International Journal of Plant & Soil Science

32(15): 65-75, 2020; Article no.IJPSS.62564
ISSN: 2320-7035

Symbiotic and Agronomic Performance of Cowpea Rhizobial Inoculants from Soil of Ethiopia under Field Conditions

Girmaye Kenasa[1*], B. C. Nandeshwar[2] and Fassil Assefa[3]

[1]*Department of Biology, Wollega University, Nekemte Campus, P.O.X-395, Ethiopia.*
[2]*Department of Plant Sciences, Wollega University, Shambu Campus, P.O.X-38, Ethiopia.*
[3]*Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, P.O.X-1176, Ethiopia.*

Authors' contributions

This work was carried out in collaboration among all authors. Author GK designed the study, performed the field experiment, generated data and wrote the whole manuscript. Author BCN performed the statistical analysis and edited the final manuscript. Author FA read and approved the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2020/v32i1530374
Editor(s):
(1) Dr. Olowoake Adebayo Abayomi, Kwara State University, Nigeria.
Reviewers:
(1) Karen Andreon Viçosi, Universidade Federal do Espírito Santo, Brazil.
(2) Shahab Ahmad Khosa, Pir Mehr Ali Shah Arid Agriculture University (PMAS-Arid University), Pakistan.
Complete Peer review History: http://www.sdiarticle4.com/review-history/62564

Original Research Article

Received 25 August 2020
Accepted 31 October 2020
Published 25 November 2020

ABSTRACT

The effect of inoculating indigenous cowpea nodule bacteria of Ethiopia on the crop performance was not tested under field condition with the ultimate goal of selecting effective inoculants. Root nodule bacteria which showed strong symbiotic effectiveness under greenhouse condition were selected for field experiment. Under field conditions, inoculation of cowpea with ECR-0+ECE-21 and ECR-101+ECE-21 resulted in a maximum grain yield (GY, 2713 kg ha^{-1}) and above ground biomass (BW, 506 g m^{-2}), respectively. The co-inoculation of cowpea with ECR-0 and ECE-21 and ECR-24 and ECE-21 significantly improved ($p<0.05$) the N-content and GY as compared to their single rhizobial inoculation. The native strains performed better than the exotic strain (Biofix). Performance of the strains was similar at the two test locations except ECR-14 and ECR-24 that resulted 13% greater of BW at Uke than at Bako. Symbio-agronomic performance of native rhizobia under field condition showed direct correlation ($r>0.5$; $p<0.01$) to each other except nodule number.

**Corresponding author: E-mail: girmayek@gmail.com;*

In general, both single and co-inoculation of the native rhizobacterial inoculants improved cowpea performance. This is a promising biofertilizer to enhance cowpea production in Ethiopia, where the farmers cannot afford to buy chemical fertilizers.

Keywords: Co-inoculation; inoculation; nitrogen fixation; relative effectiveness.

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a leguminous plant, cultivated for food, forage, and reclamation of soil fertility in different parts of the world. The crop performs well under stressed edapho-climatic condition of the sub-Saharan African regions that are characterized by acidity, poor nutrients content, low moisture content and high temperature [1]. Emphases have been given for the application of inorganic chemical fertilizers to improve productivity in these areas. In spite of its undesirable environmental ramifications, the ever-increasing cost of chemical fertilizers makes it unaffordable to the poor farmers of the sub-Saharan Africa, particularly Ethiopia.

Cowpea production is common in tropical agriculture since it fixes atmospheric dinitrogen in association with soil inhabiting rhizobia. Cowpea rhizobia are naturally promiscuous, nodulating different leguminous herb plant [2] although their degree of symbiotic efficiency varies among cross-inoculation groups [3]. The ability of cowpea rhizobia to form nodules with a wide range of legume hosts may contribute to their persistence in different agroecologies. However, host specificity is an important parameter for symbiotic efficiency, where defined species of rhizobia forms nodules on specific legumes. As a result, cowpea rhizobia are generally presumed to be poor nitrogen fixer [4] and inoculation is rarely performed or neglected under field conditions.

However, studies showed that cowpea fulfils most of its nitrogen requirements from biological nitrogen fixation [5,6]. [7] estimated that cowpea fixes up to 125 kg N $ha^{-1}yr^{-1}$ that is greater than the general amount of urea required for cereal crops production in soil of Ethiopia [8]. Several researchers also reported that inoculation of cowpea with rhizobia gave a promising response in different tropical regions [9,10,11]. However, it is established that nitrogen fixation efficiency in cowpea depends on potential of the rhizobial isolates [11,12], the type of cowpea genotypes [13,14] and edapho-climatic condition of the growing areas [15,16].

Apart from the selection of effective rhizobial endosymbiont having high capacity of symbiotic association and competitive in the rhizosphere [17] inoculation of legumes by inorganic phosphate solubilizing bacteria have been reported to improve growth and yield in legumes [18]. Rhizobacteria can contribute to plant growth by fixing atmospheric nitrogen, enhancing phosphorous availability, phytohormone production and inhibiting growth of phytopathogens [19,20]. As a result, the co-inoculation of cowpea rhizobia with phosphate solubilizing bacteria improves productivity and yield of the crop [21,22].

In Ethiopia, cost of chemical fertilizer is increasing from time to time, at which farmers cannot afford to purchase to meet the crop nutrient requirement. The cheaper and environment friendly alternative is the use rhizobacterial inoculants which has high capacity of nitrogen fixation and other plant growth promoting (PGP) properties [23,24]. However, so far no work done on rhizobacterial inoculants of cowpea in Ethiopia. Thus, evaluation of the symbiotic diversity and efficiency of native cowpea rhizobia is an important step toward developing efficient inoculants to improve the crop productivity.

2. MATERIALS AND METHODS

2.1 Sources of the Rhizobial Inoculants

Cowpea rhizobial isolates were previously collected from different parts of Ethiopia and stored with 30% glycerol (HIMEDIA, India) at -20ºC in Addis Ababa University, Applied Microbiology Laboratory for further studies. Totally, 5 cowpea rhizobial isolates were included in this study and the rhizobial isolates were previously characterized for phenotypic and genotypic properties [25]. The exotic strain (Biofix) was obtained from Holeta Agricultural Research Institute, Ethiopia.

2.2 Description of the Test Location

The field experiment was conducted at Bako and Uke agricultural research sites during the rainy season (June-September) of 2019. According to the Ethiopian agro-ecological zonation, the areas are mid-highlands characterized by unimodal rainfall pattern. Soil physico-chemical properties were analyzed according to the methods described in Table 1 at Nekemte Soil Research and Laboratory, Ethiopia.

Edapho-climatic properties of the experimental areas were almost similar except the total nitrogen that was higher in Bako soil than in Uke soil (Table 1).

2.3 Abundance of Native Rhizobia in Soils of the Test Sites

Rhizobial abundance in the test locations was determined by most probable number (MPN) using plant infection method as indicated in [26] from homogenized soil samples that were collected at the beginning of rainy season in the experimental year. The data showed that the soil samples from the two field sites harbored low number of cowpea rhizobia (Table 1) which is not sufficient for optimum cowpea production according to the study of [27] although the experimental sites are known for the production of "cowpea miscellany" crops such as peanut, cowpea, common bean, and soybean [28].

2.4 Preparation of Inoculants

Inoculants were prepared by injecting 65 ml of the actively grown broth cultures (10^9 ml^{-1}) of the rhizobial strains into 125 g of sterile peat in plastic bags. Similarly, the co-inoculants were prepared by injecting equal proportion of the rhizobial strains and the endophytic strain (65 ml of co-inoculants into 125 g of peat) into the carrier material. The bags were sealed, mixed thoroughly, and incubated at 28°C for two weeks. Viability and abundance of the inoculants were checked by MPN before field inoculation using plant infection method.

Table 1. Description of the test locations and soil physico-chemical properties of the soils

No	Parameters	Method	Bako	Uke
1	Altitude (m a.s.l)		1650	1404
2	Longitude		37º09 ºE	36º28ºE
3	Latitude		09º06ºN	09º20ºN
4	Temperature (ºC)	[29]	13-28	15-27
5	Rain fall (mm)	[29]	80-260	70-250
6	Relative humidity (%)	[29]	40-53	35-55
7	Soil order		Nitosol	Nitosol
8	Textural class		Clay	Clay
9	Organic carbon (%)	Walkley and Black [30]	2.3	3.15
10	Total Nitrogen (%)	Kjeldahl [31]	0.214	0.102
11	C/N ratio		10.74	30.8
12	Available P (ppm)	Olsen et al. [32]	0.51	0.64
13	CEC ($cmol^{(+)}$/kg soil)	NH_4Ac at pH 7	32	30
14	pH (1:2.5 H_2O)	Soil:H_2O ratio	4.98	5.03
15	Rhizobial abundance	MPN (#/ g^{-1} soil)	204	312

Table 2. Physiological and PGP properties of cowpea rhizobial strains selected for field experiment

Isolates	Species	MGT (hr)	% ST	%HC	PGP properties		
					RE (sand culture)	IAA ($\mu g ml^{-1}$)	Mobilized-P ($\mu g ml^{-1}$)
ECR-0	*Bradyrhizobium* sp.	7.1±0.2	44	69	144	-	-
ECR-14	*Bradyrhizobium japonicum*	6.8±0.3	64	55	129	-	2.7±1.0
ECR-24	*Rhizobium rubi*	27.7±0.8	40	41	106	33.2±1.5	2.2±0.9
ECR-101	*Bradyrhizobium* sp.	9.5±0.5	64	62	105	-	-
Biofix	*Bradyrhizobium* sp.	6.4±0.6	68	62	109	64.6±1.7	-
ECE-21	*Pseudomonas putida*	3.2±0.7	-	-	-	71.1±1.2	87.74±7.43

MGT, mean generation time; %ST, percentage stress tolerance; %HC, percentage heterotrophic competence; RE, relative effectiveness

2.5 Experimental Design of the Field Experiment

The experiment was set as RCBD in triplicate. The treatments included inoculation of the five-rhizobial strains individually and co-inoculation of each of the rhizobial strains with the endophytic strains (ECE-21). The controls were Biofix, nitrogen fertilizer plots (Positive controls) and untreated plots (negative controls). The positive controls were treated with 100 kg ha^{-1} urea where 25% of the fertilizer was added during planting and the left was added after 21 days of planting. All plots have received 50 kg ha^{-1} P as single superphosphate at the beginning of planting. Area of the experimental units was 3 m by 3 m with 1 m space between them. The space between plants, rows and blocks was 0.1 m, 0.4 m, and 1.5 m, respectively. During planting, 1 g of peat-based inoculants was mixed with 100 g of seed that was rinsed with 15% sucrose [26].

2.6 Data Collection from the Field Experiment

Characteristics such as Nodule number (NN), Nodule dry weight (NDW), Shoot dry weight (ShDW), nodulation index (ratio of NDW to ShDW, NI), and BW, shoot total nitrogen content (ShNC), shoot nitrogen yield, and shoot protein content (ShPC) were recorded at 50% of the crop flowering. Number of pods per plant (NP), number of seeds per pods (NS), and number of branches per plants (NB), grain yield (GY) and 100 seed weight (HuSW) were recorded at harvest. The BW and GY were measured during 13% moisture content estimated using grain moisture meter [33].

The plant ShNC of was determined by modified Kjeldahl technique as written in the manual of [31] and ShNY was obtained by multiplying ShNC by BW [34]. The ShPC was calculated by multiplying percentages of ShNC with standard conversion factor 6.25 [35].

2.7 Data Analysis

One-way ANOVA was used to compare mean symbio-agronomic performance of the inoculated crop using SAS Ver-8 at 5% of probability. In addition, Pearson correlation was tested among the symbio-agronomic characters.

3. RESULTS AND DISCUSSION

3.1 Effect of Inoculants on the Crop Performances under Field Conditions

Inoculation significantly ($p<0.01$) improved the symbio-agronomic performance of the crop except NB (Table 3). The NN per plant was in the range of 17 and 31 (Average 24.6) which was similar to the range of 10-35 NN per plant of cowpea reported from Brazil [10,33]. Inoculation has brought significant effect on the NN as spontaneous nodulation by the naturalized rhizobia was about nine times less than the maximum NN per plant formed by the inoculation of ECR-24 (Table 3). However, there was no significant difference ($p>0.05$) in NN between single inoculation and co-inoculation (Table 3).

Inoculation showed significant effect ($p<0.01$) on NDW which ranged in 367-517 mg per plant and the maximum NDW was formed by ECR-101 (Table 3). However, the data did not show significant variations ($p>0.05$) in NDW between the single inoculation and co-inoculation except a 21% increase by the co-inoculated plants with ECR-24+ECE-21. Cowpea inoculated with the native rhizobial inoculants showed significantly higher NDW than the control plants (uninoculated cowpea) and cowpea inoculated with Biofix (Table 3). In addition, NDW of cowpea inoculated with native rhizobial strains was higher than the maximum NDW (<200 mg $plant^{-1}$) reported from different countries [33,36]. This means, the symbiotic association between native rhizobial inoculants and Bole-cowpea variety was effective since NDW is one of the indicators of symbiotic effectiveness [37].

Inoculation of native cowpea rhizobial strains also showed variation in BW (394 - 506 g m^{-2}) without showing significant difference ($p>0.05$) between the single inoculated and co-inoculated plants (Table 3). The plot fertilized with urea and plot inoculated with ECR-101+ECE-21 produced 472 g m^{-2} and 506.9 g m^{-2} of BW, respectively (Table 3). Although cowpea plants treated with ECR-101+ECE-21 produced slightly higher BW than previous reports of the crop in Ethiopia (BM of uninoculated cowpea 470 g m^{-2}) [14,36] but it was much lower than the minimum BW (810 g m^{-2}) obtained from inoculation of native cowpea rhizobia of Ghana [11]. This big variation in BW may be emanated from difference in local cowpea verities used in the experiments. BW is a measure of animal feed potential in forage

Table 3. Mean performance of the inoculants for selected symbio-agronomic characters of cowpea under field condition

Isolates	NN (Plant^{-1})	NDW (mgplant^{-1})	NI (mg g^{-1})	BW (g m^{-2})	ShNC (%)	ShNY (g m^{-2})	ShPC (%)	NP	NS	GY (Kg ha^{-1})
ECR-0	17.0^{c}	487.3^{ab}	15.7^{abc}	464.2^{ab}	2.6^{b}	12.5^{b}	17.0^{bc}	263.8^{ab}	12.2^{ab}	2336.6^{bcd}
ECR-14	28.3^{ab}	438.3^{b}	17.4^{ab}	433.8^{bc}	2.3^{bc}	10.2^{bcd}	14.5^{bcd}	211.0^{bcd}	11.3^{abc}	2065.8^{de}
ECR-24	31.0^{a}	367.5^{c}	17.8^{a}	396.2^{c}	2.0^{cd}	8.1^{de}	12.7^{cd}	184.6^{cd}	10.2^{b-e}	1857.9^{efg}
ECR-101	25.3^{abc}	517.5^{a}	13.3^{c}	499.3^{ab}	3.2^{a}	15.7^{a}	19.7^{a}	307.2^{a}	12.5^{ab}	2693.7^{ab}
Biofix	24.3^{abc}	237.8^{d}	13.5^{bc}	337.7^{d}	1.7^{d}	5.9^{e}	11^{cd}	152.0^{de}	$8.5d^{e}$	1613.2^{g}
ECR-0 + ECE-21	26.6^{abc}	515.6^{a}	13.1^{c}	489.9^{ab}	3.2^{a}	15.7^{a}	20.0^{a}	315.3^{a}	13.2^{a}	2713.4^{a}
ECR-14 + ECE-21	26.6^{abc}	462.0^{ab}	15.8^{abc}	448.0^{abc}	2.6^{b}	11.5^{bc}	16^{bc}	258.0^{ab}	12.0^{ab}	2242.2^{cde}
ECR-24 + ECE-21	22.2^{abc}	463.0^{ab}	16.3^{abc}	446.5^{abc}	2.5^{b}	11.1^{bc}	18.7^{bc}	229.3^{bc}	12.0^{ab}	2131.9^{de}
ECR-101 + ECE-21	20.0^{bc}	516.2^{a}	12.8^{c}	506.9^{a}	3.2^{a}	16.5^{a}	20.0^{a}	294.6^{a}	10.7^{bcd}	2546.1^{abc}
Biofix + ECE-21	24.5^{abc}	253.8^{d}	12.9^{c}	311.4^{d}	1.8^{d}	5.8^{e}	11.7^{cd}	163.5^{cde}	9.2^{cde}	1643.5^{fg}
N+	4.5^{d}	69.8^{e}	1.4^{d}	472.9^{ab}	2.1^{cd}	9.8^{cd}	13.0^{bcd}	218.3^{bcd}	10.3^{b-e}	1990.2^{def}
N-	3.3^{d}	56.2^{e}	3.8^{d}	167.2^{e}	1.2^{e}	2.0^{f}	7.5^{e}	107.2^{e}	8.2^{e}	1169.7^{h}

NN, nodule number; NDW, nodule dry weight; NI, nodulation index; BW, above ground biomass yield; ShNC, shoot total nitrogen content; ShNY, shoot nitrogen yield; ShPC, shoot protein content; NP, number of pods per plant; NS, number of seeds per pod; GY, grain yield. Values within a column sharing the same letter are statistically non-significant at α=0.05 legumes. The significant improvement in BW by inoculation of native rhizobia (3-fold increase compared to the negative control plants and higher than the positive control) could be due to the large proportion of nitrogen source from biological fixation for the shoot dry matter gain in cowpea [6]. Previously, study also showed that 81% of the nitrogen used for growth in cowpea is obtained from biological nitrogen fixation [5].

Inoculation of cowpea with native root nodule bacterial strains resulted in significantly higher ($P<0.05$) ShNC and ShNY compared to inoculation with the commercial strain (Biofix) and the controls (Table 3). The highest ShNC of cowpea was 3.2% by inoculation of ECR-101, ECR-0+ECE-21 and ECR-101+ECE-21 that were significantly higher ($P<0.05$) than the crop treated with other strains and the controls (Table 3). Therefore, inoculation of cowpea with native rhizobial strains significantly improved nitrogen component of the plant.

The inoculation tests under field condition also revealed the increase in grain yield of cowpea by the application of the root nodule bacterial strains ranging from 1857.9 kg ha^{-1} from single inoculation with ECR-24 up to 2713 kg ha^{-1} by co-inoculation with ECR-0 +ECE-21; showing 32% increase between the treatments. The grain yield of cowpea co-inoculated with ECR-0 and ECE-21 resulted in 70% increase as compared to the positive control (treated with 100 kg ha^{-1} of urea) and 131% increase against negative control plots (Table 3). This indicates the poor performance of the resident rhizobia in soil of the field and inoculation of cowpea with elite native rhizobia could significantly enhance cowpea production.

The data also showed that the elite native cowpea root nodule bacterial strains from soil of Ethiopia improved grain yield (1857.9 to 2713.4 kg ha^{-1}) which was 2.7 to 4 times greater than the best performing inoculants in Brazil (grain yield of 693 kg ha^{-1}, equivalent to grain yield of cowpea that received 50 kg ha^{-1} of N fertilizer) [10]. They also performed better compared to the maximum grain yield of cowpea (1200 kg ha^{-1}, equivalent to grain yield of the crop that received 74 kg ha^{-1} of N as urea) reported by [38]. [39] reported a maximum of 1782 kg ha^{-1} (comparable to cowpea grain yield of the present negative control) by the application of 100 kg ha^{-1} of N P K fertilizer and using local cowpea varieties. Therefore, inoculation of elite cowpea root nodule bacteria native to soil of Ethiopia are as effective as or better than the application of chemical fertilize in increasing grain yield in cowpea.

3.2 Comparison of Single Vs Co-inoculation on the Crop Performances

The effect of co-inoculation of cowpea by rhizobial strains and the endophytic strain (ECE-21) did not show significant difference ($p>0.05$) on the plant performances compared to single inoculation except ShNC, ShNY, ShPC and GY. Co-inoculation of cowpea with ECR-0 and ECE-21, and ECR-24 and ECE-21 increased the plant ShNC, ShNY, ShPC and GY in the range of 14-27% as compared to the single rhizobial inoculation (Table 3). Previous study also showed up to 63% increment of ShNC and GY in Pigeon pea by co-inoculation of *Pseudomonas putida* and *Rhizobium* [40].

The effect of co-inoculation was insignificant ($p>0.05$) on 80% of the symbio-agronomic characters of the crop measured during this experiment. Therefore, endophytic bacteria might have specific PGP properties that have specific action on plant growth and nitrogen fixation. For instance, co-inoculation of Mung bean with *Rhizobium* and ACC deaminase producing rhizbacteria did not improve the NP, NN, and NDW of the crop [41].

3.3 Comparison of Native Vs Exotic Inoculants on the Crop Performances

The general trends in symbio-agronomic performances of cowpea inoculated with the native inoculants both as single and co-inoculation were higher than the exotic strain. Particularly, the native rhizobacterial strains formed significantly higher ($p<0.05$) NDW, BW, ShNC, NS and GY than the exotic strain both as single and co-inoculation form. In addition, ECR-101, ECR-0 +ECE-21 and ECR-101+ECE-21 performed significantly higher in ShPC and NP than the exotic strain (Table 3).

The poor symbio-agronomic performance of the exotic strain can be accounted to either the poor adaptation of the strain to the prevailing soil condition to compete with the native soil rhizobia or its general poor nitrogen fixation potential in symbiosis with the cowpea variety. As a result, bioinoculant selections are often recommended from native rhizobacterial populations that are competitive and symbiotically effective [42].

3.4 Effect of Location on the Inoculants Performances

Location showed significant effect ($p<0.05$) on NDW, ShDW and NI where the latter two were higher at Bako than at Uke. However, NN, RE, ShNC, ShPC, ShNY and NS did not show marked differences between locations (Table 4). The higher NDW at Uke than at Bako might be associated to the comparatively lower soil nitrogen content, higher organic carbon and higher soil pH at Uke compared to at Bako (Table 1). Previous studies showed the severe negative impact of excessive soil nitrogen content [43] and soil acidity [44,45] on competitiveness and persistence of rhizobacteria in soil.

Besides, the agronomic characters such as BW, NP and NB were significantly higher at Uke than at Bako that was in contrary to GY that was lower at Uke than at Bako (Table 4). The low GY at Uke could be due to the slow initiation of fungal disease at the late stage of the experiment (data not shown). The mean performance of the inoculants on NDW, ShDW, NI, BW, NP, NB, HuSW and GY of the crop showed significant difference between the two locations (Table 4). However, performance of the individual inoculants either singly or dually has insignificant effect on the symbio-agronomic characters of the crop over location except on NDW and BW (Table 5).

Table 4. Mean performance of the inoculants for attributes of symbio-agronomic performance of the crop under field locations

Characters	Mean		
	Bako	**Uke**	**Mean**
Symbiotic			
Nodule abundance (NN, # plant^{-1})	25.83^{a}	23.37^{a}	24.6
Nodule Dry Weight (NDW, g plant^{-1})	419.8^{b}	432.03^{a}	425.9
Shoot Dry Weight (ShDW, g plant^{-1})	31.82^{a}	27.14^{b}	29.48
Nodulation Index (NI, mg g^{-1})	16.25^{a}	13.54^{b}	14.89
Relative Effectiveness (RE, %)	63.36^{a}	63.04^{a}	63.2
Shoot Nitrogen Content (ShNC, %)	2.57^{a}	2.46^{a}	2.53
Shoot Protein Content (ShPC, %)	16.09^{a}	15.61^{a}	15.85
Shoot Nitrogen Yield (ShNY, g m^{-2})	10.82^{a}	10.95^{a}	10.86
Agronomic			
Biomass Weight (BW, g m^{-2})	421.3^{b}	445.5^{a}	433.4
Number of Pod on Plant (NP, # 10 plant^{-1})	221.2^{b}	254.7^{a}	237.95
Number of Seeds in Pod (NS, # pod^{-1})	11.2^{a}	11.13^{a}	11.16
Number of Branches (NB, # plant^{-1})	6.4^{b}	8^{a}	7.2
Hundred Seed Weight (HuSW, g)	17.34^{a}	15.33^{b}	16.33
Grain Yield per Hectare (GY, kg ha^{-1})	2272.93^{a}	2095.95^{b}	2184.44

Values within a row sharing the same letter are statistically non-significant at α=0.05

Table 5. Performance of the strains for attributes of the symbio-agronomic properties of the crop at the two field locations

Isolates	SDW (g plant^{-1})		NDW (mg plant^{-1})		BW (g m^{-2})		GY (kg ha^{-1})	
	Bako	**Uke**	**Bako**	**Uke**	**Bako**	**Uke**	**Bako**	**Uke**
ECR-0	37.0^{ab}	26.3^{bcd}	506^{a}	469^{ab}	444^{b-e}	484^{abc}	2527^{a-c}	2146^{cd}
ECR-14	27.0^{bcd}	24.6^{bcd}	410^{ab}	460^{ab}	401^{ef}	466^{a-d}	2028^{cd}	2103^{cd}
ECR-24	21.4^{d}	20.0^{d}	324^{c}	411^{b}	370^{f}	422^{de}	1846^{d}	1869^{d}
ECR-101	44.0^{a}	35.2^{abc}	521^{a}	514^{a}	483^{abc}	515^{a}	2828^{ab}	2559^{abc}
ECR-0+ECE-21	43.5^{ab}	36.8^{ab}	515^{a}	516^{a}	467^{a-d}	513^{a}	2879^{a}	2547^{abc}
ECR-14+ECE-21	30.1^{bcd}	28.7^{bcd}	453^{ab}	470^{ab}	413^{def}	483^{abc}	2303^{bcd}	2181^{cd}
ECR-24+ECE-21	34.2^{abc}	24.1^{cd}	474^{ab}	452^{ab}	429^{cde}	464^{a-d}	2274^{bcd}	1989^{cd}
ECR-101+ECE-21	45^{a}	36.6^{ab}	517^{a}	513^{a}	496^{ab}	518^{a}	2532^{abc}	2559^{abc}

Values within a row (for the same parameter) sharing the same letter are statistically insignificant at α=0.05. Aberrations are similar to table 3

Table 6. Correlations (r) among symbio-agronomic characteristics of cowpea inoculated with rhizobacterial strains

	BW	ShDW	ShNC	GY	RE
NN	-0.327*	-0.057	-0.166	0.000	-0.152
NDW	0.825**	0.825**	0.908**	0.844**	0.903**
BW		0.576**	0.763**	0.619**	0.774**
ShDW			0.923**	0.910**	0.954**
ShNC				0.928**	0.974**
GY					0.905**

*** Correlation is significant at the 0.01 level (2-tailed)*
** Correlation is significant at the 0.05 level (2-tailed)*
Aberrations are similar to Table 3

As shown in Table 6, the NDW of cowpea inoculated with ECR-24 was significantly higher at Uke (411 mg plant^{-1}) than at Bako (324 mg plant^{-1}). Similarly, the BW of cowpea inoculated with ECR-14, 24 and ECR-14+ECE-21 was higher at Uke that at Bako. The similar performance of most of the inoculants at the two locations (for majority of the parameters measured) may be attributed to the low abundance of cowpea rhizobia in the test locations (Table 1) and similar phosphorous pretreatment, which shows reproducibility of the results over locations. Previous studies also showed the importance of external phosphorous application for effective nitrogen fixation in cowpea irrespective of the total phosphorous content of the soil [46,47].

3.5 Correlation among Symbio-agronomic Performance of the Inoculants

The symbiotic and agronomic characteristics of cowpea inoculated with the nodule bacterial strains showed relationships. Except NN, the symbio-agronomic characteristics of the inoculated cowpea were directly correlated to each other (Table 6). NDW of the inoculated crop was strongly correlated ($r \geq 0.825$; $p<0.01$) to BW, ShDW, ShNC, GY and RE.

According to Fening and Danso [27] also reported the direct correlation ($r>0.5$) among NN, NDW, ShDW, ShNC and RE of cowpea. However, in the present experiment NN was inversely correlated ($r=-0.25$; $p<0.05$) to NDW. This could be due to nodule size which has major impact on NDW than the mere NN as reported by [32]. The strong positive correlation between BW and GY of cowpea inoculated with the rhizobacterial strains shows the importance of inoculants for food and forage production of cowpea in Ethiopia.

4. CONCLUSIONS

Cowpea rhizobia native to soil of Ethiopia vary in their symbiotic performances. Their performance particularly varied based on host varieties and edapho-climatic condition of the test locations. Co-inoculation Cowpea with rhizobia and PGP endophytic bacteria significantly improve performance of the crop compared to individual rhizobial inoculation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bado BV, Lompo F, Bationo A, Segda Z, Sédogo P, Cescas M. Contributions of cowpea and fallow to soil fertility improvement in the Guinea Savannah of West Africa, in innovations as key to the green revolution in Africa, A. Bationo, B. Waswa, J. Okeyo, F. Maina and J. Kihara, Eds. Dordrecht, Springer. 2011;859-866.
2. Hadad MA, Loynachan TE. Abundance and characterization of cowpea miscellany *Rhizobium* from Sudanese soil. Soil Biol. Biochem. 1985;17:717-721.
3. Thies JB, Bohlool B, Singleton PW. Subgroups of the cowpea miscellany: Symbiotic specificity within *Bradyrhizobium spp.* for *Vigna unguiculata, Phaseolus lunatus, Arachis hypogaea* and *Macroptilium atropurpureumt*. Appl. Environ. Microbiol. 1991;57:1540-1545.
4. Yoseph T, Baraso B, Ayalew T. Influence of Bradyrhizobia inoculation on growth, nodulation and yield performance of

cowpea varieties. African Journal of Agricultural Research. 2017;12(22):1906-1913.

5. Senaratne R, Liyanage ND, Sporer LR. Nitrogen fixation and N transfer in cowpea, mung bean and groundnut intercropped with maize. Fertil. Res. 1995;40:41-48.
6. Naab JB, Chimphango SM, Dakora FD. N_2 fixation in cowpea plants grown in farmers' fields in the Upper West Region of Ghana, measured using ^{15}N natural abundance. Symbiosis. 200;48:37-46.
7. Wani SP, Rupela DP, Lee KK. Sustainable agriculture in the semi-arid tropics through biological nitrogen fixation in grain legumes. Plant and Soil. 1995;174:29-49.
8. Crop and Food Supply Assessment Mission to Ethiopia, FAO Special Report on Global Information and Early Warning System on Food and Agriculture World Food Programme; 2007.

   Available:http://www.fao.org/3/j9325e/j9325e00.htm
9. Kyei-Boahen S, Savala CN, Chikoye D, Abaidoo R. Growth and yield responses of cowpea to inoculation and phosphorus fertilization in different environments. Front. Plant Sci. 2017;8:646.

   DOI: 10.3389/fpls.2017.00646
10. Martins LM, Xavier GR, Rangel FW, Ribeiro JR, Neves MC, Morgado LB, Rumjanek NG. Contribution of biological nitrogen fixation to cowpea: A strategy for improving grain yield in the semi-arid region of Brazil. Biol. Fertil. Soils. 2003;38:333-339.
11. Ampomah OY, Ofori-Ayeh E, Solheim B, Svenning MM. Host range, symbiotic effectiveness and nodulation competitiveness of some native cowpea *Bradyrhizobial* isolates from the Transitional Savanna Zone of Ghana. Afr. J. Biotechnol. 2008;7:988-996.
12. Guimarães AA, Jaramillo PM, Nóbrega RS, Florentino LA, Silva KB, Moreira FM. Genetic and symbiotic diversity of nitrogen-fixing bacteria isolated from agricultural soils in the Western Amazon by using cowpea as the trap plant. Appl. and Environ. Microbiol. 2012;78:6726-6733.
13. Belane AK, Dakora FD. Measurement of N_2 fixation in 30 cowpea (*Vigna unguiculata)* genotypes under field conditions in Ghana using ^{15}N natural abundance technique. Symbiosis. 2009;48: 47-57.
14. Bilatu A, Binyam K, Solomon Z, Eskinder A, Ferede A. Animal feed potential and adaptability of some cowpea (*Vigna unguiculata*) varieties in North West lowlands of Ethiopia. Wudpecker J. Agric. Res. 2012;1:478-483.
15. Doleib NH, Elsheikh EA. Modeling the effect of salt-stress on growth, nodulation and nitrogen fixation of cowpea (*Vigna unguiculata* L.) U. of K. J. Agric. Sci. 2006;14(3):354-369.
16. El-Shinnawi MM, El-Saify NA, Waly TM. Influence of the ionic form of mineral salts on growth of faba bean and *Rhizobium leguminosarum*. W. J. Microbiol. Biotechnol. 189;5:247-254.
17. Florentino LA, de Sousa PM, Silva JS, Silva KB, Maria F, Moreira S. Diversity and efficiency of *Bradyrhizobium* strains isolated from soil samples collected from around *Sesbania virgata* roots using cowpea as trap species. Res. Bras. Ci. Solo. 2010;34:1113-1123.
18. Dey R, Pal KK, Bhatt DM, Chauhan SM. Growth promotion and yield enhancement of peanut (*Arachis hypogaea*) by application of plant growth-promoting rhizobacteria. Microbiol. Res. 2004;159: 371-394.
19. Gupta A, Gopal M, Tilak KV. Mechanism of plant growth promotion by rhizobacteria. Indian J. Exp. Biol. 2000;38:856-862.
20. Ahmad M, Mulugeta K. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J. King Saud University Sci. 2014;26:1-20.
21. Qureshi MA, Shakir M, Iqbal AA, Akhtar N, Khan A. Co-inoculation of phosphate solubilizing bacteria and rhizobia for improving growth and yield of Mungbean (*Vigna radiate*). J. Anim. Plant Sci. 2011;21:491-497.
22. Costa FC, Melo IS. Endophytic and rhizospheric bacteria from *Opuntiaficus indica* mill and their ability to promote plant growth in cowpea (*Vigna unguiculata*). Afr. J. Microbiol. Res. 2012;6:1345-1353.
23. Kremer RJ, Peterson HL. Nodulation efficiency of legume inoculation as determined by intrinsic antibiotic resistance. Appl. Environ. Microbiol. 1982;43:636-642.

24. Zilli JE, Valisheski RR, Filho FF, Neves MP, Rumjanek NG. Assessment of cowpea *rhizobium* diversity in Cerrado areas of Northeast Brazil. Bras. J. Microbiol. 2004;35:281-287.
25. Girmaye K, Fassil A, Mussie HY. Phenotypic and genotypic characteristics of cowpea rhizobia from soils of Ethiopia. 201;17(42):1299-1312.
26. Handbook for Rhizobia, 2nd Ed., Springer Laboratory, Springer-Verlag, German. 1994;380.
27. Fening JO, Danso SA. Variation in symbiotic effectiveness of cowpea bradyrhizobia native to Ghanaian soils. Appl. Soil Ecol. 2002;21:23-29.
28. Alemayehu M. Country Pasture/Forage Resource Profiles, Ethiopia, FAO, Rome, Italy; 2017.
    Available:http://agris.fao.org/agris-search /search.do? recordID= ET2006000090
29. National Meteorology of Ethiopia.
    Available:http://www.ethiomet.gov.et
30. Walkley A, Black IA. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 1934;37:29-37.
31. Sahelemedihin S, Taye B. Procrdures for soil and plant analysis. National Soil Research Centre, Ethiopian Agricultural Research Organization, Addis Ababa, Ethiopia. 2000;110.
32. Olsen SR, Cole CV, Watanabe FS, Dean LA. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. In U. S. Department of Agriculture Circular No. 939. Bander is et al. (Eds.), USA. 1954;99.
33. Manufacturer of Medical, Veterinary Ultrasound Scanners and Other Electronic Devices for Agriculture.
    Available:https://www.draminski.com/
34. Gemechu K, Endashaw B, Fassil A, Muhammad I, Tolessa D, Kifle D, Emana G. Evaluation of Ethiopian chickpea (*Cicer arietinum L.*) germplasm accessions for symbio-agronomic performance. Renewable Agric. Food Syst. 2012;28:338-349.
35. Official Methods of Analysis, Association of Official Agricultural Chemists (AOAC) 11th ed. Washington, DC; 1970.
36. Kouyate MZ, Krasova-Wade T, Yattara I, Neyra M. Effects of cropping system and cowpea variety on symbiotic potential and yields of cowpea (*Vigna unguiculata*) and pearl millet (*Pennisetum glaucum*) in the Sudano-Sahelian Zone of Mali. Int. Res. J. Agric. Sci. Soil Sci. 2012;4:30-39.
37. Girmaye K, Fassil A, Nandeshwar BC. Symbiotic performance of native cowpea rhizobia of Ethiopia under greenhouse conditions. Green Farming. 2020;2&3:645-649.
38. Marra LM, Soares CR, Oliveira SM, Ferreira PM, Soares BL, Carvalho RF, Lima JM, Moreira FM. Biological nitrogen fixation and phosphate solubilization by bacteria isolated from tropical soils. Plant and Soil. 2012;357:289-307.
39. Binjola S, Kumar N, Mishra G. Differential response of cowpea (*Vigna unguiculata*) genotypes to native rhizobia in Tarai Region of Uttarakhand, India. Not Sci. Biol. 2014;6:335-337.
40. Tilak KR, Rauganayaki N, Manoharachari C. Synergistic effects of plant growth promoting rhizobacteria and *Rhizobium* on nodulation and nitrogen fixation by Pigeonpea (*Cajanus cajan*). Europ. J. Soil Sci. 2006;57:67-71.
41. Aamir M, Aslam A, Khan MY, Jamshaid MU, Ahmad AN, Zahir ZA. Co-inoculation with *Rhizobium* and plant growth promoting rhizobacteria for inducing salinity tolerance in Mung bean under field condition of Semi-arid climate. Asian J. Agric. Biol. 2013;1:1-12.
42. Kebede E, Berhanu A, Anteneh A, Solomon T. Symbiotic effectiveness of cowpea (*Vigna unguiculata* (L.) Walp.) nodulating rhizobia isolated from soils of major cowpea producing areas in Ethiopia. Cogent Food & Agriculture. 2020;6.
    DOI: 10.1080/23311932.2020.1763648
43. Anteneh A, Ayele A. *Rhizobium leguminosarum* bv. viciae sp. inoculation improves the agronomic efficiency of N of common bean (*Phaseolus vulgaris*). Environ. Syst. Res. 2015;4:11-19.
44. Keyser HH, Munns DN. Effects of calcium, manganese and aluminum on growth of rhizobia in acid media. Soil Sci. Soc. Am. J. 1979;43:500-503.
45. Keyser HH, Munns DN, Hohenberg JS. Tolerance of rhizobia in culture and in symbiosis with cowpea. Soil Sci. Soc. AM. J. 1979;43:719-723.

46. Farias TP, Trochmann A, Soares BL, Moreira FMS. Rhizobia inoculation and liming increase cowpea productivity in Maranhão State. Agronomy Maringá. 2016;38(3):387-395.

47. Nkaa FA, Nwokeocha OW, Ihuoma O. Effect of phosphorus fertilizer on growth and yield of cowpea (*Vigna unguiculata*). Int. J. Pharm. Biol. Sci. 2014;9:74-82.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/62564