American Journal of Plant Sciences, 2019, 10, 640-658
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Screening Cowpea (*Vigna unguiculata* (L.) Walp.) Genotypes for Enhanced N_2 Fixation and Water Use Efficiency under Field Conditions in Ghana

Damba Yahaya[1,2], Nicholas Denwar[3], Mustapha Mohammed[4*], Matthew W. Blair[1*]

[1]Department of Agricultural and Environmental Sciences, Tennessee State University, Nashville, TN, USA
[2]Department of Biotechnology, University for Development Studies, Tamale, Ghana
[3]Council for Scientific and Industrial Research, Savannah Agricultural Research Institute, Tamale, Ghana
[4]Department of Crop Sciences, Tshwane University of Technology, Pretoria, South Africa
Email: *mblair@tnstate.edu; *imustaph@gmail.com

How to cite this paper: Yahaya, D., Denwar, N., Mohammed, M. and Blair, M.W. (2019) Screening Cowpea (*Vigna unguiculata* (L.) Walp.) Genotypes for Enhanced N_2 Fixation and Water Use Efficiency under Field Conditions in Ghana. *American Journal of Plant Sciences*, **10**, 640-658.
https://doi.org/10.4236/ajps.2019.104047

Received: March 15, 2019
Accepted: April 23, 2019
Published: April 26, 2019

Copyright © 2019 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/
Open Access

Abstract

To explore the variations in symbiotic N_2 fixation and water use efficiency in cowpea, this study evaluated 25 USDA cowpea genotypes subjected to drought under field conditions at two locations (Kpachi and Woribogu) in the Northern region of Ghana. The ^{15}N and ^{13}C natural abundance techniques were respectively used to assess N_2 fixation and water use efficiency. The test genotypes elicited high symbiotic dependence in association with indigenous rhizobia, deriving between 55% and 98% of their N requirements from symbiosis. Consequently, the amounts of N-fixed by the genotypes showed remarkable variations, with values ranging from 37 kg·N-fixed·ha^{-1} to 337 kg·N-fixed·ha^{-1}. Most genotypes elicited contrasting symbiotic performance between locations, a finding that highlights the effect of complex host/soil microbiome compatibility on the efficiency of the cowpea-rhizobia symbiosis. The test genotypes showed marked variations in water use efficiency, with most of the genotypes recording higher $\delta^{13}C$ values when planted at Kpachi. Despite the high symbiotic dependence, the grain yield of the test cowpeas was low due to the imposed drought, and ranged from 56 kg/ha to 556 kg/ha at Kpachi, and 143 kg/ha to 748 kg/ha at Woribogu. The fact that some genotypes could grow and produce grain yields of 627 - 748 kg/ha under drought imposition is an important trait that could be tapped for further improvement of cowpea. These findings highlight the importance of the cowpea-rhizobia symbiosis and enhanced water relations in the crop's wider adaptation to adverse edaphoclimatic conditions.

Keywords

Drought, Cowpea, Carbon Isotope Discrimination, Nitrogen Fixation, Grain Yield

1. Introduction

In most parts of the world, crop yields on smallholder farms are often limited by low soil fertility resulting from continuous cropping with minimal or no fertilizer application [1]. However, besides their importance as human foods, grain legumes such as cowpea can improve overall soil nutrient status due to their ability to fix atmospheric N_2 in association with rhizobia in the soil [2] [3]. Like most legumes, the N_2-fixing trait of cowpea confers adaptation to low nutrient soils, with some genotypes deriving up to 96% of their nitrogen (N) requirements from symbiosis, which often leads to significant N contribution to cropping systems and grain yield increases [1] [4] [5]. However, due to variations in legume symbiotic performance resulting from genotypic differences and N_2-fixing efficiency of the rhizobial symbionts, there is often the need to screen for improved N_2 fixation among legume germplasm [6] [7]. Of the many techniques used to estimate legume symbiotic performance, the ^{15}N natural abundance has so far been useful in quantifying N_2 fixation in field-grown legumes [8] [9]. This technique requires the estimation of the isotopic fractionation associated with N_2 fixation in the legume (so called B value), and the selection of reference plants to estimate the soil N uptake by the legume [10] [11].

Besides low soil fertility, the impact of drought on agricultural productivity also manifests in grain yield losses [12] [13]. The occurrence of intermittent drought resulting from projected climate change is therefore expected to have negative implications on food and nutritional security owing to potential yield losses in staple crops [14]. In Africa, cowpea is widely cultivated for its edible grains and leaves, both of which are rich in proteins (22%), carbohydrates, carotenoids and micro-nutrients [15]. Consequently, Nigeria and Niger, both in sub-Sahara Africa are the major cowpea producing countries in the world [16]. The wider adaptation of cowpea to growth in marginal soils and drought makes it a preferred crop in most rural households where it serves as food security crop and an income source [17]. However, despite the reported drought tolerance in cowpea, up to 65% yield losses have been reported in the crop under drought conditions [18] [19].

Drought is a complex phenomenon [20] that is prevalent in most parts of the tropics, and is often severe in Sub-Sahara Africa where the soils are also nutrient-poor [21]. Screening and/or improvement of crops for enhanced water relations can therefore help in the selection of superior genotypes for climate change situations. Nevertheless, although the USDA cowpea germplasm comprises 1000s of genotypes, little information has on their water use efficiency, an aspect much needed for their potential utilization under water limiting conditions. Selection for drought tolerance in crops is often carried out using various approaches which include leaf gas exchange measurements, plant biomass and yield response to drought, and carbon isotope discrimination ($\delta^{13}C$) [22]. Owing to differences in mass between $^{13}CO_2$ and $^{12}CO_2$, C3 plants discriminate against the heavier isotope in favour of the lighter $^{12}CO_2$ during photosynthesis [23]. Under water limiting conditions, plants exhibit reduced discrimination (higher $\delta^{13}C$ values) leading increased water use efficiency. Conversely, plants tend to exhibit greater discrimination against $^{13}CO_2$ leading to lower $\delta^{13}C$ values under adequate water supply [23]. As a result, carbon isotope discrimination expressed as $\delta^{13}C$, is a useful surrogate in screening for water use efficiency in C3 plants such as Bambara groundnut and soybean [3] [8].

Despite the importance of cowpea in supplying human food and rejuvenating nutrient-poor soils via symbiosis, much remains to be known about the N_2-fixing potential and water use efficiency of available germplasm. The aim of this study was therefore to screen 25 USDA cowpea collections subjected to drought under field conditions for enhanced symbiotic performance and water use efficiency at two locations in the Northern region of Ghana, using the ^{15}N and ^{13}C natural abundance techniques. The study also explored variations in grain yield among the test genotypes within and between the test locations.

2. Materials and Methods

2.1. Experimental Sites

The study was conducted at two locations [Kpachi (latitude 9.4268 and longitude −0.9747) and Waribogu (latitude 9.4168 and longitude −1.0341)] in the northern region of Ghana. The Northern region receives an annual rainfall ranging from 800 mm to 1200 mm each year which occurs between April and October [24]. The rainfall distribution during this study is illustrated in **Figure 1**. Both experimental fields were fallow in the year prior to this study. However, the fields at Waribogu and Kpachi have a history of groundnut (*Arachis hypogea*) and soybean (*Glycine max*) cultivation, respectively, but without fertilizer application.

2.2. Soil Chemical Properties

Before planting, soil samples were randomly collected from 10 different points in each experimental field (0 - 30 cm depth). The soils from each experimental field were bulked together, and subsamples analyzed for chemical properties such as pH (H_2O), organic carbon [25], total nitrogen (Kjeldahl method), P using Bray-2 [26], K, Na, Ca, Mg, S, and cation exchange capacity (CEC) using ammonium acetate method (**Table S1**).

2.3. Experimental Design and Planting

The experimental treatments comprised 25 USDA cowpea genotypes. The experiment was laid in a randomized complete block design with three (3) replications

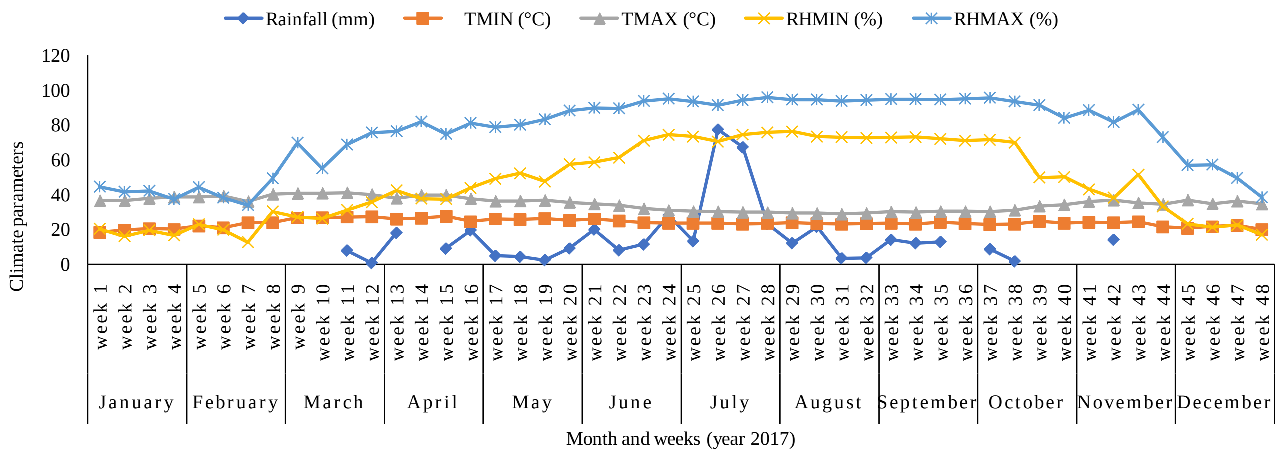

Figure 1. Average weekley rainfall distribution as well as temperatures and relative humidity in the study District (Kumbungu) during the experiment in 2017. Planting at Kpachi and Woribogu were done on 25th and 26th August 2017, respectively, coinciding with low rainfall.

per genotype at two locations (Waribogu and Kpachi) in the Kumbungu District of the Northern region, Ghana. Two seeds were sown at a spacing of 20 cm between plants and 60 cm between rows on plots measuring 2 m × 1.2 m. Planting was done on 25th and 26th August, 2017 at Woribogu and Kpachi, respectively.

2.4. Plant Sampling and Processing

At 90% flowering of all cowpea genotypes (49 days after planting), five (5) plants were sampled per plot, oven-dried (65˚C for 48 hours) in pre-labelled paper bags and weighed using a sensitive balance (A&D Engineering LLC, USA) to determine shoot dry matter (shoot DM). The oven-dried shoot samples were finely ground to pass through 0.85 mm sieve and stored in prelabelled zip-lock plastic bags prior to ^{15}N and ^{13}C isotopes analysis to assess N_2 fixation and water use efficiency, respectively. Maize plants (reference plants) that were concurrently planted along with cowpea in each experimental field were sampled and similarly oven-dried and ground for isotopic analysis to estimate soil N uptake by the legume plants [11].

2.5. $^{15}N/^{14}N$ Isotopic Analysis

The $^{15}N/^{14}N$ isotopic ratios of the ground shoots of both cowpea and maize (reference plants) were measured using mass spectrometer. For this, aliquots of 1 - 1.22 mg of each ground sample was separately weighed into tin capsules that have been pre-cleaned in toluene. Isotopic analysis was done on a Flash EA 1112 Series coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (all equipment supplied by Thermo Fischer, Bremen, Germany), at the University of Pretoria Stable Isotope Laboratory, Mammal Research. Two laboratory running standards (Merck Gel: $\delta^{13}C = -20.26‰$, $\delta^{15}N = 7.89‰$, %C = 41.28, %N = 15.29) and (DL-Valine: $\delta^{13}C = -10.57‰$, $\delta^{15}N = -6.15‰$, %C = 55.50, %N = 11.86) and a blank sample were run after every eleven (11) unknown samples. The running standards were calibrated against international standards: National Institute of Standards & amp; Technology (NIST): NIST 1557b (Bovine liver), NIST 2976 (Mussel tissue) and NIST 1547 (peach leaves). All results for nitrogen isotope values were referenced to air [8].

The $\delta^{15}N$ (‰) of shoot samples was calculated as [8]:

$$\delta^{15}N(‰)=\frac{\left[{}^{15}N/{}^{14}N\right]_{sample}-\left[{}^{15}N/{}^{14}N\right]_{atm}}{\left[{}^{15}N/{}^{14}N\right]_{atm}}\times 1000$$

where ${}^{15}N/{}^{14}N_{sample}$ is the isotopic ratio of ${}^{15}N$ and ${}^{14}N$ in the sample and ${}^{15}N/{}^{14}N_{atm}$ is the isotopic ratio of ${}^{15}N$ and ${}^{14}N$ in the atmosphere. The %N and %C in shoot samples were concurrently obtained from the mass spectrometer, and the C/N ratio was calculated as the ratio of %C and %N.

2.6. Percent of Legume Nitrogen (N) Derived from N_2 Fixation (%Ndfa)

The %Ndfa by cowpea was estimated according to Shearer and Kohl [27]:

$$\%Ndfa=\frac{\delta^{15}N_{ref}-\delta^{15}N_{leg}}{\delta^{15}N_{ref}-B}\times 100$$

where $\delta^{15}N_{ref}$ is the ${}^{15}N$ natural abundance of the reference plant, $\delta^{15}N_{leg}$ is the ${}^{15}N$ natural abundance of the test cowpea and the B value is the $\delta^{15}N$ of a cowpea plant completely dependent of symbiosis for its N requirement. The B value used in this study was −1.759‰ [5]. The mean $\delta^{15}N$ of all reference plants (maize) at Kpachi (+7.28 ± 0.15, n = 10) and Woribogu (+7.53 ± 0.15, n = 10), were respectively used as $\delta^{15}N_{ref}$ to calculate the %Ndfa by cowpeas in those locations.

2.7. Shoot N Content, N-Fixed and Soil N Uptake

The N content in shoots was estimated as the product of shoot DM and %N [28]. The amount of N-fixed was then calculated as Maskey *et al.* [29]:

N-fixed = (%Ndfa/100) × shoot N content.

Soil N uptake was calculated using the relation:

Soil N uptake = Total N content – amount of N-fixed.

2.8. ${}^{13}C/{}^{12}C$ Isotopic Analysis

To measure ${}^{13}C/{}^{12}C$, finely ground shoot samples of cowpea were similarly subjected to mass spectrometric analysis as described for ${}^{15}N/{}^{14}N$ analysis. All results for carbon isotope values were referenced to Vienna Pee-Dee Belemnite. The $\delta^{13}C$ (‰) values were calculated according to [23]:

$$\delta^{13}C=\left[\frac{\left({}^{13}C/{}^{12}C\right)_{sample}}{\left({}^{13}C/{}^{12}C\right)_{standard}}-1\right]\times 1000$$

where ${}^{13}C/{}^{12}C_{sample}$ is the isotopic ratio of the sample and ${}^{13}C/{}^{12}C_{standard}$ is the isotopic ratio of PDB, a universally accepted standard from Belemnite Pee Dee limestone formation of South Carolina [30].

2.9. Days to Flowering and Maturity

Plots were monitored from planting to observe the number of days taken for at least one flower to open (*i.e.* number of days to flowering). Physiological maturity was calculated as the number of days from sowing to physiological maturity of at least 90 percent of the plants in a plot.

2.10. Grain Yield

At physiological maturity, ten (10) plants were harvested from each plot and pods recovered. The pods were further sun-dried and threshed to obtain the seeds. The dried seeds were adjusted to 12% moisture content using a moisture meter (Dickey John corporation, USA) and weighed. Grain yield was determined per plot and expressed per hectare using plant density.

2.11. Statistical Analysis

The data gathered were tested for normality and then subjected to analysis of variance using the software Statistica version 10 [31]. A two-way ANOVA was used to compare the means of genotype × location interaction. Where the treatments showed significant differences, they were separated using Duncan's multiple range test at $p \leq 0.05$. Pearson correlation was used to explore the relationships between measured parameters.

3. Results

3.1. Soil Physicochemical Properties and Rainfall Distribution

The soils at Kpachi and Woribogu were sandy loam in texture, and slightly acidic with pH of 5.4 and 5.6, respectively (**Table S1**). The soils at Kpachi and Woribogu, respectively contained 0.54% and 0.56% organic carbon, 0.05% and 0.06% N, 5.8 and 6.5 mg/kg P, 78.0 and 93.6 mg/kg P, 2.3 and 4.6 mg/kg Na, 570 and 460 mg/kg, 64.8 and 60.0 mg/kg Mg (**Table S1**). Although soils at Woribogu were slightly higher in most nutrients, they recorded lower CEC (4.1 Cmol(+)/kg) when compared to soils at the Kpachi site (5.0 Cmol(+)/kg) (**Table S1**). The rainfall distribution, temperature (T) and relative humidity (RH) of the study District (Kumbungu) during the experiment in 2017 is shown in **Figure 1**.

3.2. Maturity Periods of the Test Cowpea

The cowpea genotypes evaluated were early maturing, with the mean number of days to flowering in the two locations ranging from 40 days after planting in genotype PI194207 to 48 days after planting in PI209971 (**Figure 2**). As to be expected, the number of days from sowing to maturity closely mirrored the patterns in flowering period (**Figure 2**). As a result, the maturity periods of the genotypes ranged from 65 days in genotype PI194207 to 75 days in PI209971 (**Figure 2**). In general, the cowpea genotypes flowered and matured earlier at Kpachi (42

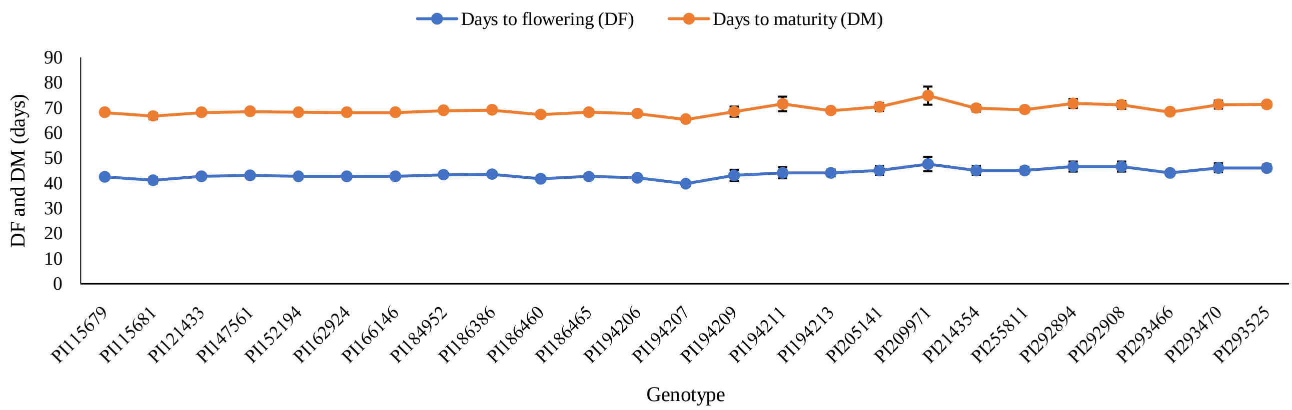

Figure 2. Average number of days to flowering and maturity of 25 cowpea genotypes planted at Kpachi and Waribogu in 2017. Error bars indicate standard errors.

and 67 days after planting, respectively) when compared to their counterparts at Woribogu which took 46 and 71 days to flower and mature, respectively (Table 1).

3.3. Shoot δ^{15}N, %Ndfa and Soil N Uptake

Subjecting the data from both locations to a two-way ANOVA revealed significant ($p \leq 0.05$) effects of genotype, location as well as their interactions on shoot δ^{15}N, %Ndfa and soil N uptake of the test cowpea genotypes (Table 1). The δ^{15}N values ranged from −1.34‰ to +2.25‰ at Kpachi, and from −1.34‰ to +2.47‰ at Waribogu (Figure 3(a)). Although genotype PI209971 recorded the least δ^{15}N at Kpachi (−1.34‰) followed by PI214354, PI293466, PI86465, PI86386, PI194213, PI194206, also with much lower (negative) δ^{15}N values (−0.71‰ to −0.46‰) when compared to other genotypes at the site, those genotypes recorded much higher(positive) δ^{15}N values at Woribogu (Figure 3(a)). Similarly, despite the reduced δ^{15}N values of genotypes PI293470, PI147561 and PI115681 at Woribogu relative to other genotypes planted at the site, their counterparts at Kpachi recorded markedly higher ($p \leq 0.05$) shoot δ^{15}N values (Figure 3(a)). Of the 25 cowpea genotypes tested, eight (8) recorded similar ($p > 0.05$) δ^{15}N in both test locations while the remaining genotypes exhibited contrasting δ^{15}N values between the two locations (Figure 3(a)).

Lower shoot δ^{15}N values were always associated with higher %Ndfa and *vice versa* (Figure 3(a) and Figure 3(b)). Consequently, all the cowpea genotypes which recorded negative δ^{15}N values also recorded markedly higher ($p \leq 0.05$) %Ndfa when compared to their counterparts with higher or positive δ^{15}N values (Figure 3(a) and Figure 3(b)). With similar δ^{15}N between the two locations therefore, genotypes PI152194, PI166146, PI186460, PI194207, PI194209, PI205141 and PI292894 recorded similar ($p > 0.05$) %Ndfa values between the two sites (Figure 3(a) and Figure 3(b)). Within both locations however, the cowpea genotypes showed high symbiotic dependence, with %Ndfa values ranging from 54.5% to 95.5% for genotypes PI115681 and PI209971, respectively, at

Table 1. A two-way ANOVA F-statistics and the main effect of location on shoot DM, symbiotic parameters and soil N uptake of 25 cowpea genotypes planted at Woribogu and Kpachi in the Kumbungu District of the Northern Region, Ghana. For locations, values (mean ± se) with dissimilar letters in a column are significantly different at ***p < 0.001.

	DF	DM	$\delta^{15}N$	Ndfa	Soil N uptake	Shoot DM	N-fixed	Shoot N concentration	
	DAP		‰	%	kg/ha	g/plant	kg/ha	%	g/plant
Location									
Waribogu	46 ± 0.4a	71 ± 0.4a	0.85 ± 0.1a	71.1 ± 1.2b	38.8 ± 3.0a	23.4 ± 1.0b	91.4 ± 3.8b	3.4 ± 0.06a	0.78 ± 0.03b
Kpachi	42 ± 0.2b	67 ± 0.2b	0.61 ± 0.1b	74.5 ± 1.4a	37.9 ± 3.0a	27.5 ± 1.8a	118.0 ± 8.4a	3.5 ± 0.05a	0.94 ± 0.06a
F-statistics									
Genotype (G)	2623.6***	2119.0***	170.6***	169.2***	45.1***	58.8***	23.8***	9.0***	24.1***
Location (L)	84159.3***	21595.3***	138.9***	232.5***	0.6^{NS}	69.5***	79.8***	0.48^{NS}	42.4***
G × L	3550.2***	2966.5***	310.1***	308.3***	33.8***	44.2***	32.6***	2.3***	24.2***

There was a significant correlation ($r = 0.52$, $r^2 = 0.27$, $p < 0.001$) between %N and %C in shoots.

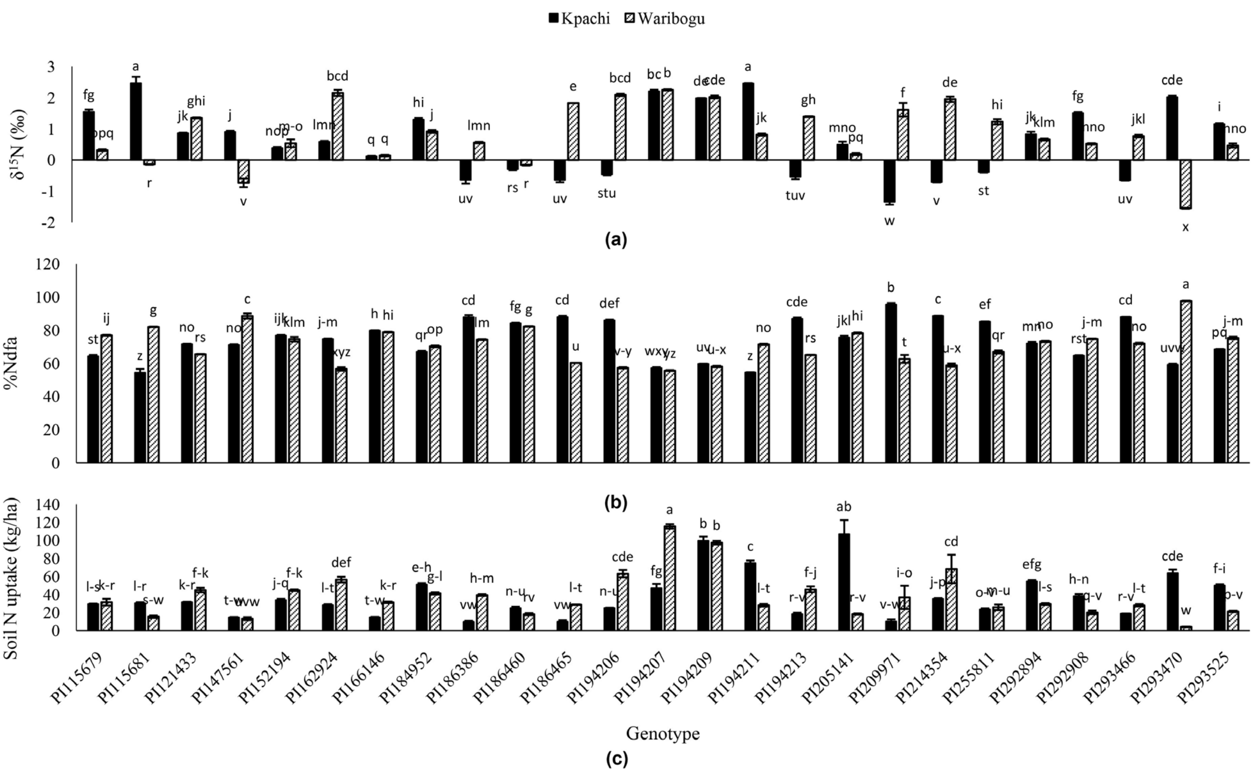

Figure 3. The interactive effects of genotype x location on (a) $\delta^{15}N$, (b) %Ndfa and (c) soil N uptake of 25 cowpea genotypes planted at Kpacchi and Woribogu in 2017. Bars with dissimilar letters are significantly different ($p < 0.001$). Error bars indicate standard errors.

Kpachi, and from 55.6% to 97.7% for genotypes PI194207 and PI293470, respectively, at Woribogu (Figure 3(b)). Genotypes PI194207 and PI194209 recorded low %Ndfa values in both locations due to their high $\delta^{15}N$ values (Figure 3(a) and Figure 3(b)).

In general, the genotypes which elicited lower $\delta^{15}N$ and higher %Ndfa values were expectedly found to exhibit lower soil mineral N uptake between the two locations, albeit a few exceptions (**Figures 3(a)-(c)**). Consequently, genotype PI293470 exhibited much lower soil N uptake at Woribogu due to its reduced δ^{15}N and greater %Ndfa at the site. Similarly, genotypes PI209971, PI214354 and several others recorded much lower soil N uptake at Kpachi due to their high %Ndfa at that location (**Figure 3(b)** and **Figure 3(c)**). Comparison between locations revealed an overall lower δ^{15}N at Kpachi, which resulted in higher %Ndfa and N-fixed by genotypes at the site when compared to those planted at Woribogu (**Table 1**).

3.4. Shoot DM, N-Fixed and N Accumulation of Cowpea Genotypes

There was a significant effect ($p \leq 0.05$) of genotype × location interaction on shoot DM of the cowpeas evaluated (**Table 1**), with values ranging from 10.1 g/plant to 73.1 g/plant at Kpachi and 10.6 g/plant to 46.0 g/plant at Woribogu (**Figure 4(a)**). At Kpachi, genotype PI205141 recorded the highest shoot DM followed by PI209971 and PI214354, also with higher shoot DM than the remaining genotypes at the site. Of the 25 genotypes evaluated, ten (10) produced higher ($p \leq 0.05$) shoot DM at Kpachi than their respective counterparts at Woribogu as opposed to seven genotypes which exhibited greater shoot DM at Woribogu when compared to their respective counterparts at Kpachi (**Figure 4(a)**). The amounts of N-fixed and shoot N content of the cowpea genotypes closely mirrored the patterns in their shoot DM between the two locations (**Figure 4(b)**). Consequently, genotypes PI205141, PI209971, PI214354 and several others coupled greater shoot DM at Kpachi with higher ($p \leq 0.05$) N-fixed and shoot N content when compared to their counterparts at Woribogu (**Figures 4(a)-(c)**). With similar shoot DM therefore, genotypes such as PI115681, PI121433, PI162924, PI184952, PI186465 and others exhibited similar N-fixed and N content between the two locations (**Figures 4(a)-(c)**). In this study, the amounts of N-fixed ranged from 36.7 kg/ha in genotype PI147561 to 336.6 kg/ha in PI205141 at Kpachi, and from 43.8 kg/ha in genotype PI86465 to 176.9 kg/ha in PI293470 at Woribogu (**Figure 4(b)**). Here, a location-wise comparison revealed greater overall shoot DM, N-fixed and shoot N accumulation in the plants at Kpachi relative to those grown at Woribogu (**Table 1**).

3.5. Shoot N and C Concentrations (%N and %C), and C/N Ratio

A two-way ANOVA also revealed significant effects of genotype, location and their interaction on the %N, %C and C/N ratio of cowpea genotypes (**Table 1** and 2). However, except for genotypes PI186386 and PI214354 which exhibited differences in N concentration (%N) between the two locations, the remaining genotypes had similar %N regardless of the test locations (**Figure 5(a)**). The %N in shoots ranged from 2.0% in genotype PI209971 to 4.1% in PI186465 at Kpachi, and from 2.5% in genotype PI209971to 3.8% in PI292908 at Woribogu (**Figure 5(a)**). Similarly, except for genotypes PI209971 and PI214354 which had higher shoot C concentration (%C) at Kpachi when compared to their respective

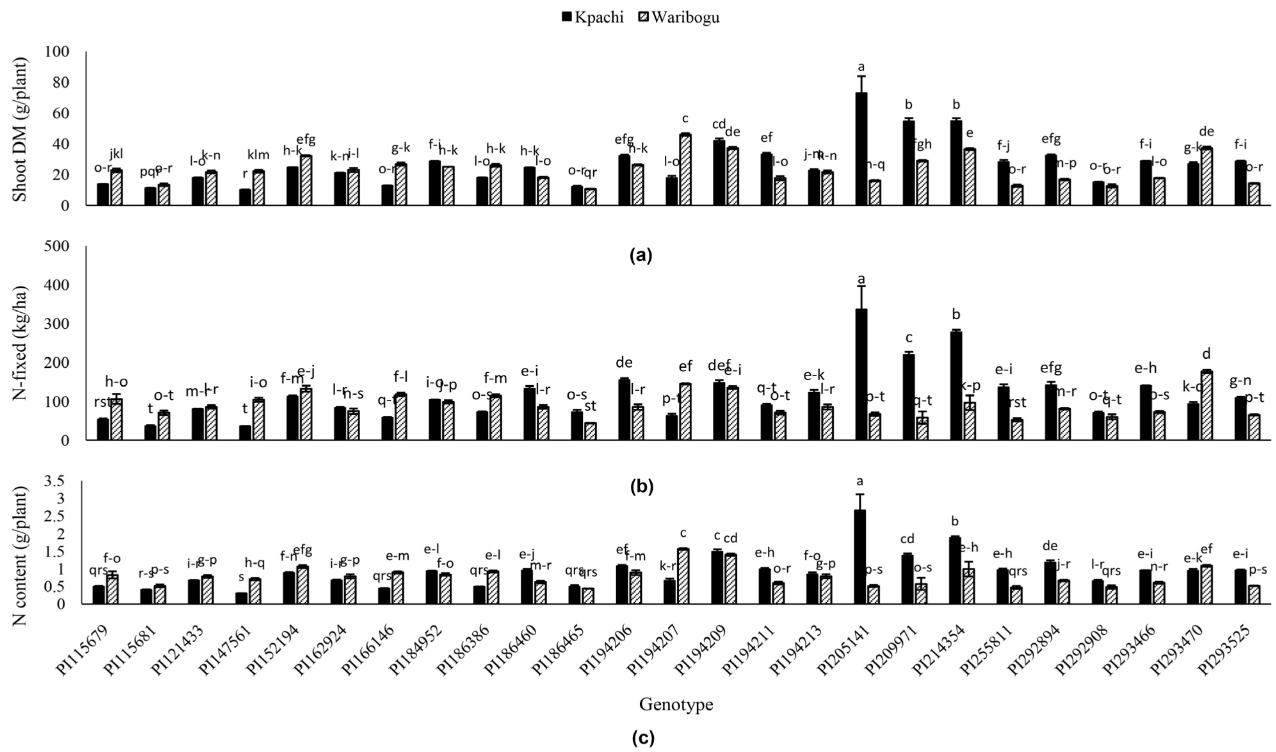

Figure 4. The interactive effects of genotype × location on (a) shoot DM (b) N-fixed and (c) N content of 25 cowpea genotypes planted at Kpacchi and Woribogu in 2017. Bars with dissimilar letters are significantly different ($p < 0.001$). Error bars indicate standard errors.

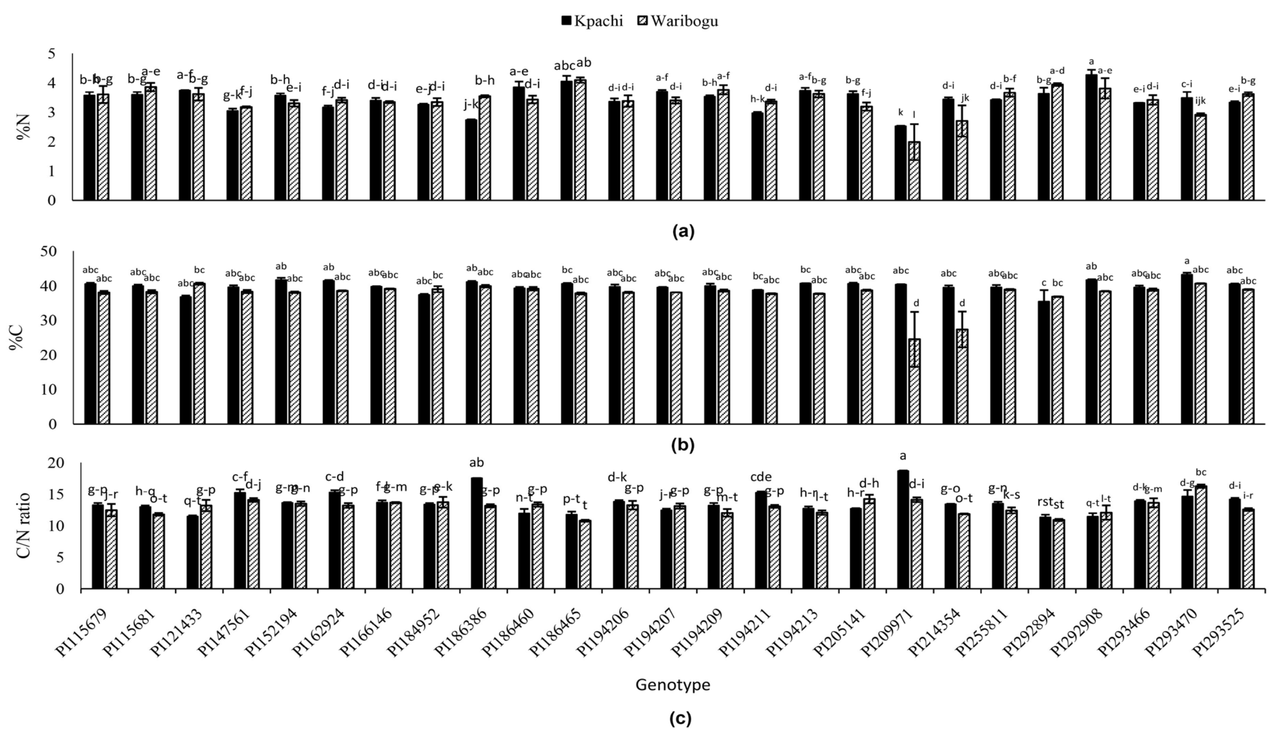

Figure 5. The interactive effects of genotype × location on (a) %N, (b) %C and (c) C/N ratio of 25 cowpea genotypes planted at Kpacchi and Woribogu in 2017. Bars with dissimilar letters are significantly different ($p < 0.001$). Error bars indicate standard errors.

counterparts at Woribogu, the remaining genotypes had similar %C between the two locations (**Figure 5(b)**). The %C in shoots ranged from 35.4 in genotype PI292894 to 43.2% in PI293470 at Kpachi, and from 24.5% in PI209971 to 40.6% in PI121433 at Woribogu (**Figure 5(b)**). Moreover, correlation and regression analysis revealed significant relationship ($r = 0.52$, $r^2 = 0.27$, $p < 0.001$) between %N and %C in shoots of the test cowpea genotypes evaluated in both locations. Of the 25 genotypes evaluated, only PI121433 recorded lower C/N ratio at Kpachi relative to its counterpart at Woribogu, as opposed to lower C/N ratio recorded by genotypes PI162924, PI186386, PI194211 and PI209971 at Woribogu when compared to their respective counterparts at Kpachi (**Figure 5(c)**). Except for the five genotypes which exhibited differences in C/N ratio between locations, the remaining genotypes recorded similar values regardless of planting location (**Figure 5(c)**). In general, the C/N ratio of the test cowpeas were low in both locations, with values ranging from 11.3 g/g to 18.6 g/g at Kpachi and 10.8 g/g to 16.3 g/g at Woribogu (**Figure 5(c)**). Although the average %N was similar between locations (**Table 1**), the plants at Kpachi recorded higher shoot C concentration leading to much lower C/N ratio relative to plants grown at Woribogu (**Table 2**).

3.6. Shoot δ^{13}C of Cowpea Genotypes

As with %N and %C, some genotypes exhibited contrasting shoot δ^{13}C values between the test locations while others recorded similar values regardless of the location (**Figure 6(a)**). For example, genotypes such as PI293470, PI292908, PI292894, PI214354, PI194213, PI194207 exhibited much higher δ^{13}C values at Kpachi when compared to their respective counterparts at Woribogu, with values ranging from −27.9‰ in genotype PI194209 to −26.2‰ in PI292908 at the former location (**Figure 6(a)**). Conversely, the cowpea genotypes PI147561, PI152194, PI162924, PI186465, PI255811 and PI293525 recorded much higher δ^{13}C at Woribogu when compared to their respective genotypes planted at Kpachi, with values ranging from −27.8‰ in genotypes PI293525 and PI162924 to −26.2‰ in PI186465 (**Figure 6(a)**). However, the remaining cowpea genotypes elicited similar δ^{13}C values between the two locations (**Figure 6(a)**). The highest δ^{13}C value was recorded in genotype PI186465 (−26.2‰) at Woribogu, followed by genotypes PI292894 (−26.3‰) and PI292908 (−26.2‰) with similarly high δ^{13}C at Kpachi (**Figure 6(a)**).

3.7. Grain Yield of Cowpea Genotypes

As with all measured parameters, there were significant effects of genotype, location as well as their interaction on grain yield of the cowpeas evaluated (**Table 2**). Grain yield ranged from as low as 56.2 kg/ha in genotype PI166146 to 555.7 kg/ha in PI293525 at Kpachi, and from 142.7 kg/ha in PI16294 to 747.8 kg/ha in PI293466 at Woribogu (**Figure 6(b)**). Of all the genotypes evaluated, seven (7) produced much higher grain yield at Kpachi when compared to their respective counterparts at Woribogu (**Figure 6(b)**). Conversely, nine (9) of the genotypes

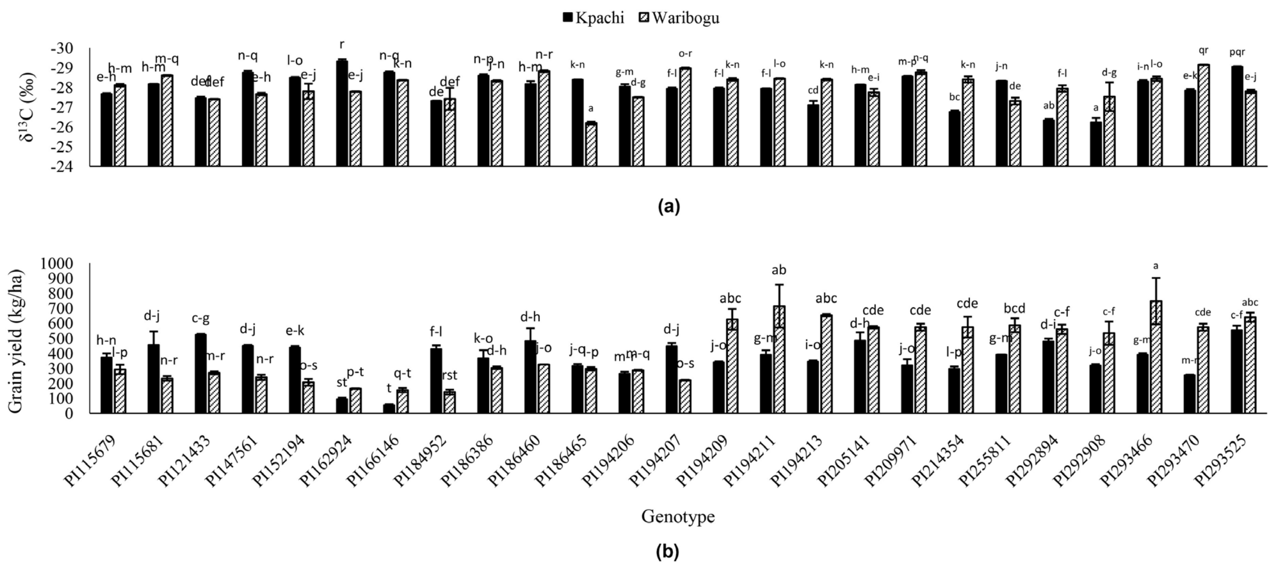

Figure 6. The interactive effects of genotype × location on (a) $\delta^{13}C$ and (b) grain yield of 25 cowpea genotypes planted at Kpacchi and Woribogu in 2017. Bars with dissimilar letters are significantly different ($p < 0.001$). Error bars indicate standard errors.

Table 2. A two-way ANOVA F statistics and the main effect of location on shoot C concentration, $\delta^{13}C$, C/N ratio, number of days to flowering and maturity, and grain yield of 25 cowpea genotypes planted at Woribogu and Kpachi in the Kumbungu District of the Northern Region, Ghana. For locations, values (mean ± se) with dissimilar letters in a column are significantly different at ***$p < 0.001$.

	Shoot C concentration		C/N ratio	$\delta^{13}C$	Grain yield
	%	g/plant	-	‰	kg/ha
Location					
Waribogu	37.6 ± 0.5b	8.7 ± 0.4b	13.0 ± 0.2b	−28.05 ± 0.08b	420 ± 24.3a
Kpachi	39.9 ± 0.2a	10.9 ± 0.7a	13.7 ± 0.2a	−27.99 ± 0.09a	371 ± 14.6b
F-statistics					
Genotype (G)	3.7***	33.8***	14.7***	18.8***	18.4***
Location (L)	29.8***	86.8***	26.3***	1.8***	17.9***
G × L	3.6***	33.9***	5.9***	19.0***	13.5***

There was a significant correlation ($r = 0.52$, $r^2 = 0.27$, $p < 0.001$) between %N and %C in shoots.

evaluated recorded much higher grain at Woribogu when compared to their respective counterparts planted at Kpachi (Figure 6(b)). The highest grain yield was recorded in genotype PI293466 (748 kg/ha) planted at Woribogu followed by genotypes PI194211, PI194209, PI194213 and PI293525, with similarly high grain yield (627 kg/ha to 714 kg/ha) at Woribogu (Figure 6(b)). As a result, the overall grain yield of the genotypes evaluated were markedly higher ($p \le 0.05$) at Woribogu (420 kg/ha) relative to Kpachi (371 kg/ha) (Table 2).

4. Discussion

4.1. Symbiotic Performance of Field-Grown Cowpea Genotypes

Cowpea is an important grain legume in most parts of the world and serves as source of protein in the diets of millions in Africa [32]. Aside the nutritional attributes of cowpea, the crop contributes to sustainable farming systems due to its nitrogen contribution to soils when in symbiotic relationship with the soil bacteria collectively termed rhizobia [1]. Owing to its drought tolerance and N_2-fixing trait, cowpea grows well and produces yield on marginal soils that characterize most parts of Africa [5] [17]. Nevertheless, low soil fertility and drought among other factors continue to limit cowpea yields. This study explored symbiotic performance and water relations among USDA cowpea genotypes to assess their suitability for growth in low nutrient soils and drought conditions.

In this study, the difference between the $\delta^{15}N$ value of reference plants and those of the test cowpeas was sufficiently large (>+2‰) [11], and therefore allowed for precise estimation of the percent of legume N derived from N_2 fixation. As found in studies of other grain legumes [3] [6] [10], the cowpea genotypes in this study exhibited high variability in N_2 fixation in association with native soil rhizobia as evidenced by the marked differences in their shoot $\delta^{15}N$ values. The $\delta^{15}N$ values of plant tissues closely reflect the N sources being assimilated, making the ^{15}N natural abundance technique a powerful tool in estimating a legume's dependence on symbiotic N_2 fixation [11]. Despite the tendency for cowpea genotypes to elicit both positive and negative $\delta^{15}N$ values in the test locations, majority of the cowpeas had much lower $\delta^{15}N$ values when planted at Kpachi, probably due to the slightly lower N concentration in soils at the site relative to Woribogu (**Table S1**). These findings are consistent with previous studies which found that high soil N inhibits N_2 fixation in symbiotic legumes [33], and that marginal differences in soil N between locations altered the $\delta^{15}N$ of symbiotic Kersting's groundnut [10]. The variations in shoot $\delta^{15}N$ values within genotypes and between locations were expectedly accompanied by differences in the percent of N derived from symbiotic N_2 fixation (%Ndfa) by the test cowpeas in this study. As to be expected, the %Ndfa by the cowpeas closely mirrored patterns of their $\delta^{15}N$ signatures, with low $\delta^{15}N$ values denoting greater symbiotic dependence, and *vice versa* (**Figure 3**), a finding consistent with known reports that legumes relying on symbiosis tend to have lower $\delta^{15}N$ values when compared to those highly dependent on mineral N for their nutrition [11]. Despite the observed variations in symbiotic dependence by cowpeas in the present study, they generally elicited high symbiotic performance, and derived between 54.5% to 95.5% of their N requirements from symbiosis at Kpachi, and from 55.6% to 97.7% at Woribogu (**Figure 3**). These findings are similar to an earlier report by Belane and Dakora [5], who found that some field-grown cowpeas can derive up to 96% of their N requirements from symbiosis. Because of the high %Ndfa values in this study, the amounts of N-fixed by the cowpeas were expectedly greater than their soil N uptake. Nevertheless, the fact that low N-fixed values were generally accompanied by relatively higher soil N uptake suggests that the two played complementary roles in supporting the growth of the test cowpeas. Consequently, increased shoot DM in the test cowpeas were mostly accompanied by high N-fixed and shoot N accumulation (**Figure 4**). The fact that the amounts of N-fixed could reach 176.9 kg/ha in genotype PI293470 at Woribogu and 336.6 kg/ha in PI205141 at Kpachi, with most other genotypes recording over 100 kg·N-fixed·ha^{-1} within the study locations (**Figure 4(b)**) suggest that, these USDA genotypes can potentially contribute high amounts of symbiotic N into cropping systems to benefit subsequent non-legumes.

4.2. C assimilation and Water Use Efficiency Cowpea Genotypes

In this study, except for genotypes PI209971 and PI214354 which exhibited dramatically low %C at Woribogu (24.5% and 27.4%), the concentrations (%) of N and C in shoots of the remaining genotypes were comparable to values previously reported for field-grown cowpeas [34]. The observed high %C in shoots of the test cowpeas possibly from increased rates of photosynthesis is a desirable trait that could be tapped for environmental carbon sequestration. Moreover, the fact that N and C nutrition are functionally linked via photosynthetic C assimilation in symbiotic legumes [34] [35] was demonstrated by a significant correlation ($r = 0.52$, $r^2 = 0.27$, $p < 0.001$) between %N and %C in this study. Furthermore, the test cowpeas in this study elicited low C/N ratio, with values ranging from 10.8% to 16.8%, agreeing with earlier studies which showed that, N_2 fixation by bacteroids in root nodules confers low C/N ratio (typically < 24) in legumes when compared to non-legume plants which often exhibit higher C/N ratio (>24) [3] [36].

With erratic rainfall resulting from climate change and its uncertainties, crop genotypes exhibiting desirable traits related to improved water relations can help to reduce the impact of drought on crop growth and yield [17]. Because stomatal closure during drought conditions results in reduced discrimination against $^{13}CO_2$ during photosynthesis in C3 plants (leading to higher $\delta^{13}C$ values), higher $\delta^{13}C$ in plant tissues denote greater water use efficiency, while increased discrimination under conditions of adequate soil moisture leads to lower $\delta^{13}C$ or water use efficiency [23]. The cowpea genotypes evaluated in this study elicited marked variations in shoot $\delta^{13}C$ values, indicating variations in their water use efficiency (**Figure 6(a)**). When planted at Kpachi, genotypes PI292908, PI292894 exhibited the highest shoot $\delta^{13}C$ values (−26.2‰ and −26.3‰, respectively) followed by PI214354 (−26.8‰), indicating higher water use efficiency relative to the other genotype at the site (**Figure 6(a)**). However, the fact that these genotypes elicited much lower $\delta^{13}C$ values relative to genotype PI186465 at Woribogu ($\delta^{13}C$ = −26.2‰) suggests that the test cowpeas responded differently to environmental variables between the two locations. The observed $\delta^{13}C$ values of the test cowpeas in this study are within the range (−25‰ to −35‰) reported for C3 plants, although some genotypes recorded higher $\delta^{13}C$ values than the average ($\delta^{13}C$ = −28‰) reported for most C3 species [37]. An earlier study by Mohale *et al.* [3] as well as Mapope and Dakora [8], respectively reported $\delta^{13}C$ values as high as −26.2‰ and −25.1‰ in field-grown Bambara groundnut and soybean in South Africa. The relatively high water use efficiency (high $\delta^{13}C$ values) elicited by most genotypes in this study could be attributed to late planting which coincided with periods of low rainfall (**Figure 2**), thus causing partial stomatal closure and reduced ^{13}C discrimination. The observed variations in water use efficiency among the test USDA genotypes offers opportunity for selection and further improvement for enhanced water relations in cowpea. The fact that overall shoot $\delta^{13}C$ of the test cowpeas was higher at Kpachi (−27.99‰) than Woribogu (−28.05‰) could be attributed to relatively lower soil moisture observed at the former location during crop establishment which also shortened the days to flowering and maturity of plants at the site (**Figure 1**).

In this study, planting was carried out in late August such that rainfall was low during the vegetative phase, and almost absent during podding (**Figure 1**). Consequently, the genotypes produced low yields (less than 1000 kg/ha) when compared to values of up to 3500 kg/ha reported for cowpea in other field studies [38]. In this study, grain yield ranged from 56 kg/ha in genotype PI166146 at Kpachi to 748 kg/ha in genotype PI293466 at Woribogu (**Figure 6(b)**). Thus, the genotypes exhibited marked variations in grain yield production between the two locations, although most of them recorded much higher values when grown at Woribogu. As a result, the overall grain yield of the test cowpeas was generally higher at Woribogu (420 kg/ha) when compared to the average grain yield of the plants at Kpachi (371 kg/ha), a finding that could also be due to the slightly higher concentrations of most of the measured nutrient elements (**Table S1**) as well as moisture in the soils at Woribogu relative to Kpachi.

5. Conclusion

In conclusion, the present study explored the symbiotic N nutrition, C assimilation and water use efficiency among USDA cowpea genotypes subjected to drought under field conditions. The findings revealed marked variations in N_2 fixation, with genotypes deriving between 55.4% to 97.7% of their N requirements from symbiosis, corresponding to N-fixed values between 36.7 kg/ha to 336.6 kg/ha. The test cowpeas were also found to show remarkable variations in water use efficiency, with most genotypes exhibiting contrasting $\delta^{13}C$ values between locations. These findings further highlight the presence of genotypic variations in symbiotic performance and water relations among cowpeas, with most genotypes in this study eliciting contrasting responses to the differences in edaphoclimatic conditions between locations. The observed high symbiotic dependence recorded in this study despite drought conditions further explains the wider adaptation of cowpea to various climatic conditions. Moreover, the fact that some genotypes could grow and produce grain yields of 627 - 748 kg/ha under imposed drought is an important trait that could be tapped for further improvement of cowpea. The results of this study offer a useful contribution to the available literature on the symbiotic performance and water relations in nodulated legumes.

Acknowledgements

The work was funded by Borlaug Higher Education for Agriculture Research Development (BHEARD) under United States Agency for Development as part of the feed for the future initiative. We acknowledge the institutional support from the Tennessee State University, University for Development Studies (UDS) and the Savannah Agriculture Research Institute (SARI). MWB is supported by an Evans Allen project of the United States Department of Agricultural (TEN-X) for breeding of photosynthetic efficiency. We are grateful for useful discussions with Dr. Lucas Mackasmiel and statistical advice from Mr. Daniel Ambachew Demissie at TSU.

Conflicts of Interest

The authors declare that there is no conflict of interest.

References

[1] Kermah, M., Franke, A.C., Adjei-Nsiah, S., Ahiabor, B.D.K., Abaidoo, R.C. and Giller, K.E. (2018) N_2-Fixation and N Contribution by Grain Legumes under Different Soil Fertility Status and Cropping Systems in the Guinea Savanna of Northern Ghana. *Agriculture, Ecosystems and Environment*, **261**, 201-210. https://doi.org/10.1016/j.agee.2017.08.028

[2] Nyemba, R.C. and Dakora, F.D. (2010) Evaluating N_2 Fixation by Food Grain Legumes in Farmers' Fields in Three Agro-Ecological Zones of Zambia, Using ^{15}N Natural Abundance. *Biology and Fertility of Soils*, **46**, 461-470. https://doi.org/10.1007/s00374-010-0451-2

[3] Mohale, K.C., Belane, A.K. and Dakora, F.D. (2014) Symbiotic N Nutrition, C Assimilation, and Plant Water Use Efficiency in Bambara Groundnut (*Vigna subterranea* L. Verdc) Grown in Farmers' Fields in South Africa, Measured Using ^{15}N and ^{13}C Natural Abundance. *Biology and Fertility of Soils*, **50**, 307-319. https://doi.org/10.1007/s00374-013-0841-3

[4] Marandu, E.T., Semu, E., Mrema, J.P. and Nyaki, A.S. (2013) Contribution of Legume Rotations to the Nitrogen Requirements of a Subsequent Maize Crop on a Rhodic Ferrasol in Tanga, Tanzania. *Tanzania Journal of Agricultural Sciences*, **12**, 23-29. https://doi.org/10.4314/eajsci.v2i2.40343

[5] Belane, A.K. and Dakora, F.D. (2010) Symbiotic N_2 Fixation in 30 Field-Grown Cowpea (*Vigna unguiculata* L. Walp.) Genotypes in the Upper West Region of Ghana Measured Using ^{15}N Natural Abundance. *Biology and Fertility of Soils*, **46**, 191-198. https://doi.org/10.1007/s00374-009-0415-6

[6] Oteng-Frimpong, R. and Dakora, F.D. (2018) Selecting Elite Groundnut (*Arachis hypogaea* L) Genotypes for Symbiotic N Nutrition, Water-Use Efficiency and Pod Yield at Three Field Sites , Using ^{15}N and ^{13}C Natural Abundance. *Symbiosis*, **75**, 229-243. https://doi.org/10.1007/s13199-017-0524-1

[7] Chibeba, M.A., Kyei-boahen, S., Guimarães, M.D.F., Nogueira, A.M. and Hungria, M. (2017) Isolation, Characterization and Selection of Indigenous *Bradyrhizobium* Strains with Outstanding Symbiotic Performance to Increase Soybean Yields in Mozambique. *Agriculture, Ecosystems and Environment*, **246**, 291-305. https://doi.org/10.1016/j.agee.2017.06.017

[8] Mapope, N. and Dakora, F.D. (2016) N_2 Fixation, Carbon Accumulation, and Plant

Water Relations in Soybean (*Glycine max* L. Merrill) Varieties Sampled from Farmers' Fields in South Africa, Measured Using ^{15}N and ^{13}C Natural Abundance. *Agriculture*, *Ecosystems and Environment*, **221**, 174-186.
https://doi.org/10.1016/j.agee.2016.01.023

[9] Samago, T.Y., Anniye, E.W. and Dakora, F.D. (2018) Grain Yield of Common Bean (*Phaseolus vulgaris* L.) Varieties Is Markedly Increased by Rhizobial Inoculation and Phosphorus Application in Ethiopia. *Symbiosis*, **75**, 245-255.
https://doi.org/10.1007/s13199-017-0529-9

[10] Mohammed, M., Jaiswal, S.K., Sowley, E.N.K., Ahiabor, B.D.K. and Dakora, F.D. (2018) Symbiotic N_2 Fixation and Grain Yield of Endangered Kersting's Groundnut Landraces in Response to Soil and Plant Associated *Bradyrhizobium* Inoculation to Promote Ecological Resource-Use Efficiency. *Frontiers in Microbiology*, **9**, 2105.
https://doi.org/10.3389/fmicb.2018.02105

[11] Unkovich, M., Herridge, D., Peoples, M., Cadisch, G., Boddey, B., Giller, K., Alves, B. and Chalk, P. (2008) Measuring Plant-Associated Nitrogen Fixation in Agricultural Systems. Australian Centre for International Agricultural Research, Canberra, Australia.

[12] Mitra, J. (2001) Genetics and Genetic Improvement of Drought Resistance in Crop Plants. *Current Science*, **80**, 758-763.

[13] Watanabe, I., Terao, T. and Tomio, T. (1998) Drought Tolerance of Cowpea (*Vigna unguiculata* (L.) Walp.) 2: Field Trial in the Dry Season of Sudan Savanna and Dry Matter Production of Potted Plants under Water-Stress. *JIRCAS Journal for Scientific Papers*, **6**, 29-37.

[14] Picasso, V.D., Casler, M.D. and Undersander, D. (2019) Resilience, Stability, and Productivity of Alfalfa Cultivars in Rainfed Regions of North America. *Crop Science*, **59**, 800-810. https://doi.org/10.2135/cropsci2018.06.0372

[15] Belane, A.K. and Dakora, F.D. (2012) Elevated Concentrations of Dietarily-Important Trace Elements and Macronutrients in Edible Leaves and Grain of 27 Cowpea (*Vigna unguiculata* L. Walp.) Genotypes: Implications for Human Nutrition and Health. *Food and Nutrition Sciences*, **3**, 377-386.
https://doi.org/10.4236/fns.2012.33054

[16] FAO (2016). http://www.fao.org/faostat/en. Accessed on 2018-05-23

[17] Hall, A.E. (2012) Phenotyping Cowpeas for Adaptation to Drought. *Frontiers in Physiology*, **3**, 155-162. https://doi.org/10.3389/fphys.2012.00155

[18] Agyeman, K., Osei-Bonsu, I. and Tetteh, E.N. (2014) Growth and Yield Performance of Improved Cowpea (*Vigna unguiculata* L.) Varieties in Ghana. *Agricultural Science*, **2**, 44-52. https://doi.org/10.12735/as.v2i4p44

[19] Cobbinah, F.A., Addo-Quaye, A.A. and Asante, I.K. (2011) Characterization, Evaluation and Selection of Cowpea (*Vigna unguiculata* (L.) Walp.) Accessions with Desirable Traits from Eight Regions of Ghana. *ARPN Journal of Agricultural and Biological Science*, **6**, 21-32.

[20] Van Loon, A.F. (2015) Hydrological Drought Explained. *Wiley Interdisciplinary Reviews*: *Water*, **2**, 359-392. https://doi.org/10.1002/wat2.1085

[21] Hadebe, S.T., Modi, A.T. and Mabhaudhi, T. (2017) Drought Tolerance and Water Use of Cereal Crops: A Focus on Sorghum as a Food Security Crop in Sub-Saharan Africa. *Journal of Agronomy and Crop Science*, **203**, 177-191.
https://doi.org/10.1111/jac.12191

[22] Medrano, H., Tomás, M., Martorell, S., Flexas, J., Hernández, E., Rosselló, J., Pou,

A., Escalona, J. and Bota, J. (2015) From Leaf to Whole-Plant Water Use Efficiency (WUE) in Complex Canopies: Limitations of Leaf WUE as a Selection Target. *The Crop Journal*, **3**, 220-228. https://doi.org/10.1016/j.cj.2015.04.002

[23] Farquhar, G.D., Ehleringer, J.R. and Hubick, K.T. (1989) Carbon Isotope Discrimination and Photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**, 503-537. https://doi.org/10.1146/annurev.pp.40.060189.002443

[24] Owusu, K. and Waylen, P.R. (2013) The Changing Rainy Season Climatology of Mid-Ghana. *Theoretical and Applied Climatology*, **112**, 419-430. https://doi.org/10.1007/s00704-012-0736-5

[25] Walkley, A. and Black, I.A. (1934) An Examination of the Degtjareff Method for Determining Soil Organic Matter, and a Proposed Modification of the Chromic Acid Titration Method. *Soil Science*, **37**, 29-38. https://doi.org/10.1097/00010694-193401000-00003

[26] Bray, R.H. and Kurtz, L.T. (1945) Determination of Total, Organic, and Available Forms of Phosphorus in Soils. *Soil Science*, **59**, 39-46. https://doi.org/10.1097/00010694-194501000-00006

[27] Shearer, G. and Kohl, D.H. (1986) N_2-Fixation in Field Settings: Estimations Based on Natural ^{15}N Abundance. *Australian Journal of Plant Physiology*, **13**, 699-756. https://doi.org/10.1071/PP9860699

[28] Pausch, R.C., Mulchi, C.L., Lee, E.H. and Meisinger, J.J. (1996) Use of ^{13}C and ^{15}N Isotopes to Investigate O_3 Effects on C and N Metabolism in Soybeans. Part II. Nitrogen Uptake, Fixation, and Partitioning. *Agriculture, Ecosystems and Environment*, **60**, 61-69. https://doi.org/10.1016/S0167-8809(96)01062-6

[29] Maskey, S.L., Bhattarai, S., Peoples, M.B. and Herridge, D.F. (2001) On-Farm Measurements of Nitrogen Fixation by Winter and Summer Legumes in the Hill and Terai Regions of Nepal. *Field Crops Research*, **70**, 209-221. https://doi.org/10.1016/S0378-4290(01)00140-X

[30] Craig, H. (1957) Isotopic Standards for Carbon and Oxygen and Correction Factors for Mass-Spectrometric Analysis of Carbon Dioxide. *Geochimica et Cosmochimica Acta*, **12**, 133-149. https://doi.org/10.1016/0016-7037(57)90024-8

[31] Statsoft Inc. (2011) Statistica (Data Analysis Software System). Version 10. https://www.Statsoft.Com

[32] Belane, A.K. and Dakora, F.D. (2011) Levels of Nutritionally-Important Trace Elements and Macronutrients in Edible Leaves and Grain of 27 Nodulated Cowpea (*Vigna unguiculata* L. Walp.) Genotypes Grown in the Upper West Region of Ghana. *Food Chemistry*, **125**, 99-105. https://doi.org/10.1016/j.foodchem.2010.08.044

[33] Mbah, G.C. and Dakora, F.D. (2017) Nitrate Inhibition of N_2 Fixation and Its Effect on Micronutrient Accumulation in Shoots of Soybean (*Glycine max* L. Merr.), Bambara Groundnut (*Vigna subterranea* L. Vedc) and Kersting's Groundnut (*Macrotyloma geocarpum Harms*.). *Symbiosis*, **2**, 205-216. https://doi.org/10.1007/s13199-017-0531-2

[34] Belane, A.K. and Dakora, F.D. (2015) Assessing the Relationship between Photosynthetic C Accumulation and Symbiotic N Nutrition in Leaves of Field-Grown Nodulated Cowpea (*Vigna unguiculata* L. Walp.) Genotypes. *Photosynthetica*, **53**, 562-571. https://doi.org/10.1007/s11099-015-0144-z

[35] Mohammed, M., Jaiswal, S.K. and Dakora, F.D. (2018) Distribution and Correlation between Phylogeny and Functional Traits of Cowpea (*Vigna unguiculata* L. Walp.)—Nodulating Microsymbionts from Ghana and South Africa. *Scientific Re-*

ports, **8**, Article No. 18006. https://doi.org/10.1038/s41598-018-36324-0

[36] Hobbie, E.A., MacKo, S.A. and Shugart, H.H. (1998) Patterns in N Dynamics and N Isotopes during Primary Succession in Glacier Bay, Alaska. *Chemical Geology*, **152**, 3-11. https://doi.org/10.1016/S0009-2541(98)00092-8

[37] O'Leary, M.H. (1988) Carbon Isotopes in Photosynthesis: Fractionation Techniques May Reveal New Aspects of Carbon Dynamics in Plants. *BioScience*, **38**, 328-336. https://doi.org/10.2307/1310735

[38] Belane, A.K., Asiwe, J. and Dakora, F.D. (2011) Assessment of N_2 Fixation in 32 Cowpea (*Vigna unguiculata* L. Walp.) Genotypes Grown in the Field at Taung in South Africa, Using ^{15}N Natural Abundance. *African Journal of Biotechnology*, **10**, 11450-11458.

Supplementary

Table S1. Physico-chemical properties of soils sampled from Kpachi and Woribogu prior to planting in 2017.

	Sand	Clay	Silt	pH	O.C	N	P	K	Na	Ca	Mg	CEC
Location		%		-	%				mg/kg			Cmol(+)/kg
Kpachi	80.28	1.81	17.91	5.40	0.56	0.05	5.8	78.0	2.3	570	64.8	5.0
Woribogu	78.2	3.67	18.13	5.62	0.61	0.06	6.5	93.6	4.6	460	60.0	4.1