

## Evaluation of cowpea (*Vigna unguiculata* (L.) Walp) genotypes for nodulation performance and biological nitrogen fixation in the lowlands of Southern and Southwestern Ethiopia

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### Abstract

Cowpea has a great potential in biological nitrogen fixation (BNF) and plays a significant role in cropping systems. However, information is scanty on the BNF potential of cowpea in Ethiopia. The study was therefore conducted to compare and select superior genotypes of cowpea for nodulation performance, BNF potential and biomass accumulation. The experiment was conducted during the 2019 main cropping season in the lowlands of southern and southwestern Ethiopia. The trial consisted of five pipeline genotypes (G) (ILRI-9333, ILRI-9334, ILRI-12713, and ILRI-12688) and released variety, Temesgen. The trial was laid out in randomized complete block design with three replications at the two locations (L). Data on nodulation, BNF and biomass yield were collected and subjected to ANOVA using SAS version 9.3. Results showed that G, L and G\*L significantly ( $p < 0.05$ ) affected nodulation, BNF and biomass yield of cowpea. But effective nodules per plant and percentage of N- derived from atmosphere (% Ndfa) were significantly ( $p \leq 0.001$ ) affected by genotype. Higher nodules dry weight per plant (1.46 and 0.82 gm per plant) and dry biomass yield (8.5 and 8 t/ha) were obtained from genotype ILRI-9334 followed by ILRI-12688, respectively. The highest number of nodules per plant (70.9), total nitrogen (116.8 kg ha<sup>-1</sup>) and N-fixed (105.5 kg ha<sup>-1</sup>) were obtained from ILRI-11114 followed by ILRI-12688. The highest Ndfa (90.4 %) was recorded for genotype ILRI-11114 followed by ILRI-12688. Generally, genotype ILRI-12688 showed better performance in biomass yield and biological nitrogen fixation potentials in the study areas. Based on this finding genotype ILRI-12688 is tentatively recommended for study areas and other places with similar agro-ecologies.

**Key words:** BNF, Genotype, Legume, Nodulation

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### INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp] is a major grain legume grown in semi-arid regions of Sub-Saharan Africa (SSA). Its protein content ranges from 27 to 43% and 21 to 33% in leaves and dry grain, respectively (Abaidoo *et al.*, 2017). Leaves and green pods are consumed as vegetables and the dried grain is used in many different food

preparations. The cowpea nutritional value and biological nitrogen fixation (BNF) potential coupled with a high plasticity to environmental conditions place this legume in a unique position in SSA in the context of food and nutritional security (Gomes *et al.*, 2019). Cowpea plays great roles in N<sub>2</sub> fixation and fixing about 29-391 kg ha<sup>-1</sup> of atmospheric nitrogen in SSA and make about 60-70 kg ha<sup>-1</sup> nitrogen available for succeeding

crops grown in rotation with it (Baijukya *et al.*, 2013), supporting cropping system by replacing chemical fertilizer (Sinclair *et al.*, 2014). Despite these advantages, cowpea production has remained very low in SSA, especially in Ethiopia (Kebede., 2020). Low soil N concentration is a major factor limiting crop production, in SSA including Ethiopia. In modern agriculture, nitrogen fertilization is widely used to improve crop yields, while much of nitrogen provided to cropping systems is in the form of industrially produced nitrogen fertilizers and it gives short sustain high yield product, but it causes a long-term negative impact on the health of farm lands (Hamza *et al.*, 2017). Hence, the N contribution by the legume-rhizobia symbiosis could be very significant to the farmers.

Although cowpea production in Ethiopia is increasing because of its importance in nutrition and agricultural system, its production was estimated to be 500 kg ha<sup>-1</sup> (Beshir *et al.*, 2019), which would have a significant contribution to the food security of farming communities. Besides, its ability to fix N and produce nodules has brought about its uniqueness. It contributes to the sustainability of cropping systems and soil fertility

improvement in marginal lands by providing ground cover, nitrogen fixation, and weeds suppression (Beshir *et al.*, 2019). Therefore, there is a need to increase cowpea production locally and this can be done through identifying cowpea genotypes that are high yielders, with high N<sub>2</sub> fixing potential and that can substantially contribute to soil N<sub>2</sub> fertility through effective symbiosis with native rhizobia (Abaidoo *et al.*, 2017). Currently information is scanty on BNF potentials of cowpea and there is scarcity of improved varieties at national and regional levels. This study was conducted to examine the performance of cowpea genotypes for nodulation performance, BNF and biomass yield in the study areas.

**MATERIALS AND METHODS**

**Description of the experimental site**

Shomba and Chano are located in south western and southern Ethiopia in Kaffa and Gamo zonal administrations in SNNPR state at 036°20’54” E and 07°26’71” N and 37°35’10.5”E and 6°06’47”N, respectively. The details are described below in Table 1.

**Table 1. Description of the experimental sites**

Description	Study areas	
	Shomba	Chano
Altitude	1200 meter above sea level	1206 masl
Soil type	Clay loam	Clay loam
RF distribution pattern	Long rainy season	Bimodal distribution pattern
Temperature Min °C	15°C	16.5°C
Temperature Max °C	29°C	31°C
Agro-ecology zone	Wet moist lowland	hot to warm sub-moist low land
Average annual rainfall	1050-1200 mm	750-818 mm
Previous land history	It was cropped with maize	It was cropped with maize

**Source:** Agriculture office of respective Zonal administrations

**Experimental design and treatments**

The experiment contained six cowpea genotypes (ILRI-9333, ILRI-9334, ILRI-11114, ILRI-12713, ILRI-12688 and Temesgen) in two locations (Shomba and Chano) with *Panicum antidotale* used as a reference crop for total nitrogen difference method and the experiment was laid out in randomized complete block design with three replications. Spacing of 30 cm between plants and 60 cm between rows were used. The gross plot size

(m) of 3.6 × 3.9 (14.04 m<sup>2</sup>) with a net plot area 3.3 × 2.4 (7.92 m<sup>2</sup>) were employed. Spacing between plots and blocks were 1m and 1.5m, respectively. The selected experimental land was prepared using oxen and plowed four times, and then the seed bed preparation was done. The treatments (genotypes) were assigned randomly following the randomization principles to their respective plots. NPS fertilizer at the rate of 100 kg ha<sup>-1</sup> was applied as recommended by (MoA., 2013). The reference crop (*Panicum antidotale*)

was sown at the same time with cowpea on August 11, 2019 and UREA was added at rate of 100 kg ha<sup>-1</sup>.

**Soil sampling and analysis**

The soil of experimental sites at 0-30 cm depth has been tested in Jimma University soil laboratory to

determine selected physicochemical properties of the soil. The laboratory analysis for soil texture, pH, cation exchange capacity (CEC), organic carbon (OC), organic matter (OM), total nitrogen (TN) and available phosphorus (P) were done (Table 2).

**Table 2. Soil physicochemical property of the experimental sites**

Parameters	Shomba	Chano
pH:H <sub>2</sub> O (1:2.5)	6	6.7
CEC (me/100 g)	23	32.4
Organic carbon (%)	2.34	2.73
Organic matter (%)	4.03	4.7
Total Nitrogen (%)	0.183	0.203
Available P (ppm)	21.28	65.14
Texture	Clay loam	Clay loam
Sand (%)	40	30
Clay (%)	30	36
Silt (%)	30	34

**Data collection and analysis**

Crop phenology, nodulation, biomass yield and biological nitrogen fixation traits of the cowpea genotypes were recorded. Days to 50% flowering was visually determined by counting the number of days from emergence to the time when 50% of the plants in the plot set. Assessment of nodulation was done at 50% flowering by randomly and carefully uprooting five plants from each plot. The nodules were detached from the roots, washed and counted and the proportion of effective nodules per plant was determined by color through cutting the nodules. The nodules dry weight per plant was determined using an electronic scale after oven drying at 65°C for 48 hours. Five plant samples were taken randomly from two middle rows from

each plot at 50% flowering to determine biomass yield and BNF.

**Nitrogen content of plant tissues**

The samples were oven dried at 65°C for 48 hours and ground and sieved through 0.1 mm sieve size. Nitrogen content was determined by the micro-Kjeldahl digestion, distillation and titration method (Fageria *et al.*, 2009). The total nitrogen difference method (TND) was used to determine the amount of N fixed. Fixed Nitrogen (kg ha<sup>-1</sup>) was estimated by subtracting total nitrogen in non-N fixed crop from total nitrogen in legumes. N-fixed was calculated using the equation of Recous *et al.*, (1995):

$N \text{ fixed (kg ha}^{-1}\text{)} = \text{Total N in legumes} - \text{Total N in reference crop,}$

$$\text{Total N in plants} = \frac{\text{dry matter (kg ha}^{-1}\text{)} \times \% \text{ of N in plants}}{100}$$

$$\%Ndfa = \frac{\text{Total N in legumes} - \text{Total N in reference crop}}{\text{Total N in legumes}} \times 100$$

Where %Ndfa is the percentage of N<sub>2</sub> derived from the atmosphere

All the collected data were checked for meeting basic assumption of ANOVA before they were subjected to analysis using general linear model procedure of SAS version 9.3 (SAS, 2011). The mean comparison was done using the LSD test at 5% probability levels. Correlation analyses between the collected parameters were carried out using Pearson's correlation coefficient.

## RESULTS AND DISCUSSION

### Days to 50% flowering

Days to 50% flowering were significantly affected by genotype by location (G\*L) interaction ( $p \leq 0.01$ ) and genotypes ( $p \leq 0.001$ ) but not significantly affected by locations alone (Table 3). Genotype ILRI-12713 took shortest time (48 days) to reach 50% flowering at Chano, while, genotype ILRI-9334 took longer (68.6 and 63 days) to reach 50% flowering at both Shomba and Chano sites, respectively, but significant variation in days to 50% flowering was not observed among genotypes ILRI-9333, ILRI-12688 and ILRI-11114 at both locations. The observed variation among genotypes in days to 50% flowering could be due to inherent genetic variation among genotypes. Similar findings were reported by Agza *et al.*, (2012), where variation among cowpea varieties could be due to genetic or environment effect. The longer maturity periods at Shomba might have been caused by the promoted vegetative growth due to high soil moisture and high rainfall than at Chano. The shortest to medium time of flowering and maturity in crops is agronomical preferable traits to adjust the cropping season with climatic conditions especially in areas with short rainy seasons to reduce food shortage and hunger among small-holder farmers. The results are similar with that of (Atsbha *et al.*, 2018), where genotype ILRI-12713 reached 50% flowering earlier than others. ILRI-12713 reached 50% flowering even earlier than the report by Atsbha, which might be due to environmental variations. Results of the current work were comparable to those reported cowpea genotypes in the literature (Agza *et al.*, 2012; Fantaye *et al.*, 2017).

### Number of nodules per plant

Number of nodules per plant was significantly affected by the genotypes ( $p < 0.001$ ) and locations

( $P < 0.05$ ) separately and combined (G\*L interaction ( $p < 0.001$ )). Genotype ILRI-11114 produced significantly higher ( $p \leq 0.001$ ) number of nodules per plant (71.9) than other genotypes at Chano. Whilst Temesgen produced the least number of nodules per plant (25). Comparing the locations, genotype ILRI-11114, ILRI-12688, ILRI-12713 and ILRI-9333 produced higher number of nodules per plant at Chano than Shomba. Generally, the results showed that cowpea genotypes nodulated freely with the naturally-occurring nodulating bacteria in the soil. The differences revealed among genotypes in nodule numbers could be due to variation in genetic makeup of genotypes or climatic effect (Table 3). Most of tested genotypes produce higher number of nodules at Chano than Shomba; which could be due to low soil acidity, high cation exchange capacity and high available phosphorus (Table 2). Inherent genetic variation exists among genotypes and could be explored for increased productivity (Appiah *et al.*, 2015; Ado., 2017).

Many researchers have reported significant differences in nodule numbers among different varieties of legumes. However, increased number of nodules may not necessarily signify efficiency as many factors affect nitrogen fixation (Ado., 2017). The process of nodulation in legumes is controlled by efficiency of the local rhizobia, environmental, nutritional and endogenous plant factors such as phyto-hormones, plant nodulation reception systems and auto regulation of nodulation (Ado., 2017). Lawrence *et al.*, (2018) stated that genotypes significantly influenced biological nitrogen fixation. The marked variation in nodule numbers per plant among the varieties could be attributed to difference in the genetic makeup of the individual varieties (Ayodele and Oso., 2014).

### Effective nodules per plant (%)

Effective nodules per plant was significantly ( $p < 0.001$ ) affected by genotypes and locations but not affected by interaction effect. The proportion of effective nodules per plant was significantly ( $p \leq 0.001$ ) different among the genotypes. Genotype ILRI-12688 produced significantly ( $p \leq 0.001$ ) the highest proportion of effective nodules per plant followed by ILRI-9334 and

Temesgen than others at Shomba and Chano sites, respectively (Table 3). There was no significant difference among genotypes ILRI-9334, ILRI-9333, ILRI-11114 and ILRI-12713 at Chano site (Table 3). The observed variation in the proportion of effective nodules per plant among cowpea genotypes in study areas might be due to variation in genetic makeup of tested genotypes or climatic condition of the experimental sites (Table 3 and Table 1). Similar range was reported by Ado., (2017), where the highest effective nodules per plant was 80%. Hungria and Vargas., (2000) reported that the plant nitrogenase activity reduced dramatically as a result of formation of ineffective nodules at low soil moisture and high temperature (40°C).

On the other hand, Solomon *et al.* (2012) and Saha *et al.* (2017) reported that the legumes grown in soils having high available nitrogen reduces the nitrogen fixation rates, whereas soils with low to medium available nitrogen levels enhance the nodulation and nitrogen fixation of the legumes and may increase the yield without reducing the amount of nitrogen fixed. In contrary, there were reports that showed deficiency in phosphorous supply and availability leading to severe limitations on nitrogen fixation and nodulation (Mmbaga *et al.*, 2014), resulting in reduction of nodule mass, nitrogen fixation and yield.

#### Nodules dry weight per plant

Nodules dry weight per plant was significantly affected by genotypes ( $p < 0.001$ ) and locations ( $p < 0.01$ ) separately and in combination (G\*L interaction ( $p < 0.05$ )). The results showed that genotype ILRI-9334 produced significantly ( $p \leq 0.001$ ) higher nodules dry weight per plant followed by genotype ILRI-12688, whilst ILRI-9333 and Temesgen produced the least nodules dry weight per plant (Table 3). The observed variation among cowpea genotypes in nodules dry weight could present potentials for selection among genotypes for varying climatic or edaphic conditions (Table 1 and Table 2).

Ado., (2017) reported significant differences in number of nodules per plant among cowpea

varieties. ILRI-11114 produced the highest number of nodules per plant, but did not produce the highest nodules dry weight. The highest nodules dry weight was obtained in ILRI-9334 which did not produce highest number of nodules per plant. Even if number of nodules positively correlated with nodules dry weight, it did not show strong correlation ( $r = 0.16$ ). This could be probably because of genotypes that produced a greater number of nodules per plant produced smaller sized nodules, whilst those that produced fewer number of nodules produced larger sizes. Similar observations were reported in literature (Appia *et al.*, 2015). Yoseph *et al.*, (2017) reported that variation in cowpea genotypes for nodules dry weight per plant present opportunities for selecting better performing cultivars. Ayisi *et al.*, (2004) reported that nodulation parameters vary in cowpea genotypes intercropped with maize and sorghum.

#### Dry biomass yield

Dry biomass yield was significantly ( $p < 0.001$ ) affected by separate and interactions of genotypes and locations. ILRI-9334 was found to be the highest ( $p \leq 0.001$ ) yielder (8.5 t ha<sup>-1</sup>) at Shomba followed by ILRI-12688 (8.06 t ha<sup>-1</sup>) at Chano, while ILRI-9333 was the least yielder ( $p \leq 0.001$ ) of dry matter at Shomba (Table 4). Generally, the result implied that the variation in dry biomass yield among genotypes across the locations might give opportunities for selecting better performing one for varying climatic conditions (Table 1 and 4). Simunji *et al.*, (2019) reported that there was variation among cowpea varieties in dry biomass yield. Atsbha *et al.*, (2018) observed highest dry matter yield from Temesgen variety which in the present finding was found to be among the low dry biomass yielders. Fortunately, the genotypes which gained more dry matter yield produced more grain yield and also fixed more nitrogen from the atmosphere. Kouyaté *et al.* (2012) reported that genotypes that yield higher dry biomass could help farmers to improve the soil fertility status if selected to be used in rotation systems.

**Table 3. Number of days to 50% flowering & nodulation parameters of cowpea genotypes at Shomba and Chano.**

Genotypes	DTFF		NNPP		NDWPP (g)		ENPP (%)	
	Shomba	Chano	Shomba	Chano	Shomba	Chano	Shomba	Chano
ILRI-9333	56.33 <sup>dc</sup>	53.67 <sup>dc</sup>	42.33 <sup>ed</sup>	53.67 <sup>cb</sup>	0.61 <sup>cde</sup>	0.5 <sup>e</sup>	52.80 <sup>c</sup>	34.00 <sup>c</sup>
ILRI-9334	68.66 <sup>a</sup>	63.00 <sup>b</sup>	57.00 <sup>b</sup>	36.67 <sup>fe</sup>	1.46 <sup>a</sup>	0.97 <sup>b</sup>	59.90 <sup>b</sup>	38.46 <sup>c</sup>
ILRI-11114	52.67 <sup>d</sup>	52.68 <sup>d</sup>	48.00 <sup>cbd</sup>	70.93 <sup>a</sup>	0.65 <sup>cde</sup>	0.67 <sup>cde</sup>	48.90 <sup>c</sup>	36.70 <sup>c</sup>
ILRI-12713	54.00 <sup>dc</sup>	48.5 <sup>e</sup>	37.00 <sup>fe</sup>	52.86 <sup>cb</sup>	0.57 <sup>de</sup>	0.56 <sup>ed</sup>	53.00 <sup>c</sup>	38.20 <sup>c</sup>
ILRI-12688	55.66 <sup>dc</sup>	55.67 <sup>dc</sup>	38.00 <sup>fed</sup>	44.2 <sup>ced</sup>	0.82 <sup>cb</sup>	0.76 <sup>cd</sup>	77.60 <sup>a</sup>	62.50 <sup>a</sup>
Temesgen	57.33 <sup>c</sup>	54.66 <sup>dc</sup>	29.33 <sup>fg</sup>	25.33 <sup>h</sup>	0.67 <sup>cde</sup>	0.51 <sup>e</sup>	61.90 <sup>b</sup>	51.10 <sup>b</sup>
LSD <sub>0.05</sub>	3.9		10		0.21		5.9	
CV (%)	3.7		13.9		17.7		7.5	

Where: DTFF = days to 50% flowering; NNPP = nodule numbers per plant; NDWPP = nodules dry weight per plant and ENPP = percent of effective nodules per plant; Means followed by the same letters within a column are not significantly different ( $p \leq 0.001$ ).

### Total nitrogen content

Total nitrogen was significantly affected by genotypes ( $p \leq 0.001$ ) and their interaction location (G\*L interaction effect ( $p \leq 0.01$ )). The genotype ILRI-11114 significantly ( $p \leq 0.01$ ) produced highest total nitrogen (116.86 kg ha<sup>-1</sup>) followed by genotype ILRI-12688 (108.53 kg ha<sup>-1</sup>) at Chano as compared with other genotypes, whilst Temesgen produced the lowest total nitrogen (65.16 kg ha<sup>-1</sup>) at Chano (Table 4). Generally, the genotypes ILRI-11114 and ILRI-12688 had better total nitrogen at both locations; which could be due to genetic differences that can be selected for breeding purposes. According to (Simunji *et al.*, 2019) the highest total nitrogen gained was 141.5 kg ha<sup>-1</sup> which is 17% higher than the results from our report; likely due to variation in climatic or genetic factors. Coskan & Dogan. (2011) stated that establishment of effective N<sub>2</sub> fixing symbiosis between legumes and their N<sub>2</sub> fixing bacteria is dependent upon many environmental factors, and can be greatly influenced by farm management practices. The total nitrogen is crucial requirement for plant growth because it is returned to the soil through mineralization of plant nutrients and it could benefit the succeeding cereal crops (Simunji *et al.*, 2019). Cowpea varieties vary in nitrogen fixation potential due to differences in the number, weight, and efficiency of nodules and farming systems (Makoi *et al.*, 2009). Lawrence *et al.*, (2018) showed that genotypes significantly influenced biological nitrogen fixation.

### Nitrogen fixed from the atmosphere

Nitrogen fixed from the atmosphere was significantly affected by genotypes ( $p \leq 0.001$ ) and

their interaction location (G\*L interaction effect ( $p \leq 0.01$ )). Genotype ILRI-11114 was observed to have greater potential of accumulating nitrogen fixed regardless of locations followed by ILRI-12688, whilst Temesgen fixed significantly ( $p \leq 0.01$ ) the lowest total nitrogen (50.93 kg ha<sup>-1</sup>) on study areas (Table 4). The observed variation among cowpea genotypes in nitrogen fixation from the atmosphere could be due to low soil acidity, high cation exchange capacity and high available phosphorus at Chano that enhanced the process by providing conducive environment for microbial activity compared to Shomba (Table 2). Inherent genetic differences of genotypes could also contribute to the variations as reported by Simunji *et al.*, (2019), where legumes generally take more than half of their nitrogen requirements from the atmosphere and therefore take less N from the soil compared to the non-N-fixing crops. Munjonji *et al.*, (2018) reported that cowpea genotype with low grain yield performing better for BNF which could be due to genetic variation among genotypes as responses to climatic or edaphic effects. Environmental conditions can influence the nitrogen fixation characteristics and this influence can vary among genotypes (Barbosa *et al.*, 2018). Montañez., (2000) stated that the amount of nitrogen fixed by legumes varies widely with host genotype, *Rhizobium* efficiency, soil and climatic conditions.

### Percentage of N derived from atmosphere

Percentage of nitrogen derived from atmosphere was significantly affected by genotypes and locations ( $P \leq 0.001$ ), but not by their interaction. The genotype ILRI-11114 significantly ( $p \leq 0.001$ )

derived 90.4% and 86.4% of the nitrogen from the atmosphere, followed by ILRI-12688 (88.8% and 86.8%) at Chano and Shomba respectively (Table 4). Temesgen derived the least percentages of nitrogen from the atmosphere (78% and 82.5%) at both Shomba and Chano sites, respectively. Approximately similar results (89.2% and 89.85%) were reported by (Ulzen., 2013). Solomon *et al.* (2012) and Saha *et al.* (2017) reported that the legumes grown in soils with high available nitrogen reduced the nitrogen fixation rates, whereas soils with low to medium available nitrogen enhanced the nodulation and nitrogen fixation of the legumes and may increase the yield without reducing the amount of nitrogen fixed, supporting the present results. According to Dawson *et al.* (2008) BNF is influenced by the

symbiont, genotypic characteristics and depends on host range and also it varies depending on biological and environmental factors and this effectiveness can be measured by determining the concentration of the compound involved in this process (Liu *et al.*, 2011). The rate of BNF is highly variable and depends on bacterial strain in the soil, legume cultivar, soil, and environmental conditions (Shantharam and Mattoo., 1997). Generally, BNF is varying among genotypes depending on biological, edaphic and environmental factors (Table 4), presenting potentials for selecting genotypes for different climatic and soil conditions.

**Table 4. Mean biomass yield and biological nitrogen fixation traits of cowpea genotypes the two sites**

Genotypes	DBMY (t ha <sup>-1</sup> )		TN (kg ha <sup>-1</sup> )		NF (kg ha <sup>-1</sup> )		%Ndfa(%)	
	Shomba	Chano	Shomba	Chano	Shomba	Chano	Shomba	Chano
ILRI-9333	3.6 <sup>c</sup>	6.4 <sup>c</sup>	79.6 <sup>fg</sup>	86.56 <sup>fe</sup>	68.1 <sup>dc</sup>	72.33 <sup>c</sup>	83.47 <sup>cb</sup>	85.46 <sup>c</sup>
ILRI-9334	8.5 <sup>a</sup>	7.26 <sup>bc</sup>	90.56 <sup>de</sup>	97.1 <sup>fg</sup>	76.32 <sup>c</sup>	85.6 <sup>b</sup>	84.2 <sup>b</sup>	88.1 <sup>b</sup>
ILRI-11114	4.6 <sup>ed</sup>	6.7 <sup>c</sup>	105.4 <sup>bc</sup>	116.86 <sup>a</sup>	91.12 <sup>b</sup>	105.5 <sup>a</sup>	86.4 <sup>a</sup>	90.4 <sup>a</sup>
ILRI-12713	5.03 <sup>d</sup>	7.16 <sup>bc</sup>	70.8 <sup>hg</sup>	103.7 <sup>bc</sup>	59.3 <sup>de</sup>	67.27 <sup>dc</sup>	82.4 <sup>c</sup>	83.56 <sup>dc</sup>
ILRI-12688	7.03 <sup>bc</sup>	8.0 <sup>ba</sup>	103.7 <sup>bc</sup>	108.53 <sup>ba</sup>	92.23 <sup>b</sup>	94.3 <sup>b</sup>	86.8 <sup>a</sup>	88.8 <sup>ba</sup>
Temesgen	5.03 <sup>d</sup>	4.97 <sup>d</sup>	65.76 <sup>h</sup>	65.16 <sup>h</sup>	54.26 <sup>e</sup>	50.93 <sup>e</sup>	78.1 <sup>d</sup>	82.5 <sup>d</sup>
LSD <sub>0.05</sub>	1.13		9.2		9.1		1.69	
CV (%)	8.3		5.8		6.8		1.2	

Where, DBMY = Dry biomass yield, TN = total nitrogen, NF = nitrogen fixed, %Ndfa = percentage of nitrogen derived from the atmosphere; means followed by the same letters within a column are not significantly different at (p <0.05)

**Correlation analysis**

Correlation analysis showed that nodulation and biomass yield of cowpea genotypes were significantly associated (Table 5). Correlation analysis showed that the number of nodules had significant, strong and positive correlation with dry biomass yield (r = 0.46\*\*), total nitrogen (r = 0.5\*\*\*), nitrogen fixed from the atmosphere (r = 0.49\*\*) and percentage of nitrogen derived from the atmosphere (r = 0.41\*\*). This indicates that the higher nodulation performance of the genotypes would enhance nitrogen fixation. Hence the results showed that a rise in any of these variables would

result in a corresponding increase in the other and vice versa. Generally, positive correlation was observed among selected parameters of cowpea genotypes. Seido *et al.*, (2019) reported that plants that produced higher nodules mass resulted in higher dry biomass yield and more N<sub>2</sub> fixation in cowpea genotypes, confirming the present results. Hungria and Bohrer. (2000), reported also that the higher the nodule weight, the higher efficiency of the fixed N<sub>2</sub>, with the vegetative and the yield tending to increase as shown also in the present results.

**Table 5. Correlation matrix for the selected parameters of cowpea genotypes**

	NNPP	NDPP	DBM	TN	NF	%Ndfa
NNPP	1					
NDPP	0.16 <sup>NS</sup>	1				
DBM	0.46 <sup>**</sup>	0.55 <sup>***</sup>	1			
TN	0.5 <sup>***</sup>	0.3 <sup>NS</sup>	0.44 <sup>**</sup>	1		
NF	0.49 <sup>**</sup>	0.31 <sup>NS</sup>	0.41 <sup>**</sup>	0.99 <sup>***</sup>	1	
%Ndfa	0.41 <sup>**</sup>	0.37 <sup>*</sup>	0.32 <sup>*</sup>	0.84 <sup>***</sup>	0.88 <sup>***</sup>	1

**CONCLUSIONS**

The results revealed a significant effect in cowpea genotypes in nodulation, BNF and biomass yield across the locations except effective nodules per plant and percentage of N- derived from atmosphere (%Ndfa). The highest nodules dry weight per plant was obtained from ILRI-9334. Much greater biomass yield was obtained from genotype ILRI-9334 and ILRI-12688. The highest total nitrogen, N-fixed and %Ndfa was obtained from genotype ILRI-11114 followed by ILRI-12688. The nodulation performance by cowpea genotypes were positively and significantly correlate with biomass yield. Generally positive correlation was observed among selected parameters. The study also showed significant genotypic variation among the cowpea cultivars in nodulation performance, biological nitrogen fixation potential and biomass yields. Genotype ILRI-12688 is performed best in terms of BNF and biomass yield from tested genotypes at both the study areas. As this study was conducted for only one season across the locations further study should be needed in various environments to select and recommend superior genotypes with high BNF potential, dry biomass yield as a variety.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest regarding the publication of this article.

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