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# Development of environmental friendly bioinoculate for peanut (*Arachis hypogea* L.) production in Eastern Ethiopia

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## Abstract

**Background:** Bacterial bioinoculate is one of environmentally friendly input for crop production. Several works revealed that effectiveness of *Rhizobium* bioinoculant have been affected by the native rhizobia population. Hence, this study was initiated to evaluate the effect of selected *Bradyrhizobium* in nodulating peanut in the major growing areas of Ethiopia.

**Methods:** The effect of eight effective *Bradyrhizobium* vis-a-vis 20 kg N ha<sup>-1</sup> and the control check on the nodulation and yield of two peanut cultivars (Sedi and Werer 942) at Babile and Fedis, eastern Ethiopia, during 2013 cropping season was evaluated.

**Results:** The results showed that the inoculation, location and cultivar and their two and three ways interaction had a significant influence on the nodulation and grain yield. Inoculation of *Bradyrhizobium* increased the nodule number, nodule dry weight, number of seeds per plants, total pods weight, hundred seeds weight, total biomass yield, grain yield and shelling % up to 37, 24.9, 22.4, 22.5, 5.5, 26.2, 35.8 and 24.1%, respectively. Isolate HUGNR-29 was found to be more effective as it scored the highest nodule dry weight and grain yield. Application of inorganic N increased the yield and yield components of peanut in either of locations. Though the statistically better effective nodule % was recorded at Babile, significantly higher nodule number, nodule dry weight, and total N accumulation were obtained from Fedis than Babile site. Significantly higher total pods weight, hundred seeds weight, total biomass weight, grain yield and total plant N accumulation was recorded with the cultivar werer-962 than those produced with Bati-Sedi.

**Conclusions:** The result showed that *Bradyrhizobium* inoculation is essential to improve the peanut production through environmentally and economically sustainable ways though the soil native rhizobia nodulating peanut was high. Peanut cultivar and study site based rhizobia inoculant development is also important.

**Keywords:** Babile, *Bradyrhizobium*, Fedis, HUGNR-29, Inoculation

## Background

The peanut is the fourth most widely cultivated oilseed in the America continents, Africa and Asia. Peanut plants grow best in well-drained sandy soils and sunny warm temperatures with moderate rainfall. In Ethiopia, the total planted area was 443000 ha and a production of 103.7 M ton of grain as it has been reported in the 2014 cropping

season (FAOSTAT 2014). This report also showed that the productivity of peanut has been 1604.1 kg ha<sup>-1</sup>. To increase this productivity, plant breeder has tried to release new high yielder varieties of peanut in Ethiopia (Kebede and Bushra 2012). So far, peanut varieties improving program in Ethiopian has obtained the peanut variety yielding up to 2.2 ton ha<sup>-1</sup>. When its productivity is comparing to the productivity (3.25 ton ha<sup>-1</sup>) that has been reported in North Carolina is very low (Jones 2003). Hence, improving the production and productivity of peanut in Ethiopia using environmental friendly technologies is essential.

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Peanut is considered a highly promiscuous species because it is nodulated by rhizobia able to nodulate a diverse group of legumes and a large group of unrelated rhizobia (Alwi et al. 1989). For instance, up to 18% of the tested fast and slow growing rhizobia isolated from cowpea are able to form effective nodules with peanut (Thies et al. 1991; Mpeperekki et al. 1996). Several studies have also already shown phenotypically and genetically diverse bradyrhizobia isolates nodulating peanut (Li et al. 1999; Zhang et al. 1999). Most commonly reported isolates of bradyrhizobia nodulating peanut are *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* (Willems et al. 2001; Yang et al. 2005; Nievas et al. 2012; Bogino et al. 2010). Peanut is also nodulated by fast-growing rhizobia such as *Rhizobium giardini* and *Rhizobium tropici* (Huang 1990; Taurian et al. 2006). The contribution of biological nitrogen fixation (BNF) through peanut-rhizobia symbiosis has been reported as 40.9 kg ha<sup>-1</sup> (Okito et al. 2004).

Abundant types of indigenous rhizobia nodulating peanut are widely distributed in various geographical and ecological areas of the world (Xu et al. 1995; Chen et al. 1997; Tan et al. 1999; Peng et al. 2002). Most cultivated soils of the tropics appear to have relatively large populations (> 10<sup>2</sup> g<sup>-1</sup> dry soil) of cowpea miscellany which can induce nodules on peanut root (Allen and Allen 1981). My unpublished work showed the presence of native rhizobia nodulating peanut in Ethiopian soils. Hence the indigenous rhizobia may interfere the effectiveness of inoculated effective isolate thereby causes lack of success of inoculation in improving nodulation and N<sub>2</sub> fixation of peanut (Hitbold et al. 1983; Kvien and Pallas 1986). However, if the native rhizobia is symbiotically effective, increase in native rhizobia population increased the yield of pulses (Thrall et al. 2007).

The presence of nodules on roots of the peanut plant does not necessarily mean that sufficient N<sub>2</sub> is being fixed for maximum growth of the host plant (Weaver 1974; Nambiar et al. 1982a). Inoculation is a major strategy for the development of sustainable input of nitrogen into agricultural soils (Lindström et al. 2010). Inoculation of legume seed is an efficient and convenient way of introducing highly efficient rhizobia to the soil to improve the peanut production (Deaker et al. 2004). Inoculation of *Bradyrhizobium* peanut resulted in the nitrogen fixation amounting up to 186 kg N ha<sup>-1</sup> (Moawad et al. 1986). Castro et al. (1999) found that peanut roots have been well nodulated by native strain rather than the exotic one. Hence, selection of efficient strains based on their local ecologic adaptation can increase grain production of crops (Denton et al. 2003; Lindström et al. 2010; Bianco et al. 2013). On top of differing the rhizobia in their ability to fix N<sub>2</sub>, the effective peanut-rhizobia symbiosis is the product of rhizobial and

plant cultivars as well as of environmental conditions (Nelson 1987; Sessitsch et al. 2002). Manipulating the peanut cultivar and *Bradyrhizobium* strain together in one system would be the most important for a productive approach to optimizing N<sub>2</sub> fixation and increase the peanut productivity (Chen et al. 2003). Hence, the hypothesis of this work was stated that inoculation of rhizobia isolated from native soil improves the nodulation and yield of peanut even though the soil native rhizobia population has been > 10<sup>2</sup> g<sup>-1</sup> soil. Our approach has been to utilize indigenous rather than exotic rhizobia, since the former, already adapted to the existing ecological conditions, can provide very satisfactory results (Douka and Xenoulis 1987; Hungria et al. 1996; Makatiani and Odee 2007). Therefore, the objective this work was to evaluate the symbiotic effectiveness of selected indigenous *Bradyrhizobium* sp. nodulating peanut in eastern Ethiopia.

## Methods

### Description of the study sites

Field studies were conducted in 2013 cropping season at Fedis (09°06.941'N and 042°04.835'E at an altitude of 5476 ft above sea level [asl]) and Babile (09°13.234'N and 042°19.407'E at 5478 ft asl) Agricultural Research Sub-center, Haramaya University, located in the eastern part of Ethiopia. The soils of the study sites (Babile and Fedis) were sandy loam and silty clay loam, respectively. Fedis site receives a mean annual rainfall of 714.3 mm (Ethiopia Meteorological Report, unpublished), with a mean maximum and minimum temperature of 27.8 and 8.8 °C, respectively. The area has a bi-modal pattern, falling in two seasons, with the short rain season occurring between March and May and the long rain season from between June through October. At Babile site, the annual means maximum and minimum temperatures are 20 and 90 °C, respectively with annual mean rainfall of 540 mm which has been calculated based on the weather data between 2000 and 2014. The soil of Babile site before planting the experiment showed the following properties: pH 6.66; EC, 0.04 dS m<sup>-1</sup>; organic carbon content, 0.56%; total N, 0.06% available P content (Olsen), 2.22 mg kg<sup>-1</sup>; contents of exchangeable K<sup>1+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and Na<sup>1+</sup>, 0.34, 4.18, 3.5 and 0.15 cmol(+) kg<sup>-1</sup>, respectively; cation exchange capacity, 8.17 cmol(+) kg<sup>-1</sup>, number of rhizobia, 5.8 × 10<sup>2</sup> cells g<sup>-1</sup> of dry soil. The top 20 cm of Fedis soil contained 1.32% soil organic C, 0.12% total N, 1.78 mg kg<sup>-1</sup> Olsen-P, cation exchange capacity, 37.2 cmol(+) kg<sup>-1</sup> and 23.12, 12.87, 0.12 and 1.09 exchangeable Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>1+</sup> and K<sup>1+</sup>, respectively, and had a pH value (in water) of 7.76 and EC value of 0.06 dS mat<sup>-1</sup> the start of the experiment. The number of rhizobia nodulating peanut at Fedis soil was < 10<sup>2</sup> viable cell g<sup>-1</sup> dry soil.

### Sources of the *Bradyrhizobium isolates*

Rhizobial isolates used were recommended *Bradyrhizobium* sp. for Peanut (*Arachis hypogea* L.). The bacteria used in this study were HUGNR-1, HUGNR-2, HUGNR-3, HUGNR-4, HUGNR-5, HUGNR-6, HUGNR-7 and HUGNR-8. These isolates were selected because they were previously found efficient isolates in N<sub>2</sub> fixation under greenhouse conditions (Akuma 2010).

### Sources of the cultivars

Recently released and high yielder cultivars of peanut (Werer-924 and Sedi) were obtained from lowland oil crop, Haramaya University, Ethiopia.

### Preparation of inoculant

Purity was assured by routine plating on YEM agar supplemented with Congo red and white colony was selected for further use for inoculant production. White colony were transferred into 100 ml containing YEM (yeast extract mannitol) medium in 250 ml Erlenmeyer flasks and incubated at 28 °C on a shaker incubator at 150 rpm. After 48 h incubation, the number of viable cells in the culture broth was determined by the drop-plate method by counting colony-forming units (CFU) on YEMA plates (Somasegaran and Hoben 1994).

The carrier used for this experiment was decomposed filter cake after sterilized in autoclave at 121 °C. Filter cake based inoculant was prepared by mixing sterilized Filter cake and culture broth at 1:1 v w<sup>-1</sup> under aseptic conditions and placed in an incubator at 25 °C for 1 week. After inoculation, the population of viable rhizobia cell was reached at 10<sup>9</sup> rhizobia g<sup>-1</sup> of dry peat.

### Experimental design and treatments

Ten treatments comprised of eight elite *Bradyrhizobium*, N fertilized 20 kg N ha<sup>-1</sup>) and an absolute control where no external inputs were applied were laid out as a randomized complete block design (RCBD) with three replications. The seeds were planted in 3 m rows with 10 cm spacing between plants and 40 cm between rows. The gross plot size was 3 × 3 m. Seeds which had been surface wetted, were mixed thoroughly with the peat culture and sown immediately. Triple-superphosphate (46 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) was uniformly applied for experimental plots. Inorganic N (20 kg N ha<sup>-1</sup>) as starter N was applied only for N-fertilized treatment. All inorganic fertilizer were incorporated in the soil before planting.

### Indigenous rhizobial count

Undamaged and uniform seeds of peanut was sterilized using 0.2% HgCl<sub>2</sub> and 95% ethanol (Vincent 1970) and then the germination was done in petri-dishes containing wetted filter paper. Three healthy seedlings were

transferred to each of pot containing autoclaved sand (Vincent 1970) and thereafter thinned to one seedling per pot. From each soil sample, serial dilutions, ranging from 10<sup>-1</sup> to 10<sup>-10</sup> were prepared and added at a rate of 1 ml per pot. After 6 weeks, the numbers of nodulated plants were recorded and the most probable number (MPN) of rhizobia nodulating peanut was estimated (Brockwell 1963).

### Agronomic data collection

At the late flowering stage at the R<sub>2</sub> stage, five plants from internal rows were uprooted for nodulation parameters assessment. The soil adhering to roots and other plant parts was removed under running tap water. The nodules were collected and the dry weight of the plants and nodules were measured after drying the samples at 70 °C for 72 h. At harvest, plants obtained from 1 m<sup>2</sup> were analyzed for yield determinations and the results expressed in kg ha<sup>-1</sup>.

### Data analysis

Analysis of variance (ANOVA) was carried out using the randomized block design method. Least significance difference (LSD) was calculated to discriminate the effect treatment on the measured plant traits at 5% probability.

## Results

### Nodulation

This field inoculation experiment was conducted at Fedis and Babile sites where the native rhizobia of peanut were 2.6 × 10<sup>2</sup> and 3.3 × 10<sup>3</sup> rhizobia of peanut g<sup>-1</sup> soil, respectively as estimated by the MPN method. The result of the ANOVA showed that the main effect of inoculation (I), locations (L) and cultivar (C) and their two and three ways interaction significantly affected the pooled nodule number (NN) and nodule dry weight (NDW) at P < 0.05 (Table 1). However, the effect of cultivar on pooled NN and NDW across locations and L × C interaction effect on pooled NDW were not significant at P > 0.05, implying that the performance of the tested cultivar in terms of nodulation in these two locations are similar. This study showed that both cultivars performed equally in NDW production in both experimental locations. The result revealed that *Bradyrhizobium* inoculation did not improve significantly the pooled NDW compared with not inoculated.

The main effect of location and C × I significantly influenced the % of effective nodules. This result suggests the presence of different effectiveness of native and inoculated *Bradyrhizobium* with the cultivar Werer-962 and Bati-Sedi. It was also found various effective nodules % at Babile and Fedis. Inoculating HUGNR-10 resulted in significantly increase the pooled NN over the uninoculated

**Table 1 Analysis of variance of measured traits of peanut as affected by the main effect of *Bradyrhizobium* inoculation, location, and cultivar of peanut their two and three ways interaction**

Sources of variation	df	F values	NN	NDW	EffN (%)	SDW	NSP	TPW	100 seed wt (g)	TBY	GY	Shelling (%)	Tot plant P	Tot plant N
Inoculation (I)	9	3.30**	2.28*	1.15 <sup>ns</sup>	1.46 <sup>ns</sup>	2.24*	4.64***	3.04**	2.20*	10.24***	1.70 <sup>ns</sup>	0.31 <sup>ns</sup>	0.28	
Location (L)	1	57.46***	15.18***	7.15**	14.97***	0.71 <sup>ns</sup>	16.23***	754.45***	0.32 <sup>ns</sup>	0.09 <sup>ns</sup>	4.23*	0.91 <sup>ns</sup>	7.61**	
Cultivar (C)	1	2.28 <sup>ns</sup>	1.27 <sup>ns</sup>	0.10 <sup>ns</sup>	4.57*	201.02***	217.81***	67.91***	354.12***	198.44***	5.53*	2.30 <sup>ns</sup>	42.24***	
L × I	9	8.23***	3.16**	1.83 <sup>ns</sup>	2.76**	0.70 <sup>ns</sup>	5.49***	2.18*	1.35 <sup>ns</sup>	2.87**	3.99***	1.18 <sup>ns</sup>	1.50	
C × I	9	2.02*	2.87**	2.40*	1.40 <sup>ns</sup>	0.97 <sup>ns</sup>	3.31**	2.03*	1.52 <sup>ns</sup>	3.18**	3.71**	0.40 <sup>ns</sup>	0.83	
L × C	1	20.18***	1.89 <sup>ns</sup>	1.19 <sup>ns</sup>	11.28**	2.18 <sup>ns</sup>	44.74***	274.37***	88.19***	0.02 <sup>ns</sup>	19.92***	0.00 <sup>ns</sup>	3.77	
L × C × I	9	3.49**	4.03***	2.07*	1.36 <sup>ns</sup>	0.38 <sup>ns</sup>	1.77 <sup>ns</sup>	2.16*	3.03**	3.99***	1.54 <sup>ns</sup>	0.62 <sup>ns</sup>	2.24*	

ns non significant, NN nodule number per plant, NDW nodule dry weight (g plant<sup>-1</sup>), EffN (%) effective nodule number percentage, SDW shoot dry weight (g plant<sup>-1</sup>), NSP number of seeds per peg, TPW total pegs weight (kg ha<sup>-1</sup>), TBY total biomass yield (kg ha<sup>-1</sup>), GY grain yield (kg ha<sup>-1</sup>)

\* Significant at 0.05; \*\* significant at 0.01; \*\*\* significant at 0.001

treatment (Table 2). However, inoculation did not affect the % of the effective nodule, indicating that the indigenous soil *Bradyrhizobium* sp. was able to induce effective nodules on peanut roots.

In general, the result revealed that significantly higher NN and NDW were found at Fedis than Babile site (Table 2). This could be associated the presence of high peanut rhizobia population and conducive environmental condition for nodulation at Fedis soil when compared to Babile soil. However, significantly higher % of effective nodules was recorded at Babile site than Fedis site. Both cultivars induced the statistically at par amount of NN, NDW and % of effective nodules.

All inoculated isolates including N fertilization except HUGNR-18 significantly increased the effective nodule % at Babile site (Fig. 1c). It was also found that only HUGNR-13 inoculation resulted in significant increase in effective nodule % at Fedis site. The present work also found that all isolates tested except HUGNR-18 inoculation caused the significant increased in effective nodules % with Bati-sedi cultivar (Fig. 2c). With Werer-962, a significant increase in effective nodules % was found due to HUGNR-11 and consortium inoculation and N fertilization.

The tested *Bradyrhizobium* showed different performance on NN and NDW production in both locations. At Babile site, except for HUGNR-11, all isolates including N fertilized plant gave the higher NN than the uninoculated treatment (Fig. 1a). At Fedis site, only HUGNR-11 inoculation resulted in increase in NN when compared to uninoculated treatment. This different result obtained at two locations may have arisen from higher native rhizobia population at Fedis than Babile site. Beside this, better NDW at Babile site were recorded due to Consortium isolates and HUGNR-29 inoculations but higher NDW than uninoculated at Fedis site due to all inoculation treatments, except HUGNR-10 and HUGNR-19 (Fig. 1b).

When the data stratified by cultivar, most of the tested *Bradyrhizobium* isolates except HUGNR-11, HUGNR-13 and HUGNR-29 improved the NN of the cultivar Werer-962 whilst all isolates inoculation with the cultivar Bati-Sedi cultivar resulted in higher NN than the control check (Fig. 2a). Only HUGNR-29 with werer-962 improved the NDW over the uninoculated treatment (Fig. 2b). The isolates which were resulted in a significantly increased NDW with Bati-Sedi were Consortium isolates, HUGNR-13, HUGNR-18 and HUGNR-29. This

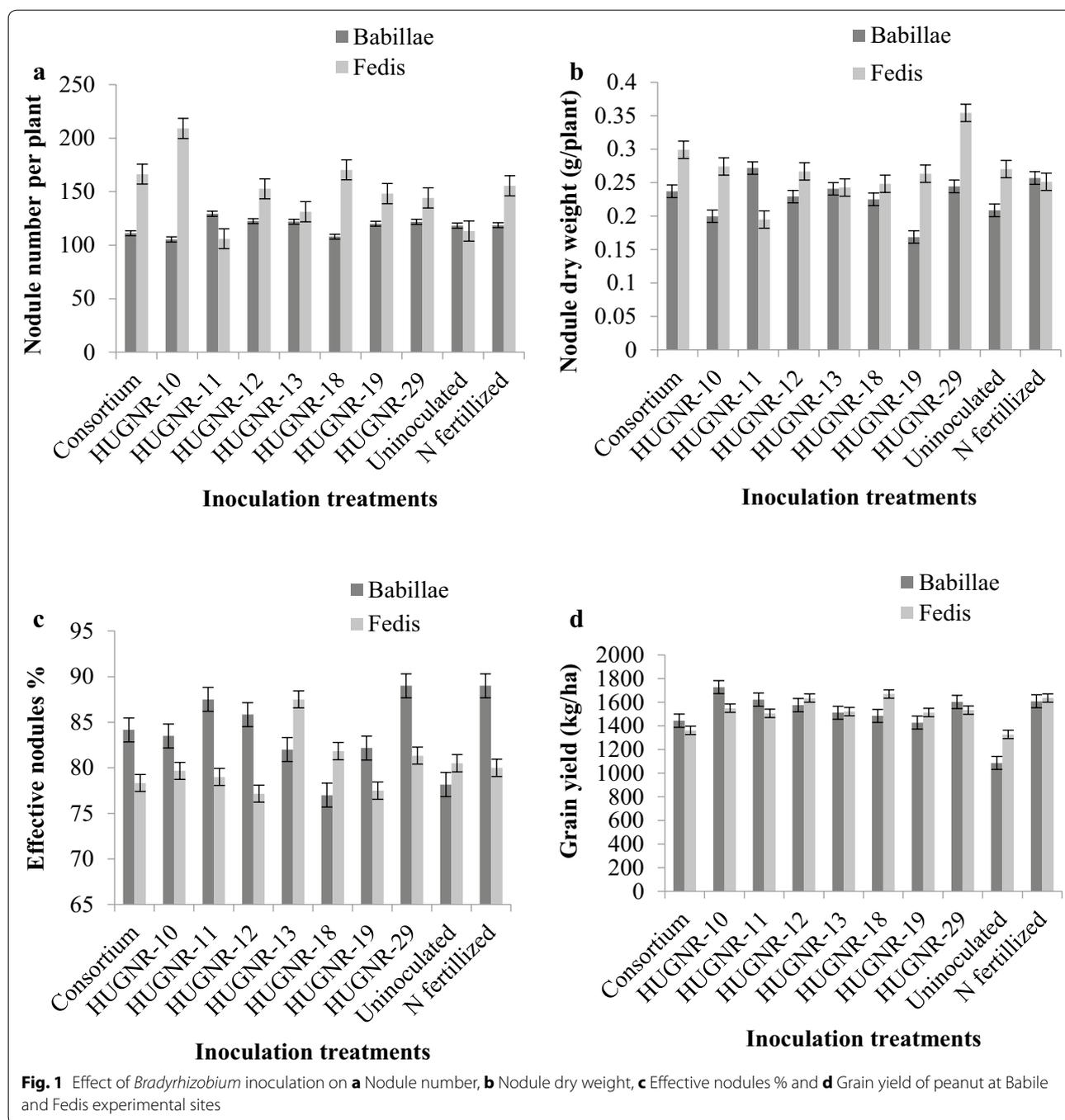
**Table 2** Nodulation traits, shoot dry weight, number of seeds per pg and total pegs weight as affected by *Bradyrhizobium* inoculation, cultivar, and location

Treatments	NN	NDW	EffNN (%)	SDW	NSP	TPW
Consortium	138.75ab	0.2679ab	81.25a	51.7a	2.89ab	3384.3abc
HUGNR-10	158.83a	0.2703ab	83.25a	59.48a	2.72ab	3701.4a
HUGNR-11	121.83b	0.2332ab	83.25a	51.58a	2.72ab	3634.3a
HUGNR-12	137.58ab	0.2478ab	81.50a	53.97a	2.70ab	3752.3a
HUGNR-13	126.58b	0.2417ab	84.75a	55.03a	2.64ab	3493.1abc
HUGNR-18	139.17ab	0.2365ab	79.42a	54.81a	2.58ab	3613.4a
HUGNR-19	134.08ab	0.2160b	79.83a	50.35a	3.00a	3011.6c
HUGNR-29	133.00ab	0.2991a	85.17a	57.85a	2.92ab	3553.2ab
Uninoculated	115.75b	0.2394ab	79.33a	50.76a	2.45b	3062.5bc
N fertilized	137.08ab	0.2539ab	84.50a	43.84a	2.86ab	3518.5abc
F value	3.30**	2.28*	1.15 <sup>ns</sup>	1.46 <sup>ns</sup>	2.24*	4.64***
LSD (P < 0.05)	29.54	0.0715	9.66	16.73	0.52	539.46
Babile	118.883b	0.2314b	84.00a	57.38a	2.78a	3323.15b
Fedis	149.65a	0.2697a	80.45b	48.49b	2.72a	3621.76a
F value	57.46***	15.18***	7.15**	14.97***	0.71 <sup>ns</sup>	16.23***
LSD (P < 0.05)	8.077	0.0196	2.64	4.575	0.1428	147.52
Werer 962	137.33a	0.2561a	82.43a	50.48b	2.24b	4019.44a
Bati-Sedi	131.20a	0.2450a	82.02a	55.39a	3.26a	2925.46b
F value	2.28 <sup>ns</sup>	1.27 <sup>ns</sup>	0.10 <sup>ns</sup>	4.57*	201.02***	217.81***
LSD (P < 0.05)	8.08	0.0196	2.64	4.58	0.14	147.52
CV (%)	16.56	21.49	8.84	23.79	14.30	11.69

Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test

<sup>ns</sup> non-significant, NN nodule number per plant, NDW nodule dry weight (g plant<sup>-1</sup>), EffNN (%) effective nodule number percentage, SDW shoot dry weight (g plant<sup>-1</sup>), NSP number of seeds per peg, TPW total pegs weight (kg ha<sup>-1</sup>)

\* Significant at 0.05, \*\* significant at 0.01, \*\*\* significant at 0.001

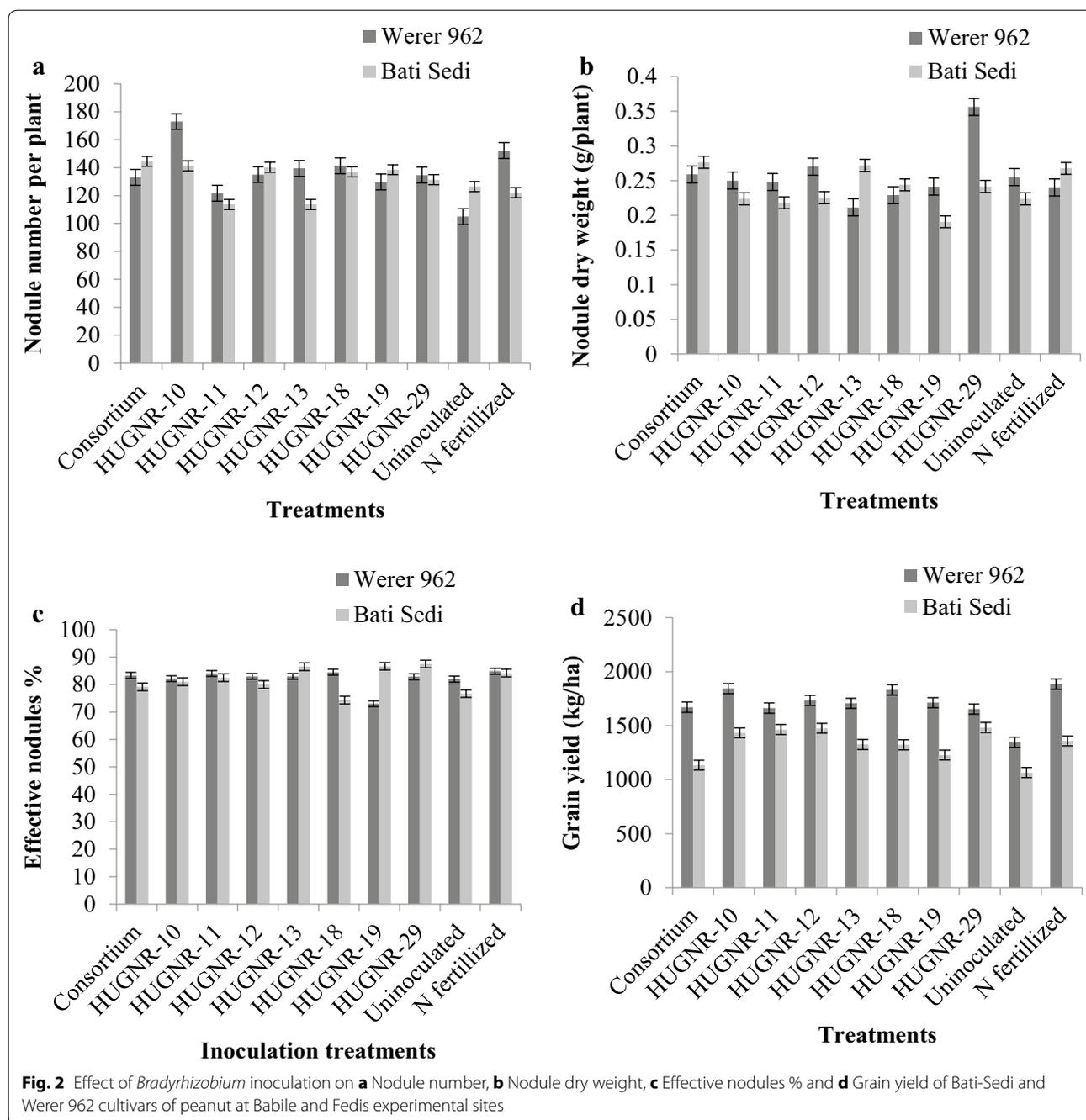


result indicates the permissiveness of the cultivar werer-962 for most of the tested isolates.

**Shoot dry weight**

The main effect of location and cultivar, and L × I and L × C on the pooled shoot biomass yield produced at late flowering stage across locations were significant at P < 0.05 (Table 1). Inoculation did not significantly

(P > 0.05) increase the pooled shoot biomass of peanut (Table 2). Significantly higher pooled shoot biomass at the late flowering stage was recorded at Babile site than Fedis site, indicating Babile site could have a favorable condition for peanut as compared to the Fedis. The cultivar Bati-Sedi has recorded significantly higher pooled shoot biomass than the cultivar Werer-962.



**Number of seeds per pod**

The *Bradyrhizobium* inoculation and cultivar had significant effect on average number of seeds per pod across locations (Table 1). The lower number of seed per pod was measured in the uninoculated treatment. This indicates the need of N in the form of either inorganic or organic to improve the number of seeds per pod. A significantly higher average number of seeds per pod was recorded with the cultivar Bati-Sedi than the cultivar

Werer-962. However, a number of seed per pod of peanut was not significantly differed which were obtained from two locations.

**Total pods weight per hectare**

The main effect of inoculation, cultivar and locations and their two ways interaction significantly varied the pooled total pods weight per hectare across locations (Table 1). Plants inoculated with HUGNR-10, HUGNR-11,

HUGNR-12 and HUGNR-18 resulted in significantly increased total pod biomass ( $\text{kg ha}^{-1}$ ) as compared uninoculated treatment but did not differ statistically with N-fertilized treatment (Table 2). This implies that these isolate may provide a similar amount of N as it had been applied N in the N fertilized treatment. Inoculating HUGNR-12 increased the total pod's weight by 22.5% over uninoculated treatment. Significantly higher average total pod weight was recorded at Fedis than Babile site. The cultivar werer-962 produced significantly higher average total pods weight than the cultivar Bati-Sedi.

#### 100 seeds weight

ANOVA revealed that the main effect and their two and three interaction effect of three factors significantly influenced the pooled hundred seeds weight across locations (Table 1). However, none of the tested *Bradyrhizobium* isolates did improve significantly ( $P < 0.05$ ) the pooled hundred seeds weight of peanut. Across the inoculation treatments, Babile site recorded significantly higher hundred seeds weight than Fedis site. The cultivar Werer-962 recorded significantly better average hundred seeds weight than the cultivar Bati-Sedi.

#### Total biomass yield

The ANOVA revealed that the main effects of inoculation, cultivar, and location and their two ways interaction effect were significant on the pooled total biomass yield per hectare of peanut across locations (Table 1). Inoculating HUGNR-29 significantly increased pooled total biomass yield by 26.2% compared with uninoculated but this biomass was statistically at par with N-fertilized treatment (Table 3). Both locations produced statistically at par amount of average total biomass yield of peanut. Significantly higher average total biomass yield across inoculation treatments was produced with the cultivar Werer 962 than Bati-Sedi.

#### Grain yield

The main effect of inoculation, cultivar and locations and their two and three ways interaction significantly affected the pooled grain yield of peanut across locations (Table 1). However, the main effect of location and  $L \times C$  did not influence the pooled grain yield, showing that location different did not influence the grain yield production of the tested cultivars of peanut. All *Bradyrhizobium* inoculation including N fertilized treatments

**Table 3 Hundred seed weight, total biomass yield, grain yield, total plant N and P accumulation as affected by *Bradyrhizobium* inoculation, cultivar, and location**

Treatments	100 seed wt	TBY	GY	Shelling (%)	Tot plant P	Tot plant N
Consortium	44.01ab	7927.1ab	1402.69b	41.64ab	1.4728a	3.0489a
HUGNR-10	45.56ab	8146.9ab	1638.56a	45.85ab	1.6368a	3.0733a
HUGNR-11	44.39ab	7864.8ab	1563.75ab	44.85ab	1.4471a	3.0722a
HUGNR-12	45.30ab	8051.7ab	1605.16a	45.10ab	1.4656a	2.9494a
HUGNR-13	42.62b	8217.5ab	1516.45ab	45.33ab	1.4259a	2.9762a
HUGNR-18	42.13a	8070.7ab	1577.31ab	44.86ab	1.4812a	3.0177a
HUGNR-19	46.26a	7228.0ab	1470.41ab	50.59a	1.5505a	2.9732a
HUGNR-29	44.92ab	8560.3a	1609.71a	46.42ab	1.4259a	3.0078a
Uninoculated	43.82ab	6782.5b	1206.95c	40.77b	1.3763a	2.9594a
N fertilized	44.87ab	7391.9ab	1622.07a	47.11ab	1.3935a	3.0506a
F-value	3.04**	2.20*	10.24***	1.70 <sup>ns</sup>	0.31 <sup>ns</sup>	0.28 <sup>ns</sup>
LSD ( $P < 0.05$ )	2.95	1657.9	191.42	9.65	0.6426	0.4103
Babile	50.36a	7729.8a	1517.25a	46.61a	1.4255a	2.9351b
Fedis	39.22b	7858.5a	1525.30a	43.89b	1.5096a	3.0907a
F-value	754.45***	0.32 <sup>ns</sup>	0.09 <sup>ns</sup>	4.23 <sup>†</sup>	0.91 <sup>ns</sup>	7.61**
LSD ( $P < 0.05$ )	0.81	453.36	52.34	2.64	0.1757	0.1122
Werer 962	46.46a	9937.6a	1706.54a	43.69b	1.4006a	3.1961a
Bati-Sedi	43.12b	5650.7b	1336.02b	46.81a	1.5345a	2.8297b
F-value	67.91***	354.12***	198.44***	5.53*	2.30 <sup>ns</sup>	42.24***
LSD ( $P < 0.05$ )	0.81	453.36	52.34	2.64	0.1757	0.1122
CV (%)	4.96	16.00	9.47	16.04	32.96	10.25

Means in the same column followed by the same letter are not significantly different at the 5% probability level by Tukey's test

<sup>ns</sup> non-significant, TBY total biomass yield ( $\text{kg ha}^{-1}$ ); GY grain yield ( $\text{kg ha}^{-1}$ )

\* Significant at 0.05; \*\* significant at 0.01; \*\*\* significant at 0.001

produced significantly higher pooled grain yield than the uninoculated treatment (Table 3). In both experimental locations, all *Bradyrhizobium* inoculation including N fertilization were found to increase the grain yield production (Figs. 1d, 2d). Moreover, all inoculation treatment including N application with both cultivars gave the higher grain yield than uninoculated.

Inoculating HUGNR-10 and HUGNR-29 plants were found to increase the pooled grain yield by 35.8 and 33.45% over the uninoculated treatment, respectively. When compared to the uninoculated, fertilization with N increased the grain yield by 34.5%. The difference in pooled grain yield production between two locations was not statistically significant at  $P > 0.05$ . The cultivar Werer-962 recorded significantly higher pooled grain yield than the cultivar Bati-Sedi.

### Shelling %

Except for the main effect of inoculation, and three-way interaction, the remaining main and interaction effect on pooled shelling % of peanut was significant at  $P < 0.05$  (Table 1). Inoculations and N application increased significantly the shelling percentage of a peanut compared with uninoculated (Table 3). Averaged across the inoculation treatments, significantly higher shelling % was recorded at Babile site than Fedis site. The cultivar Bati-Sedi was found to have significantly higher shelling % than Werer-962.

### Total plant N and P concentration

The ANOVA revealed that none of the main and their interactions was affect significantly ( $P > 0.05$ ) the total plant P concentration (Table 1). However, the main effect of location, cultivar and three ways interaction effect on pooled total plant N concentration was significant ( $P < 0.05$ ). Inoculation had no significant effect on pooled plant N concentration, showing that indigenous and inoculated *Bradyrhizobium* could have been a similar efficacy in  $N_2$  fixation. Averaged total N plant concentration of peanut across the inoculation treatments at Fedis site was found to be significantly higher than that obtained from Babile site. The cultivar werer-962 accumulated significantly higher average plant tissue N than Bati-Sedi.

### Discussion

Use of inoculants in Ethiopia for food legume production including peanut is very infant. Due to low productivity of peanut in Ethiopia, enhancing the peanut-rhizobia symbiosis thereby improve the productivity using inoculation of effective rhizobia is essential. The native rhizobia population, the host genotypes and environmental condition are the major factors affecting

the efficacy of inoculated rhizobia. The soil of the study sites had the native rhizobia capable of nodulating peanut greater than  $10^2 \text{ g}^{-1}$  of soil. Large number of native rhizobia in the two soils could be because of the presence of conducive soil properties (Yousef et al. 1987; Thrall et al. 2007; Drew et al. 2012) and cultivating the host plant for long time (Chemining'wa and Vessey 2006; Kimiti and Odee 2010). However, the previous experiment conducted this region showed that inoculation of locally isolated rhizobia improved the yield of common bean (Anteneh 2016), Fababea (Argaw et al. 2012a) and Field pea (Argaw et al. 2012b), though the soil had  $> 10^3$  rhizobia  $\text{g}^{-1}$  soil. Therefore, this study was initiated to evaluate locally isolated *Bradyrhizobium* effectiveness on the productivity of peanut at major production areas of eastern Ethiopia.

Despite the natural rhizobia population has been large enough in the experimental soils, inoculation of selected isolated of native *Bradyrhizobium* significantly increased the pooled nodule number. However, the result revealed that inoculation did not affect significantly the pooled nodule dry weight and % of effective nodules of peanut. Similarly, effective indigenous *Bradyrhizobium* isolate did not increase nodulation when the native rhizobia were abundant (Okogun and Sanginga 2003; Denton et al. 2009). On top of this, the present study found the non-significant effect of inoculation on pooled shoot dry weight, total plant N accumulation, and total P concentration. This result clearly indicated that the native rhizobia effective in supplying N to host plants as the inoculated isolates did. In line with this result, Kremer and Peterson (1983) found that the  $N_2$  fixation by peanut has been only increased when the inoculation improved the nodule weight.

When the data was stratified by location, at Fedis site, of tested isolates, consortium isolates and HUGNR-29 inoculating plants had significantly higher NDW than the uninoculated plants. At Babile site, except HUGNR-18, all tested isolates including N application increased significantly the NDW and % of the effective nodule. In line with this finding, inoculation of *Rhizobium* on promiscuous legume plants increased nodulation (Valladares et al. 2002). However, few numbers of tested rhizobia found effective at Fedis site could be associated with the presence of competitive and high rhizobia population nodulating peanut in the experimental soil. Similarly, Wielbo et al. (2012) highlighted that soil type and the host plant identity influenced the competitiveness of rhizobia. Competitive ability of rhizobia strains also determined by the metabolic potential in utilizing a diversity carbon and energy sources of tested isolates and the responsiveness of nod genes to flavonoid activation (Wielbo et al. 2007; Maj et al. 2010).

All isolates of *Bradyrhizobium* significantly increased the nodule number of the cultivar Werer-962 while a significant increase of this trait for the cultivar Bati-Sedi was found when inoculated with Consortium isolates, HUGNR-10, HUGNR-12, HUGNR-18 and HUGNR-19. Only HUGNR-29 inoculation increased the significantly nodule dry weight of the cultivar Werer-962. Effective nodules % of the cultivar Werer-962 significantly improved by isolate HUGNR-18 inoculation. The difference in strain survival, growth, and competition in the rhizosphere, the production of appropriate plant and bacterial signals might have been the causes of the difference in compatibility of tested isolated with tested cultivars of peanut (Wollum 1998). This observation was in agreement with the findings of Chen et al. (2003) that there has been peanut cultivars-*Bradyrhizobium* strain specificity in Argentina soil. With cultivar Bati-Sedi, most of the tested *Bradyrhizobium* increased the nodule dry weight and % of effective nodules. With regards to inorganic N application, the significant increased nodule dry weight and % of effective nodules found with Bati-Sedi but did not found with Werer-962. This indicates the variance effect of N application on nodulation of the two tested cultivars of peanut. This finding is confirmed by Abdel Wahab and Abd-Alla (1996) with soybean and showed that Soybean cv. Crawford showed a greater response to the low rate of N than cv. Clark.

The result of the present work has highlighted that inoculating HUGNR-29 was effective in significantly improving the pooled NDW and % of effective nodules. This isolate also improved the pooled total biomass and grain yield of peanut, indicating the need of inoculation for improving the effective nodule and production of peanut in the study sites as has been reported by Huang (1987). On top of this, all tested *Bradyrhizobium* inoculation including inorganic N application significantly increased the grain yield of peanut at Fedis and Babile sites. The positive effect of N application might be associated the presence of low concentration of nitrate in both soils which could act as an additional source of nitrogen until the plant start  $N_2$  fixation (Becana and Sprent 1987). However, the result showed that yield increases with inorganic N application was not statistically at par with HUGNR-29 inoculation, implying that *Rhizobium* inoculation can substitute the external inorganic N application. Elkan (1992) reported that the rhizobia-peanut symbiosis can derive N from the atmosphere as much as  $152 \text{ kg N ha}^{-1}$  which is 80% of the plant of the protoplasmic nitrogen. Moreover, about 55% of the plant N needs of peanut are delivered from the air via nitrogen fixation (Hardarson 1993).

The averaged nodule number and dry weight and total plant N accumulation across the inoculation treatments

at Fedis were found to be significantly higher than those obtained from Babile. This difference could be because of the environmental conditions such as the effectiveness and competitiveness of the native rhizobia, soil condition and weather condition prevailed during the experimental time at both locations. Räsänen and Lindström (1999) found the different ability of isolate HAMB12180 nodulating *Acacia Senegal* due to different soil temperature. The result the current work found the non-significant difference in averaged % of effective nodules, total biomass and grain yield across the inoculation treatments which were recorded at Fedis and Babile. This indicates that indigenous rhizobia in Fedis and Babilale sites may have been similar effectiveness in  $N_2$  fixation and supplying N to the plant.

It was also found the non-significant difference in nodule number and dry weight and % of effective nodules recorded between the cultivar Werer-962 and Bati-Sedi. This finding is in line with the study on peanut cultivars (van Rossum et al. 1994) that had similar nodulation in neutral soil pH condition. It has been reported previously by Alwi et al. (1989) who tested 29 *Bradyrhizobium* against 16 cultivar of peanut and found that some of the isolates were effective with varieties and other showed a degree of specificity with cultivar. Chen et al. (2003) demonstrated that *Bradyrhizobium* displayed a varying degree of effectiveness, with some isolates being fairly effective with all tested cultivars and others with selected ones.

Despite the variation in nodulation, significantly higher averaged total pods weight, 100 seeds weight, total biomass and grain yield and shelling % across the inoculation treatments were found with Werer-962 than Bati-Sedi. These differences could have been due to inherent productivity potential difference between the two tested cultivars of peanut. Barbour et al. (1992) found that nodulation and  $N_2$  fixation by peanut varieties are found to be heritable and controlled by genetic make of the host plant. In the current work, the cultivar Werer-962 accumulated significantly higher plant N concentration than the cultivar Bati-Sedi. This N concentration difference between the cultivars could have been because of the fact that biomass production of the cultivar werer-962 was greater than the cultivar Bati-Sedi which cause variation in  $N_2$  fixation. Peoples et al. (2001) highlighted that 20–25 kg of shoot N has been fixed for every tone of legume shoot dry weight accumulated. Ankomah et al. (1996) found out that the good correlation between shoot N concentration and  $N_2$  fixation. In addition, the variation of N accumulation might also be related with inherent property of the tested peanut cultivars as has been reported by Nambiar and Dart (1980).

## Conclusions

It is evident from the results of this study that inoculation of *Bradyrhizobium* is necessary in order to improve nodulation and seed yields of peanut. This result also revealed that the need of location and cultivar specific selection of *Bradyrhizobium* for better production of peanut in the study locations. It has been also found that N applied at starter rate requires to improve the yield of peanut. Further work is needed to determine whether combined application of N with *Bradyrhizobium* or/and their sole application can improve the peanut productivity in the study site. Moreover, multi-locational trials are required to check their effectiveness for the other agro-climatic conditions to give concrete conclusive to use the isolate as a candidate for national biofertilizer production.

## Acknowledgements

This work was funded by the Ethiopian Institute Agricultural Research, Peanut Improvement Project. A special thanks to the field assistant for invaluable help with data collection and processing in both the field and the laboratory.

## Competing interests

The author declare no competing interests.

## Availability of data and materials

Not applicable.

## Consent for publication

Not applicable.

## Ethics approval and consent to participate

Not applicable.

## Publisher's Note

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Received: 11 February 2017 Accepted: 19 September 2017

Published online: 29 September 2017

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