic tree of *nodC* gene (521 bp) sequences showing the relationships between faba  
724 bean (F) and lentil (L) test strains and close rhizobial species. The tree was constructed using Tamura  
725 3-parameter model plus discrete gamma distribution. Bootstrap values  $\geq 50\%$  are presented at the  
726 branch nodes. The scale bar, 0.05 indicates estimated nucleotide substitution rates. Gene sequence  
727 accession numbers of the references are in parenthesis, and type strains are indicated with superscript  
728 "T". *B.*, *Bradyrhizobium*; *R.*, *Rhizobium*; sv., symbiovar.

729  
730 Figure 4. Phylogenetic tree of *nifH* gene (293 bp) sequences showing the relationships between faba  
731 bean (F) and lentil (L) test strains and close rhizobial species. The tree was constructed using Tamura  
732 3-parameter model plus discrete gamma distribution. Bootstrap values  $\geq 50\%$  are presented at the  
733 branch nodes. The scale bar, 0.1 indicates estimated nucleotide substitution rates. Gene sequence  
734 accession numbers of the references are in parenthesis, and type strains are indicated with superscript  
735 "T". *B.*, *Bradyrhizobium*; *R.*, *Rhizobium*; sv., symbiovar.

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737 Figure 5. Distance-based redundancy analysis (dbRDA) showing the relationship between nine  
738 genospecies and environmental factors of the sampling sites (available P, Ca, and K), (exchangeable  
739 Al), rainfall, and elevation. The longer the vector pointing toward the genospecies is, the greater the  
740 influence of the environmental factors on the distribution of the genospecies.

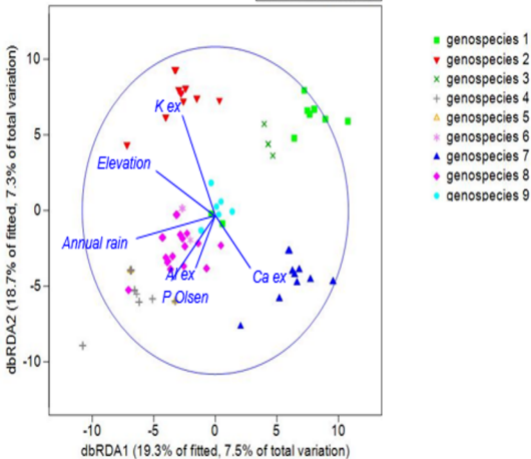
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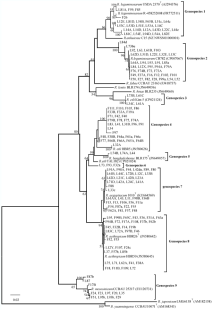
- A- Alem-gena
- B- Holetta
- C- Fiche
- D- Debre-brehan
- E- Akaki
- F- Debre-zeit
- G- Chefe-donsa
- H- Alagae
- I- Butajira
- J- Hawassa



Resemblance: S7 Jaccard









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**Supplementary Table 1. List of primers used for PCR amplification.**

Gene code	Primer combination and their target gene position*	Primer sequence	Reference
<i>recA</i>	recA-6F (6-31 bp)	5'-CGK CTS GTA GAG GAY AAA TCG GTG GA-3'	Gaunt et al. 2001
	recA-555R (555 – 530 bp)	5'-CGR ATC TGG TTG ATG AAG ATC ACC AT-3'	
<i>nodC</i>	nodCfor540 (544 – 566 bp)	5'-TGA TYG AYA TGG ART AYT GGC T-3'	Sarita et al. 2005
	nodCrev1160 (1164 – 1184 bp)	5'-CGY GAC ARC CAR TCG CTR TTG-3'	
<i>nifH</i>	nifH-1F (367 – 389 bp)	5'- GTC TCC TAT GAC GTG CTC GG-3'	Rivas eta al. 2002
	nifH-1R (794 – 774 bp)	5'- GCT TCC ATG GTG ATC GGG GT-3'	

F- Forward, R- reverse; \*Primer position relative to the full gene sequences of the complete genome of *S. meliloti* 1021.

## References

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Rivas R, Velazquez E, Willems A et al. A new species of *Devosia* that forms a unique nitrogen-fixing root-nodule symbiosis with the aquatic legume *Neptunia natans* (L.f.) druce. *Appl Environ Microbiol* 2002; 68: 5217-22.

Sarita S, Sharma PK, Priefer UB et al. Direct amplification of rhizobial *nodC* sequences from soil total DNA and comparison to *nodC* diversity of root nodule isolates. *FEMS Microbiol Ecol* 2005; 54: 1-11.

Supplementary Table S2. Distance-based Linear Models (DistLM) marginal tests result of each predictor variable.

Predictor Variables	SS(trace)	Pseudo-F	P	Proportion
Electrical Conductivity	1930.8	10.113	0.001	0.049788
pH	2759.3	14.784	0.001	0.071153
Olsen Phosphorus	1881.9	9.8434	0.001	0.048527
Total Nitrogen	2505.6	13.331	0.001	0.064611
Total Carbon	2462	13.083	0.001	0.063486
Exchangeable Calcium	2647.7	14.142	0.001	0.068274
Exchangeable Potassium	2478.5	13.177	0.001	0.063911
Exchangeable Magnesium	2290.5	12.115	0.001	0.059063
Exchangeable Sodium	2338	12.382	0.001	0.060289
Exchangeable Aluminum	2746.9	14.713	0.001	0.070832
Cation Exchange Capacity potential	2603.4	13.889	0.001	0.067133
Base Saturation	2786.3	14.94	0.001	0.071849
Elevation	2445.1	12.988	0.001	0.063051
Average Annual Temperature	2329.5	12.335	0.001	0.06007
Annual rainfall	2831.4	15.201	0.001	0.073012

Supplementary Table S3. The sampling sites, and host plants of the rhizobial strains and th

<b>Code</b>	<b>Close reference species, reA accession, recA sequence % of identity</b>	<b>Geonospecies</b>	<b>nodC group</b>
F26	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 99	1	2
F59	<i>R. leguminosarum</i> USDA 2370 <sup>T</sup> (AJ294376), 100	1	
F65b	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 98	1	
F85	<i>R. leguminosarum</i> USDA 2370 <sup>T</sup> (AJ294376), 100	1	1
L12a	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 97	1	
L12e	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 98	1	
L14a	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 97	1	2
L14c	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 98	1	1
L14d	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 97	1	2
L22c	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 97	1	1
L43d	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 97	1	
L44e	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 97	1	
L51c	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 98	1	
L51e	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 98	1	1
L53a	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 98	1	
L53c	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 98	1	1
L53d	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 98	1	1
L54a	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 97	1	1
L54b	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 98	1	1
L54e	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 97	1	1
L64c	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 98	1	1
L81a	<i>R. leguminosarum</i> USDA 2370 <sup>T</sup> (AJ294376), 100	1	
L81c	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 97	1	1
L81d	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 98	1	1
L82e	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 97	1	1
L94d	<i>Rhizobium leguminosarum</i> R-458252008 (FR772511), 97	1	4
F12	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	1
F16	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	1
F27	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
F28	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
F37a	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	2
F49	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	1
F50	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
F73	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	3
F73a	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
F74b	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
F76	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	3
F79a	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	2
F82	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
F94a	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	1
F95	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	3
F99a	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	1
F101	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
F102	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	2
F103	<i>R.leguminosarum</i> CB782 (CP007067), 99.6	2	

L12x	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	1
L13c	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	3
L22e	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
L32e	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	3
L51d	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
L52	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
L54	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
L62b	<i>R.leguminosarum</i> CB782 (CP007067), 99.6	2	2
L62d	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	1
L63	<i>R.leguminosarum</i> CB782 (CP007067), 99.6	2	2
L64a	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
L73bu	<i>R.leguminosarum</i> CB782 (CP007067), 99	2	
L84	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	4
L84d	<i>R.leguminosarum</i> CB782 (CP007067), 98	2	
L85a	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
L91	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
L92	<i>R.leguminosarum</i> CB782 (CP007067), 99.6	2	
L93	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	
L94	<i>R.leguminosarum</i> CB782 (CP007067), 100	2	1
L34c	<i>R. etli</i> Kim 5 (CP021124), 100	3	
L61a	<i>R. etli</i> Kim 5 (CP021124), 100	3	2
L61c	<i>R. etli</i> Kim 5 (CP021124), 100	3	
L73b	<i>R. etli</i> Kim 5 (CP021124), 100	3	
F19a	<i>R. etli</i> HBR5 (JN580626), 96	4	1
F33a	<i>R. etli</i> HBR5 (JN580626), 96	4	4
F33b	<i>R. etli</i> HBR5 (JN580626), 96	4	4
F38b	<i>R. etli</i> HBR5 (JN580626), 97	4	2
F40	<i>R. etli</i> HBR5 (JN580626), 96	4	1
F42	<i>R. etli</i> HBR5 (JN580626), 96	4	4
F48	<i>R. etli</i> HBR5 (JN580626), 97	4	
F61a	<i>R. etli</i> HBR5 (JN580626), 97	4	
F64a	<i>R. etli</i> HBR5 (JN580626), 97	4	
F64b	<i>R. etli</i> HBR5 (JN580626), 97	4	
F65a	<i>R. etli</i> HBR5 (JN580626), 97	4	4
F66a	<i>R. etli</i> HBR5 (JN580626), 97	4	
F66b	<i>R. etli</i> HBR5 (JN580626), 97	4	
F66c	<i>R. etli</i> HBR5 (JN580626), 97	4	
F71	<i>R. etli</i> HBR5 (JN580626), 96	4	4
F74a	<i>R. etli</i> HBR5 (JN580626), 96	4	
F75	<i>R. etli</i> HBR5 (JN580626), 96	4	2
F77	<i>R. etli</i> HBR5 (JN580626), 97	4	
F78	<i>R. etli</i> HBR5 (JN580626), 96	4	
F79b	<i>R. etli</i> HBR5 (JN580626), 96	4	4
F86	<i>R. etli</i> HBR5 (JN580626), 96	4	4
F91	<i>R. etli</i> HBR5 (JN580626), 96	4	
F96	<i>R. etli</i> HBR5 (JN580626), 96	4	
F97	<i>R. etli</i> HBR5 (JN580626), 96	4	
F105	<i>R. etli</i> HBR5 (JN580626), 96	4	1
F110	<i>R. etli</i> HBR5 (JN580626), 96	4	
F111	<i>R. etli</i> HBR5 (JN580626), 96	4	

L31b	<i>R. etli</i> HBR5 (JN580626), 96	4	3
L32a	<i>R. etli</i> HBR5 (JN580626), 100	4	
L34	<i>R. etli</i> HBR5 (JN580626), 96	4	
L41	<i>R. etli</i> HBR5 (JN580626), 96	4	
L83	<i>R. etli</i> HBR5 (JN580626), 96	4	
L44	<i>R. bangladeshense</i> BLR175 <sup>T</sup> (JN649057), 99	5	2
L74a	<i>R. bangladeshense</i> BLR175 <sup>T</sup> (JN649057), 99	5	
L74b	<i>R. bangladeshense</i> BLR175 <sup>T</sup> (JN649057), 99	5	2
F32a	<i>R. etli</i> TAL182 (CP021024), 95	6	3
F93	<i>R. etli</i> TAL182 (CP021024), 95	6	
L73	<i>R. etli</i> TAL182 (CP021024), 95	6	
F13	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 99	7	
F15	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 99	7	1
F22	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.6	7	3
F36b	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 99	7	
F44	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	
F54	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.6	7	
F55	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.6	7	1
F56	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 99	7	1
F57	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.6	7	1
F62a	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.6	7	
F80	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	
F81	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.6	7	1
F84	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.6	7	1
F87a	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.6	7	3
F88	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.5	7	
F89	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	1
F98a	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	3
F98b	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 99	7	
F106	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 99	7	1
L11	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 99	7	1
L12c	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	2
L21c	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	
L23a	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	1
L24c	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	
L33b	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	5
L33c	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.5	7	1
L41a	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	2
L42a	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	2
L42b	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	2
L42d	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	2
L42du	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	
L43	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 99	7	2
L44c	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	
L61aa	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 99	7	
L61b	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	
L71d	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	
L72b	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	2
L91a	<i>R. aegyptiacum</i> 1010 <sup>T</sup> (KU664569), 98.3	7	3
F11a	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	1



F11b	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	1
F14	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 100	8	1
F17a	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.6	8	1
F18	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	1
F19b	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 100	8	1
F24c	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	1
F31a	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	
F32b	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 100	8	
F36	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	1
F37b	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.6	8	2
F37c	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	
F38a	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	2
F41	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	1
F43	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	1
F45	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 100	8	
F46	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 100	8	3
F52	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	3
F53	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	3
F57b	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	
F62b	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.6	8	
F63a	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	
F65c	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	
F72	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.6	8	3
F94b	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.6	8	
F97b	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 100	8	4
F99b	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	3
F107	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	1
F108	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.6	8	1
F109	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	
L12y	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	3
L37	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	
L62a	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	5
L71	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	2
L72	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	
L72a	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 100	8	1
L75	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	
L83c	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 100	8	2
L85b	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	3
L95	<i>R. aethiopicum</i> HBR26 <sup>T</sup> (JN580642), 99.8	8	1
F17b	<i>R. mesosinicum</i> CCBAU 25217 (EU120731), 97	9	
F20	<i>R. mesosinicum</i> CCBAU 25217 (EU120731), 97.4	9	
F23	<i>R. mesosinicum</i> CCBAU 25217 (EU120731), 97.4	9	
F24	<i>R. mesosinicum</i> CCBAU 25217 (EU120731), 97.4	9	
F29	<i>R. mesosinicum</i> CCBAU 25217 (EU120731), 97.4	9	
F51	<i>R. mesosinicum</i> CCBAU 25217 (EU120731), 97.4	9	
F87b	<i>R. mesosinicum</i> CCBAU 25217 (EU120731), 96	9	
L35	<i>R. mesosinicum</i> CCBAU 25217 (EU120731), 97.4	9	
L87	<i>R. mesosinicum</i> CCBAU 25217 (EU120731), 96.3	9	
L93b	<i>R. mesosinicum</i> CCBAU 25217 (EU120731), 97.4	9	
L95b	<i>R. mesosinicum</i> CCBAU 25217 (EU120731), 97.4	9	

L97 *R. mesosinicum* CCBAU 25217 (EU120731), 97.4

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**Code** Close reference species, reA accession, recA sequence % **Geonospecies** *nodC* group

their genospecies and symbiotic gene groups.

<i>nifH</i> group	Host legume	Site	Latitude	Longitude
	Faba bean	Debre-brehan	9.4021N	39.3357E
	Faba bean	Debre-zeit	8.4128N	39.0218E
5	Faba bean	Alagae	7.364N	38.2744E
	Faba bean	Fiche	9.4643N	38.4324E
3	Lentil	Alem-gena	8.554N	38.3919E
	Lentil	Alem-gena	8.554N	38.3919E
3	Lentil	Alem-gena	8.554N	38.3919E
2	Lentil	Alem-gena	8.554N	38.3919E
3	Lentil	Alem-gena	8.554N	38.3919E
3	Lentil	Debre-brehan	9.4021N	39.3357E
	Lentil	Akaki	8.5248N	38.5006E
	Lentil	Akaki	8.5248N	38.5006E
5	Lentil	Debre-zeit	8.4128N	39.0218E
2	Lentil	Debre-zeit	8.4128N	39.0218E
2	Lentil	Debre-zeit	8.4128N	39.0218E
	Lentil	Debre-zeit	8.4128N	39.0218E
2	Lentil	Debre-zeit	8.4128N	39.0218E
	Lentil	Debre-zeit	8.4128N	39.0218E
2	Lentil	Debre-zeit	8.4128N	39.0218E
	Lentil	Debre-zeit	8.4128N	39.0218E
2	Lentil	Alagae	7.364N	38.2744E
	Lentil	Fiche	9.4643N	38.4324E
2	Lentil	Fiche	9.4643N	38.4324E
2	Lentil	Fiche	9.4643N	38.4324E
2	Lentil	Fiche	9.4643N	38.4324E
	Lentil	Holetta	9.0452N	38.3017E
1	Faba bean	Alem-gena	8.554N	38.3919E
1	Faba bean	Alem-gena	8.554N	38.3919E
3	Faba bean	Debre-brehan	9.4021N	39.3357E
1	Faba bean	Debre-brehan	9.4021N	39.3357E
3	Faba bean	Butajira	8.0747N	38.2158E
	Faba bean	Akaki	8.5248N	38.5006E
1	Faba bean	Debre-zeit	8.4128N	39.0218E
3	Faba bean	Chefe-donsa	8.5759N	39.0851E
3	Faba bean	Chefe-donsa	8.5759N	39.0851E
1	Faba bean	Chefe-donsa	8.5759N	39.0851E
1	Faba bean	Chefe-donsa	8.5759N	39.0851E
3	Faba bean	Chefe-donsa	8.5759N	39.0851E
	Faba bean	Fiche	9.4643N	38.4324E
1	Faba bean	Holetta	9.0452N	38.3017E
	Faba bean	Holetta	9.0452N	38.3017E
	Faba bean	Holetta	9.0452N	38.3017E
	Faba bean	Hawassa	7.5036N	38.3019E
3	Faba bean	Hawassa	7.5036N	38.3019E
	Faba bean	Hawassa	7.5036N	38.3019E

	Lentil	Alem-gena	8.554N	38.3919E
	Lentil	Alem-gena	8.554N	38.3919E
1	Lentil	Debre-brehan	9.4021N	39.3357E
	Lentil	Butajira	8.0747N	38.2158E
	Lentil	Debre-zeit	8.4128N	39.0218E
	Lentil	Debre-zeit	8.4128N	39.0218E
1	Lentil	Debre-zeit	8.4128N	39.0218E
	Lentil	Alagae	7.364N	38.2744E
1	Lentil	Alagae	7.364N	38.2744E
3	Lentil	Alagae	7.364N	38.2744E
1	Lentil	Alagae	7.364N	38.2744E
	Lentil	Chefe-donsa	8.5759N	39.0851E
1	Lentil	Fiche	9.4643N	38.4324E
	Lentil	Fiche	9.4643N	38.4324E
	Lentil	Fiche	9.4643N	38.4324E
1	Lentil	Holetta	9.0452N	38.3017E
	Lentil	Holetta	9.0452N	38.3017E
	Lentil	Holetta	9.0452N	38.3017E
1	Lentil	Holetta	9.0452N	38.3017E
3	Lentil	Butajira	8.0747N	38.2158E
3	Lentil	Alagae	7.364N	38.2744E
6	Lentil	Alagae	7.364N	38.2744E
6	Lentil	Chefe-donsa	8.5759N	39.0851E
1	Faba bean	Alem-gena	8.554N	38.3919E
1	Faba bean	Butajira	8.0747N	38.2158E
1	Faba bean	Butajira	8.0747N	38.2158E
3	Faba bean	Butajira	8.0747N	38.2158E
2	Faba bean	Akaki	8.5248N	38.5006E
1	Faba bean	Akaki	8.5248N	38.5006E
	Faba bean	Akaki	8.5248N	38.5006E
5	Faba bean	Alagae	7.364N	38.2744E
5	Faba bean	Alagae	7.364N	38.2744E
	Faba bean	Alagae	7.364N	38.2744E
5	Faba bean	Alagae	7.364N	38.2744E
	Faba bean	Alagae	7.364N	38.2744E
1	Faba bean	Alagae	7.364N	38.2744E
	Faba bean	Alagae	7.364N	38.2744E
1	Faba bean	Chefe-donsa	8.5759N	39.0851E
	Faba bean	Chefe-donsa	8.5759N	39.0851E
3	Faba bean	Chefe-donsa	8.5759N	39.0851E
	Faba bean	Chefe-donsa	8.5759N	39.0851E
	Faba bean	Chefe-donsa	8.5759N	39.0851E
	Faba bean	Chefe-donsa	8.5759N	39.0851E
	Faba bean	Fiche	9.4643N	38.4324E
	Faba bean	Holetta	9.0452N	38.3017E
	Faba bean	Holetta	9.0452N	38.3017E
	Faba bean	Holetta	9.0452N	38.3017E
	Faba bean	Hawassa	7.5036N	38.3019E
	Faba bean	Hawassa	7.5036N	38.3019E
	Faba bean	Hawassa	7.5036N	38.3019E

1	Lentil	Butajira	8.0747N	38.2158E
1	Lentil	Butajira	8.0747N	38.2158E
	Lentil	Butajira	8.0747N	38.2158E
3	Lentil	Akaki	8.5248N	38.5006E
1	Lentil	Fiche	9.4643N	38.4324E
5	Lentil	Akaki	8.5248N	38.5006E
5	Lentil	Chefe-donsa	8.5759N	39.0851E
5	Lentil	Chefe-donsa	8.5759N	39.0851E
3	Faba bean	Butajira	8.0747N	38.2158E
	Faba bean	Holetta	9.0452N	38.3017E
5	Lentil	Chefe-donsa	8.5759N	39.0851E
1	Faba bean	Alem-gena	8.554N	38.3919E
1	Faba bean	Alem-gena	8.554N	38.3919E
	Faba bean	Debre-brehan	9.4021N	39.3357E
	Faba bean	Butajira	8.0747N	38.2158E
	Faba bean	Akaki	8.5248N	38.5006E
2	Faba bean	Debre-zeit	8.4128N	39.0218E
1	Faba bean	Debre-zeit	8.4128N	39.0218E
	Faba bean	Debre-zeit	8.4128N	39.0218E
1	Faba bean	Debre-zeit	8.4128N	39.0218E
	Faba bean	Alagae	7.364N	38.2744E
	Faba bean	Fiche	9.4643N	38.4324E
1	Faba bean	Fiche	9.4643N	38.4324E
1	Faba bean	Fiche	9.4643N	38.4324E
1	Faba bean	Fiche	9.4643N	38.4324E
	Faba bean	Fiche	9.4643N	38.4324E
	Faba bean	Fiche	9.4643N	38.4324E
1	Faba bean	Holetta	9.0452N	38.3017E
	Faba bean	Holetta	9.0452N	38.3017E
	Faba bean	Hawassa	7.5036N	38.3019E
	Lentil	Alem-gena	8.554N	38.3919E
3	Lentil	Alem-gena	8.554N	38.3919E
	Lentil	Debre-brehan	9.4021N	39.3357E
3	Lentil	Debre-brehan	9.4021N	39.3357E
3	Lentil	Debre-brehan	9.4021N	39.3357E
6	Lentil	Butajira	8.0747N	38.2158E
	Lentil	Butajira	8.0747N	38.2158E
3	Lentil	Akaki	8.5248N	38.5006E
3	Lentil	Akaki	8.5248N	38.5006E
3	Lentil	Akaki	8.5248N	38.5006E
3	Lentil	Akaki	8.5248N	38.5006E
3	Lentil	Akaki	8.5248N	38.5006E
5	Lentil	Akaki	8.5248N	38.5006E
	Lentil	Akaki	8.5248N	38.5006E
	Lentil	Alagae	7.364N	38.2744E
3	Lentil	Alagae	7.364N	38.2744E
3	Lentil	Chefe-donsa	8.5759N	39.0851E
3	Lentil	Chefe-donsa	8.5759N	39.0851E
	Lentil	Holetta	9.0452N	38.3017E
1	Faba bean	Alem-gena	8.554N	38.3919E

1	Faba bean	Alem-gena	8.554N	38.3919E
1	Faba bean	Alem-gena	8.554N	38.3919E
1	Faba bean	Alem-gena	8.554N	38.3919E
1	Faba bean	Alem-gena	8.554N	38.3919E
1	Faba bean	Alem-gena	8.554N	38.3919E
	Faba bean	Debre-brehan	9.4021N	39.3357E
5	Faba bean	Butajira	8.0747N	38.2158E
3	Faba bean	Butajira	8.0747N	38.2158E
5	Faba bean	Butajira	8.0747N	38.2158E
3	Faba bean	Butajira	8.0747N	38.2158E
3	Faba bean	Butajira	8.0747N	38.2158E
3	Faba bean	Butajira	8.0747N	38.2158E
	Faba bean	Akaki	8.5248N	38.5006E
	Faba bean	Akaki	8.5248N	38.5006E
	Faba bean	Akaki	8.5248N	38.5006E
	Faba bean	Akaki	8.5248N	38.5006E
	Faba bean	Debre-zeit	8.4128N	39.0218E
1	Faba bean	Debre-zeit	8.4128N	39.0218E
	Faba bean	Debre-zeit	8.4128N	39.0218E
3	Faba bean	Alagae	7.364N	38.2744E
5	Faba bean	Alagae	7.364N	38.2744E
	Faba bean	Alagae	7.364N	38.2744E
4	Faba bean	Chefe-donsa	8.5759N	39.0851E
	Faba bean	Holetta	9.0452N	38.3017E
1	Faba bean	Holetta	9.0452N	38.3017E
1	Faba bean	Holetta	9.0452N	38.3017E
1	Faba bean	Hawassa	7.5036N	38.3019E
1	Faba bean	Hawassa	7.5036N	38.3019E
	Faba bean	Hawassa	7.5036N	38.3019E
	Lentil	Alem-gena	8.554N	38.3919E
	Lentil	Butajira	8.0747N	38.2158E
6	Lentil	Alagae	7.364N	38.2744E
5	Lentil	Chefe-donsa	8.5759N	39.0851E
	Lentil	Chefe-donsa	8.5759N	39.0851E
5	Lentil	Chefe-donsa	8.5759N	39.0851E
3	Lentil	Chefe-donsa	8.5759N	39.0851E
	Lentil	Fiche	9.4643N	38.4324E
	Lentil	Fiche	9.4643N	38.4324E
	Lentil	Holetta	9.0452N	38.3017E
	Faba bean	Alem-gena	8.554N	38.3919E
	Faba bean	Debre-brehan	9.4021N	39.3357E
	Faba bean	Debre-brehan	9.4021N	39.3357E
	Faba bean	Debre-brehan	9.4021N	39.3357E
	Faba bean	Debre-brehan	9.4021N	39.3357E
	Faba bean	Debre-zeit	8.4128N	39.0218E
	Faba bean	Fiche	9.4643N	38.4324E
	Lentil	Butajira	8.0747N	38.2158E
	Lentil	Fiche	9.4643N	38.4324E
	Lentil	Holetta	9.0452N	38.3017E
	Lentil	Holetta	9.0452N	38.3017E

<i>nifH</i> group	Lentil Host legume	Holetta Site	9.0452N Latitude	38.3017E Longitude
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