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Variation in winged bean (*Psophocarpus tetragonolobus*) growth parameters, seed yield, nodulation, and nitrogen fixation

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Abstract. *Adegboyega TT, Abberton MT, Abdelgadir AH, Mahamadi D, Olaniyi OA, Ofodile S, Babalola OO. 2021. Variation in winged bean (*Psophocarpus tetragonolobus*) growth parameters, seed yield, nodulation, and nitrogen fixation. Asian J Agric 5: 61-71.* Underutilized legumes are widely distributed in tropical agriculture and associated with low yield. Thus they have not really been fully explored due to lack of research investment, breeding programs targeting crop improvement, marketing, and low awareness of nutritional benefits. This study was conducted to determine the variation in growth parameters, seed yield, nodulation, and nitrogen fixation in winged bean (*Psophocarpus tetragonolobus*) germplasms. High genotypic and phenotypic coefficients of variances were observed in traits evaluated. The combined analysis of variance for five variables of 25-winged bean accessions showed that replication by year interaction was statistically significant ($p \leq 0.0001$) for nodule parameters and dry shoot weight while it was not significant ($p = > 0.05$) for dry root weight, and total biomass. Significant variations ($p = \leq 0.05$) were observed among the accessions on some growth parameters. The genetic variability of winged beans could be carefully exploited to provide higher grain yield, as well as other economic and important traits to boost food security and conservation of the plant genetic materials.

Keywords: Crop improvement, genetic variability, nitrogen fixation, sub-Saharan Africa, winged bean

INTRODUCTION

Winged bean (*Psophocarpus tetragonolobus* (L.) DC.,) is a member of the underutilized or orphan legumes in the tropics but with proven ability for vigorous twining habit, Nitrogen fixation capacity and tuberous root system (Eagleton 2020; Vatanparast et al. 2016). It grows in the world's most common agro-ecologies (the hot, humid equatorial countries) (Afridatul and Syukur 2021; Lepcha et al. 2017). The United States Academy of Science gave the limelight to the crop in a 1975 publication and since then research attention has focused on the crop particularly on its agronomy, nutritional composition, and other aspects of crop improvements but subsequently, research interest dwindled but current evidence showed renewed interest at crop promotion and molecular breeding activities being exploited amongst other interventions (Eagleton 2020; Lepcha et al. 2017). The research could focus on germplasm characterization, exploration of genetic resources, employment of modern breeding tools/technologies and soil-related strategies (nodulation, nitrogen fixation, etc.).

The crop is a member of the Fabaceae family, Papilionoidea subfamily, a dicotyledonous plant consisting of a diploid genome and six pairs of long chromosomes estimated at around 1.22 Gb (Giga base pair) in size Vatanparast et al. (2016). Winged beans grow indeterminately, with intertwining vines and lateral branches to support plant growth (Schiavinato and Válio 1996). Certain variations exist in the seed coat color, ranging from cream to black, brown to purple, and mottled colors (Klu and Kumaga 1999; Mohanty et al. 2020). It's a multipurpose crop with extensive culinary uses with different plant parts (flowers, green pods, tubers, seeds, and leaves) (Massawe et al. 2016). It requires less input and produces reasonable amount of food to provide adequate dietary support from the plant parts (Lepcha et al. 2017).

In areas where winged bean is cultivated, particularly in India, New Papua Guinea, Thailand, Ghana, Indonesia, and Burma, various parts of the plant have been used for one meal preparation, confectionaries or the other to suit the need of the local communities (either as snack or whole meal) (Lepcha et al. 2017). Khalili et al. (2013) and Nazri et al. (2011) reported their antimicrobial efficacy in treating common bacterial infections. Wan et al. (2014) have also demonstrated that extracts from winged bean seeds could be useful for industrial applications.

In general, this crop is grown traditionally. It is very common to find winged beans associated with other crops as part of a farming system. It is also part of a rotation, especially with sweet potato (*Ipomoea batatas*) as an alternate crop. Rice, followed by winged bean and sugar cane, is a common rotation in Southeast Asia (Myanmar). In Papua New Guinea, it is very common to find winged beans in association with maize. The maize and bean are planted simultaneously, or the legume is planted later and uses the dry stalks for support. The winged bean is also associated with *Leucaena leucocephala* (Eagleton 2020; Rahman et al. 2014a). The nutrients, mineral composition, and physicochemical properties of winged bean have considerable potential. The seeds, pods, swollen roots, flowers, and foliage are rich in macronutrients (Makeri et al. 2017) and micronutrients (Lepcha et al. 2017). The seed oil content and fatty acids composition may be explored for different industrial uses. Oil obtained from winged beans has been described as better than soybean oil owing to its efficient heating capacity which makes it suitable for frying food. What needs to be done now is to scale up its utilization (Makeri et al. 2016). Winged beans are suitable for cultivation in soils with low fertility as it combines with and fixes nitrogen, useful as a cover crop, and is also used for intercropping systems (Wong et al. 2017). Adegboyega et al. (2019) and Esan et al. (2020) variable levels of nutrients and antinutrients were recorded in the seeds and tubers irrespective of the state in which samples were processed. The consumption of winged beans could help minimize malnutrition and provide alternative substitutes for other legumes. Consequently, there is an urgent need to support the increasing population of the world in the face of poor distribution and recycling of agricultural commodities to further boast food and agricultural sustainability (Tanzi et al. 2019).

This study investigated genetic variability for seed yield, nodulation, and N fixation of field-grown accessions of winged bean without application of fertilizers and inoculants, using ^{15}N and^{13}C natural abundance to identify potential traits accessions for future breeding programs and farming systems.

MATERIALS AND METHODS

Study area

Field experiments were conducted at IITA (latitude 7˚30ˆ8”; longitude 3˚ 54’37”) Ibadan, Southwest Nigeria, on an Alfisol soil of the Egbeda series (Omotoso and Aluko 2016). Every month, rainfall ranged between 0.05 and 86.5 mm, while the minimum and maximum temperatures ranged between 20 and 27°C and from 24 to 35.2 °C (Figure 1).

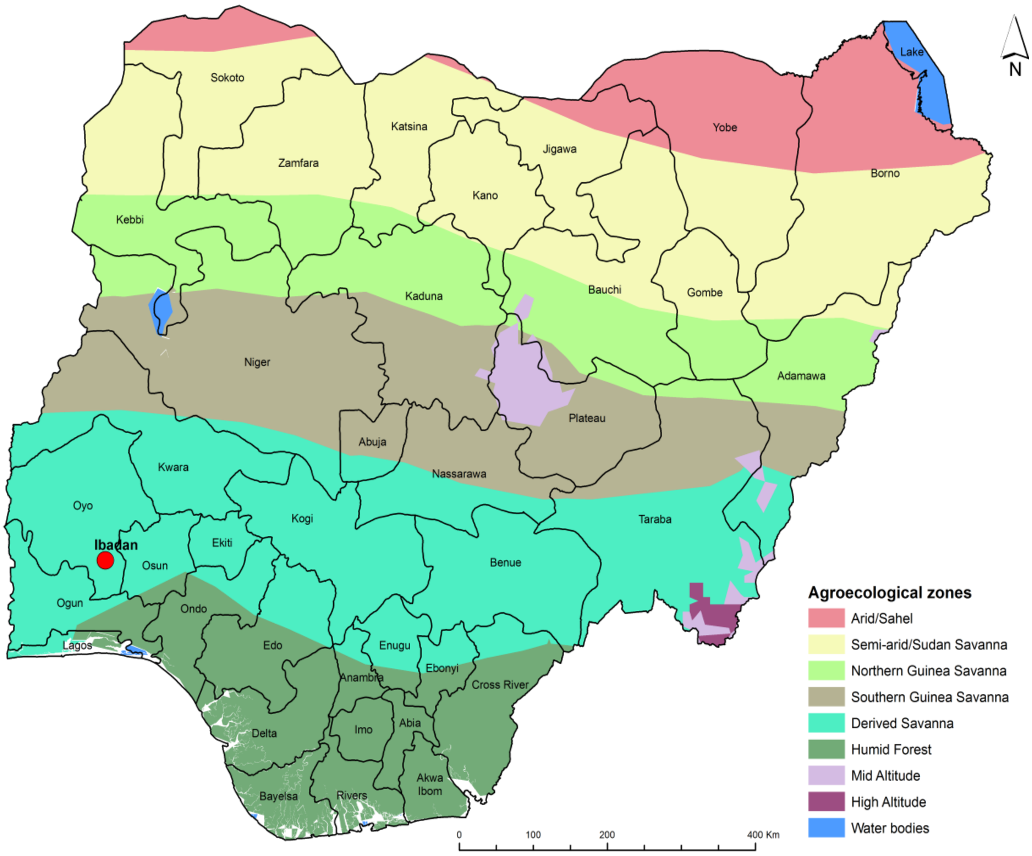

Figure 1. Map of Nigeria showing the various agroecological zones and the study location (Ibadan, in red). Source: Geospatial Information System (GIS) Unit, International Institute of Tropical Agriculture (IITA), Nigeria.

Planting materials and experimental design

Twenty-five accessions of the crop were obtained from the IITA germplasm collection center (Table 1). Soil samples were collected from a depth of less than 20 cm (0-15) analyzed (Table 2) according to Juo (1978). Seeds were planted with 1m spacing between plants on 5m long ridges using standard agronomic procedures for the field experiment conducted in randomized block design in three replicates. The seedlings were staked with plastic-coated stakes four weeks after planting. To keep the experimental field weed-free, manual weeding was done regularly. Neither fertilizers nor bacterial inoculants were applied. Morphological traits measured were terminal petiole length, leaf rachis, terminal leaf length and width, flower width and dry pod weight, and other seed parameters (seed length and thickness, 100-seed fresh weight, seed width, total seed) weight, and total number of seeds) including the pod length. The plant growth and nodulation parameters evaluated included nodule number per accession, nodule dry weight, dry shoot weight, dry root weight, and total biomass. Methuen Handbook of Color was used for seed coat description (Kornerup and Wanscher 1978).

Data collection and sample processing

Morphological measurements were taken from five individual plants from each accession. When the plants were at 50% flowering, shoots were cut off at the base with the roots specifically dug out and washed under flowing tap water. Nodule number, fresh and dry weights, fresh and dry shoot weights, fresh and dry root weights were recorded. Plant shoots were separated into leaves, stems, and petioles. The plant shoots and roots were then oven-dried at 60°C for 48 h. After that, they were measured, and processed to smooth powder in a 0.85-mm sieve to determine the amount of nitrogen fixed and estimates of the carbon and nitrogen ratio in the test plants. Mass spectrometry was carried out the investigation at the Department of Earth and Environmental Sciences, Katholieke Universiteit Leuven, Belgium. The non-fixing plant, *Eleusine indica,* was sampled concurrently from the field and processed for ^{15}N and ^{13}C isotope analysis as described above.

Measurement of N_2 fixation

This was carried out to determine the amount of N fixed by each accession plant. The milled material was carefully dispensed into aluminum tin capsules (0.5–1 mg) and analyzed for %N and $^{15}N/^{14}N$ ratio using a Thermo EA-1110 elemental analyzer (FORNO EA/NA ThermoQuest, Italia, S.P.A). Natural abundance of ^{15}N is expressed as δ (delta) notation and is the per mile deviation of the ^{15}N natural abundance of the sample from atmospheric (atm) N_2 (0.36637 atom % ^{15}N).

The isotopic composition ($\delta^{15}N$) was calculated as described by Unkovich et al. (2008).

$$\Delta^{15}N = \frac{(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{atm}}{(^{15}N/^{14}N)\ atm} \times 1000$$

Where: $^{15}N/^{14}N$ is the abundance ratio of ^{15}N and ^{14}N in the shoot and root samples while the $^{15}N/^{14}Natm$ is the abundance ratio of ^{15}N and ^{14}N in the atmosphere.

The percentage N derived from atmospheric N_2 was calculated by the equation below Unkovich et al. (2008).

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

Where: $\delta^{15}N$ ref represents ^{15}N natural abundance of reference plants, $\delta^{15}N$ leg is the ^{15}N of the legume, and the B value is the ^{15}N of the test legume solely reliant on N_2 fixation. In this study, the B value used was −1.54 as Unkovich et al. (2008) explained.

The quantity of N-fixed was estimated as described by Unkovich et al. (2008);

$$N\ fixed = amount\ of\ N \times \%\ NdfA$$

Analysis of 13C/12C

This was carried out to determine the amount of C and N ratio in the plants of each tested accession. To analyze the $^{13}C/^{12}C$ ratio, shoot and root samples (0.5–1.0 mg) were used following the same procedures described for the $^{15}N/^{14}N$ isotopic ratio. The ratio of $^{13}C/^{12}C$ in the sample was used to calculate the ^{13}C ($\delta^{13}C$) as described by Stout and Rafter (1978).

$$\Delta^{13}C = \frac{((^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{standard-1})}{(^{13}C/^{12}C)_{standard}} \times 1000$$

Where: $^{13}C/^{12}C$ is the isotopic ratio of the sample, and $^{13}C/^{12}C$ standard is the isotopic ratio of the known universally accepted standard for C.

Table 1. Twenty-five accessions of winged bean sourced from Genetic Resources Center, IITA.

Accession	Origin	Seed color
Tpt2	No passport data	Light brown
Tpt4	Costa Rica	Dark brown
Tpt6	Indonesia	Light brown
Tpt10	Sri Lanka	Brownish grey
Tpt11	Nigeria	Greyish orange
Tpt12	Sri Lanka	Brown
Tpt14	No passport data	Brown
Tpt15	No passport data	Dark brown
Tpt16	Indonesia	Greyish orange
Tpt17	Trinidad and Tobago	Light brown
Tpt18	No passport data	Brown
Tpt19	Nigeria	Dark brown
Tpt30	No passport data	Brownish orange
Tpt32	Liberia	Brown
Tpt33	No passport data	Light brown
Tpt42	No passport data	Reddish-brown
Tpt43	Bangladesh	Dark brown
Tpt48	No passport data	Greyish yellow
Tpt51	Bangladesh	Greyish orange
Tpt53	Nigeria	Dark brown
Tpt125	No passport data	Light brown
Tpt126	Nigeria	Brown
Tpt154	No passport data	Greyish orange
Tpt15-4	No passport data	Reddish blond brownish orange
Tpt3-B	No passport data	Yellowish dark blond

Genotypic and phenotypic components of variance

These components of variance were carried out according to Burton and Devane (1953):

Genotypic Variance ($\sigma^2 g$) = (MSg-Mse)/r

Where: MSg: Mean square of genotype, Mse: Mean square of error, r: number of replications

Phenotypic Variance, $\sigma^2 p = \sigma^2 g + \frac{\sigma^2 e}{r}$

Where: $\sigma^2 e$: Mse and $\sigma^2 g$: genotypic variance

Genotypic Coefficient of Variation (GCV)

$$\mathbf{GCV} = \frac{\sqrt{\sigma^2 \mathbf{g}}}{\bar{\mathbf{X}}} x \mathbf{100}$$

Where: $\sigma^2 g$: genotypic variance and $\bar{x}$: mean of the character

Phenotypic Coefficient of Variance (PCV)

$PCV = 100 (\sigma^2 P)^{½}/x$

Where: $\sigma^2 P$: phenotypic variance, and $\bar{x}$ = mean of a character

Broad Sense Heritability (h^2): This refers to the extent of the total phenotypic variation due to all genetic effects. This was calculated as recommended by Hanson et al. (1956) as follows:

$h^2 = 100 (\sigma^2 g/\sigma^2 p)$

Data analysis

Statistical Analysis System computer software version 9.4 (Institute 2015) was used for data analysis for the general linear model (GLM) procedure and least significant differences were estimated at $P \leq 0.05$ for mean separation with mean values obtained from two years of field data. Genotypic and phenotypic components of variance were calculated by the formula of Burton and Devane (1953), broad-sense heritability was computed as a percentage of the mean following the methods of Hanson et al. (1956). For principal component axis (PCA), eigenvalues (a measure of the extent of variation observed in variables), difference, cumulative variance, and principal scores (eigenvectors, which makes the understanding of the linear transformation in an easy way) of the first three components of genetic divergence in winged bean shoots and roots were also generated using SAS software as well as dendrogram.

RESULTS AND DISCUSSION

Results

In this study, a total of thirty-one (31) traits were measured, consisting of various agro-morphological characteristics using the standard winged bean descriptor and ^{15}N natural abundance for plant shoots and roots analysis. Table 1 describes the list of accessions used for the study in relation to number, origin, and seed color. Prominent seed colors were: light brown, brown, dark brown, reddish-brown, greyish brown, and reddish-blond brownish orange. Soil analysis results of the study site and map of the study location were given in Table 2 and Figure 1, respectively. The site had moderate levels of the nutrients measured. Table 3 shows the estimates of variance for yield characters. The pod length, dry pod weight, total seed weight, total number of seeds, seeds per pod, and 100-seed fresh weight exhibited high genotypic ($s^2 g$) and phenotypic ($s^2 p$) variances. The coefficient of variation guide provides a quantification of the variance among the different traits and accessions studied. The total number of seeds per accession, total seed weight, terminal leaf length, terminal leaf width, pod length, dry pod weight, and number of seeds per pod exhibited a high genotypic (GCV) and phenotypic (PCV) coefficient of variances. The phenotypic coefficient of variation (PCV) values were 7.14 for flower width to 71.82, for dry pod weight while the GCV values were from 2.22 for flower width to 43.12 for dry pod weight. Furthermore, PCV values were observed to be more than the GCV for all the traits evaluated. Based on the classification, the PCV value was low for flower width; medium for terminal leaf length, leaf rachis, and seed parameters (seed length, seed thickness, seed width, and 100-seed fresh weight). It was high for terminal leaf length, terminal leaf width, pod length, dry pod weight, and number of seeds per pod, total seed weight, and total number of seeds.

The GCV values were low for terminal petiole length, leaf rachis, terminal leaf length, flower width, seed length, seed thickness, and 100-seed fresh weight; no trait was in the medium range. However, values were high for dry pod weight, number of seeds per pod, pod length, total seed weight, and total number of seeds. The high GCV values of these characters may imply that the traits mentioned above can be improved through selection. The PCV values were low for flower width; medium for terminal leaf length, leaf rachis and seed parameters (seed length, seed thickness, seed width and 100-seed fresh weight).

Table 2. Soil analysis of study site.

Soil parameters	**Value**
Sand (%)	78.00
Silt (%)	9.00
Clay (%)	13.00
pH (H_2O)	7.30
Organic carbon (%)	1.97
Total N (%)	0.04
Available nutrient (mg kg^{-1})	
P ^{1}	2.36
Ca^{2+}	472.00
Mg^{2+}	82.80
K^+	54.60
Na^+	131.10
Zn	5.10
Cu	1.35
Mn	168.95
Fe	31.40

It was high for terminal leaf length, terminal leaf width, dry pod weight, number of seeds per pod, pod length, total seed weight, and total number of seeds (Table 3). The broad-sense heritability ranged from 83.05% for total seed weight to 1.64% for leaf rachis (Table 3). However, for traits with low heritability, 40% or less, selection may be difficult due to environmental influence. Based on these criteria, the broad-sense heritability estimate was high for total seed weight (83.05); moderate (75-55) for total number of seeds, seed per pod, and pod length; low with less for 55 for dry pod weight, seed length, and other traits.

The mean number of nodules was 359.62, with mean nodule dry weight being 3.69 g and the dry shoot weight was 239. 97 g. While the dry root weight g and total biomass were 19. 38 g and 250.93 g, respectively. The combined analysis of variance for five variables of 25-winged bean accessions show that replication by year interaction was statistically significant ($P \leq 0.0001$) for number of nodules per accession (NON), nodule dry weight (NDW) and dry shoot weight (DSW) while it was not significant p = > 0.05 for dry root weight (DRW), and total biomass (TBM) (Table 4).

The interaction of the year of planting and weeks after planting showed highly significant results for all traits measured while the accession by year interaction was not statistically significant (p = > 0.05) for the traits measured. Significant differences (p =) were observed among the accessions on nodulation parameters of winged beans. Tpt15 had a significantly (p= ≤ 0.0001) higher number of nodules (607.42) than other accessions while Tpt3-B had the lowest (207.33). Tpt126 had the highest nodule dry weight at 5.03 g and Tpt3-B had the lowest at 2.54 (Table 5). Significant differences existed among the accessions (Tpt15, Tpt126, Tpt18, Tpt42, and Tpt154) on some growth parameters of winged bean, as shown in Table 5. The highest and the least total biomass were found in Tpt18 (428.83 g) and Tpt19 (168.07 g), respectively. Statistically significant (p = ≤ 0.05) variations were recorded in the dry root weights among the accessions with Tpt42 (36.47 g) and Tpt154 (11.32 g) having the highest values. The means of % N derived from the atmosphere (Ndfa) and N fixed of the studied accessions are shown in Table 6. The amount of N fixed (Kg ha^{-1}) in the shoots varied among the accessions with Tpt32 fixing 25.76 Kg ha^{-1}, followed by Tpt15-4 (24.23 Kg ha^{-1}). The accession fixing the least N was Tpt30 (9.02) with considerably lower amounts fixed in the roots. The amount of N derived from the atmosphere also ranged from 24.97% (Tpt30) to 62.13% (Tpt32). This result suggested that winged beans derived a fair quantity of Nitrogen from the atmosphere (Table 6).

Table 3. Estimates of variance for seed yield and its component characters in winged bean.

Traits	**s^2g**	**s^2p**	**GCV (%)**	**PCV (%)**	**h^2 (%)**
Terminal petiole length (cm)	0.90	10.07	7.11	23.78	8.94
Leaf Rachis	0.19	11.60	2.41	18.82	1.64
Terminal leaf length (cm)	0.40	4.61	5.62	18.97	8.79
Terminal leaf width (cm)	0.00	5.76	0.00	24.66	0.00
Flower width (cm)	0.01	0.06	2.22	7.14	9.64
Dry pod weight (g)	11455.07	31779.21	43.12	71.82	36.05
No of Seeds/pod	17.47	24.18	28.50	33.54	72.23
Seed Length (mm)	0.40	1.29	6.90	12.40	30.97
Seed Thickness (mm)	0.29	1.32	7.07	15.22	21.59
Pod Length (cm)	20.43	36.66	28.02	37.54	55.74
100-Seed Fresh Weight (g)	1.38	20.60	3.59	13.86	6.69
Seed Width (cm)	0.18	1.07	4.88	11.97	16.63
Total seed weight (g)	1,097,451.14	1321501.84	41.99	46.07	83.05
Total Number of seeds	8,568,620.93	11484969.33	37.73	43.68	74.61

Note: s^2g: Genotypic variance; s^2p: Phenotypic variance; GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation; h^2: Broad sense heritability

Table 4. Summary of combined analysis of variance for 5 variables of 25 accessions of winged bean accessions.

Parameter	**NON**	**NDW (g)**	**DSW (g)**	**DRW (g)**	**TBM (g)**
Mean	359.62	3.69	239.97	19.38	250.93
Minimum	42.00	0.01	6.52	1.06	10.78
Maximum	1032.00	19.24	2195.04	207.11	2237.34
DF	291.00	291.00	279.00	290.00	270.00
Rep (Year)	762542.29***	42.59***	103339.74*	447.78^{ns}	68981.54^{ns}
WAP (Year)	546630.91***	762.27***	350379.82***	7956.37***	363741.64***
Year	15215065.31***	76.79***	4528622.964***	2245.70*	4293374.95***
Accession	92292.92*	4.55^{ns}	28871.50^{ns}	328.28^{ns}	29143.77^{ns}

Note: NON: Number of nodules; NDW: Nodule dry weight; DSW: Dry shoot weight; DRW: Dry root weight; TBM: Total biomass. DF: Degree of freedom; WAP: Weeks after planting; Rep: Replicate; ns: not significant *** and *Significant probability level at $P \leq 0.0001$ and 0.05 respectively.

Table 5. Means of nodulation and plant growth characteristics of winged bean accessions.

Accession	NON	NDW (g)	DSW (g)	DRW (g)	TBM (g)
Tpt10	290.67	3.29	253.93	19.71	275.38
Tpt11	401.55	4.21	226.80	19.52	246.33
Tpt12	352.17	4.15	188.92	15.45	204.49
Tpt125	312.08	2.86	177.49	24.00	200.85
Tpt126	469.42	5.03	285.72	20.22	303.79
Tpt14	322.73	3.21	232.87	24.11	246.31
Tpt15	607.42	4.54	329.38	17.06	346.44
Tpt15-4	400.50	3.10	285.17	16.61	254.13
Tpt154	239.18	3.45	163.40	11.32	176.61
Tpt16	326.83	3.79	260.47	14.85	275.32
Tpt17	293.25	3.00	192.91	14.20	207.96
Tpt18	477.75	4.14	403.23	25.60	428.83
Tpt19	381.08	4.20	163.34	16.98	168.07
Tpt2	332.17	3.54	275.99	18.32	281.80
Tpt3-B	207.33	2.54	168.49	28.75	198.15
Tpt30	464.50	4.77	279.37	19.50	298.87
Tpt32	434.92	3.67	223.81	16.79	239.45
Tpt33	329.36	3.48	259.36	19.46	209.34
Tpt4	427.73	4.11	258.72	20.98	279.70
Tpt42	320.83	2.90	209.39	36.47	248.85
Tpt43	288.58	4.06	256.50	16.77	273.27
Tpt48	392.09	3.25	255.10	16.22	252.07
Tpt51	251.07	3.48	183.06	20.36	181.04
Tpt53	301.27	3.86	165.39	14.98	180.64
Tpt6	357.27	3.69	229.51	15.05	211.37
C.V	94.71	85.73	87.70	103.52	83.50
F value	4.08***	4.27***	3.12***	1.48**	3.26***

Note: NON: Number of nodules; NDW: Nodule dry weight; DSW: Dry shoot weight; DRW: Dry root weight; TBM: Total biomass. Mean values of accessions are significantly different at $P \leq 0.05$, ns: not-significant and ***Significant probability level at $P \leq 0.0001$.

Table 6. Means of % N derived from the atmosphere and N fixed in shoots and roots of winged bean accessions.

Accession	RNfAT (%)	RTN (Kg/ha)	SNfAT (%)	STN (Kg/ha)
Tpt10	27.79	1.67	43.49	15.77
Tpt11	28.11	2.81	47.43	18.14
Tpt12	34.01	1.57	58.86	24.11
Tpt125	33.12	2.99	54.17	20.11
Tpt126	29.67	2.79	32.43	11.72
Tpt14	47.56	5.46	54.09	19.29
Tpt15	16.66	1.51	30.75	13.64
Tpt15-4	26.59	1.82	60.03	24.23
Tpt154	42.58	1.02	54.85	21.09
Tpt16	44.04	3.61	41.59	14.97
Tpt17	40.57	1.56	32.93	13.81
Tpt18	35.11	3.18	24.31	9.34
Tpt19	59.19	0.79	51.15	19.29
Tpt2	27.86	0.90	42.06	17.47
Tpt3-B	39.35	2.30	62.81	21.13
Tpt30	27.01	2.77	24.97	9.02
Tpt32	69.54	3.21	62.13	25.76
Tpt33	31.03	2.77	33.09	12.95
Tpt4	23.57	2.44	47.34	17.88
Tpt42	42.34	3.61	35.67	13.77
Tpt43	49.90	3.65	45.56	18.69
Tpt48	32.09	2.52	51.26	19.28
Tpt51	26.48	1.47	55.07	21.96
Tpt53	46.84	2.91	52.88	22.68
Tpt6	24.67	2.57	32.04	12.63
C.V	72.56	77.64	37.13	38.93
F value	1.54^{ns}	1.66^{ns}	1.69*	1.54^{ns}

Note: RNfAT: Root N derived from the atmosphere; RTNfixed (kg/ha): Root N fixed; SNfAT: Shoot N derived from the atmosphere; STNfixed (kg/ha): Shoot N fixed

The principal component analysis (PCA) is a statistical procedure that can provide a summary of large data into a form that can easily be observed. The PCA derived from the analysis of the plant growth and nodulation revealed differences in eigenvalues were 4.23 (PC I), 2.23 (PC II) and 2.15 (PC III) indicating the first three PCAs when the cut-off was at 10%; other components 4-8 were less than 4% (Table 7) while the total contribution of the principal component was 72%. The first PCA contributed the most towards variability and was highly related to plant growth parameters. The second PCA was related to variation among accessions owing to total N fixed in roots, shoot C/N and shoot ɗ ^{13}C while the third PCA showed variation in the S ɗ^{15}N and shoot N.

The results showed that Tpt15, Tpt18, Tpt126, Tpt30, Tpt32, and Tpt15-4 all had a very high mean number of nodules, important for N fixation and nodulation. The significant mean squares of numbers of nodules indicated variability among the accessions regarding nodulation potentials. Tpt18 had the highest biomass production, which was reflected in its high value for dry shoots and dry roots weights. A comparison of growth between the studied accessions showed differences in shoot and root dry weights, but these were not statistically ($p = > 0.05$) different. The dry shoot weight ranged from 403.23g in Tpt18 to 163.40 g in Tpt15-4.

The carbon and nitrogen ratio (C/N ratio) indicates the C and N relationships in plants used in measuring plant water use capacity. Significant differences ($p = \leq .05$) were recorded in C and N ratio among the accessions studied.

Overall, the C/N ratio ranged from 15.87 (Tpt3-B) to 11.97 (Tpt15) for the shoots; it ranged from 14.03 (Tpt4) to 18.33 (Tpt12) for the roots (Table 8). The δ^{15}N value varied from 3.34 (Tpt18) to 0.86 (Tpt3-B) in the shoots and from 3.07 (Tpt15) to 0.49 (Tpt32) for roots (Table 8). The δ^{13}C of C3 plants explained their water-use ability (Lawson and Pike 2017). Analysis of variance of δ^{13}C from shoots and roots revealed highly significant variation ($p= \leq .0001.$) among accessions under investigation. The δ^{13}C values of shoots were higher, while the values for the roots also varied considerably. Therefore, the δ^{13}C values of shoots ranged from -30.60 (Tpt48) to -29.62 (Tpt19) and from -30.17 (Tpt53) to -19.19 (Tpt6) for the roots (Table 8) could indicate variations in the pattern of water use potential of the studied accessions.

Clustering analysis produced three clusters at a similarity index of 0.6 (Figure 2). Cluster I contained 10 accessions (41.6 %) followed by cluster II with 8 accessions (33.4 %) and cluster III contained 6 accessions (25.0%). Of the 25 accessions, Tpt32 was observed not to belong to any of the three clusters formed. Accessions with high values of the parameters measured in the roots and shoots were grouped in Cluster I while other Clusters (II and III) contained relatively low values. The clustering relationship also corresponds to the nodulation, plant growth parameters (Table 5), Ndfa and estimated amount of Nitrogen fixed (Table 6). If accessions from derived clusters are crossed, it may produce a desirable genetic diversity in subsequent generations (Figure 2). Three of the 15 principal components generated explained more than 72% of the variations encountered (Table 7). The PC I accounted for 28.0% of the variation and illustrated primarily the variations in shoot N; shoot and root N fixed. The PC II accounted for an additional 23.0% of the total variation and primarily described the variation patterns in root C/N, ɗ13 C Corr, ɗ^{15}N, Ndfa and shoot ɗ^{15}N. The PC III emphasized the root N (Table 7). The PCA showed that the first three PCs accounted for more than 72% of the total variation; 54.0% was contributed by the first two PCs (PC I and PC II) (Figure 3).

Table 7. Eigenvalues, difference, cumulative variance, and principal scores (eigenvectors) of the first three components of genetic divergence in winged bean shoots and roots.

Characters	**Component score**		
	PC I	**PC II**	**PC III**
RCN	0.327	–	–
Rd13Ccorr	–	–	–
Rd15N	–0.368	–	–
RN	–0.310	–	–
RNfAT (%)	0.368	–	–
RTNfixed (kg/ha)	–	0.348	–
SCN	–	0.552	–
Sd13Ccorr	–	0.247	–
Sd15N	–	–	0.282
SN	–	–	0.347
SnfAT (%)	0.41		–
STNfixed (kg/ha)	0.405	–	–
Eigenvalue	4.23	2.23	2.15
Proportion (%)	0.35	0.19	0.18
Cumulative (%)	0.35	0.54	0.72

Note: RnfAT: Root N derived from the atmosphere; RTNfixed (kg/ha): Root N fixed; SnfAT: Shoot N derived from the atmosphere; STNfixed (kg/ha): Shoot N fixed, PCs: Principal components

Table 8. Means estimation of C/N, ɗ13 Corr, ɗ15N and %N in shoots and roots of winged bean.

Accession	**RCN**	**Rɗ13Ccorr**	**Rɗ 15N**	**RN**	**SCN**	**Sɗ13Ccorr**	**Sɗ15N**	**SN**
Tpt10	14.94	–29.29	2.52	2.75	15.07	–29.94	2.10	2.69
Tpt11	15.08	–28.99	2.51	2.65	14.12	–30.18	1.85	2.84
Tpt12	18.33	–28.84	2.22	2.19	12.98	–30.00	1.11	3.09
Tpt125	15.41	–29.60	2.26	2.60	14.58	–30.41	1.42	2.75
Tpt126	15.77	–29.10	2.43	2.55	14.68	–30.03	2.82	2.73
Tpt14	16.37	–28.93	1.56	2.55	14.90	–30.06	1.42	2.68
Tpt15	14.31	–29.23	3.07	2.82	11.97	–30.11	2.93	3.34
Tpt15-4	14.21	–29.65	2.58	2.85	13.21	–30.24	1.04	3.03
Tpt154	17.41	–29.21	1.80	2.33	13.91	–30.43	1.37	2.88
Tpt16	15.64	–29.61	1.73	2.61	14.87	–30.39	2.23	2.70
Tpt17	17.47	–29.20	1.90	2.31	12.50	–30.12	2.79	3.21
Tpt18	15.38	–29.19	2.17	2.60	13.84	–29.95	3.34	2.89
Tpt19	15.68	–29.08	0.99	2.56	14.22	–29.62	1.61	2.82
Tpt2	14.81	–29.54	2.52	2.71	12.84	–30.16	2.20	3.15
Tpt3-B	17.66	–29.39	1.96	2.33	15.87	–30.20	0.86	2.53
Tpt30	14.95	–29.67	2.56	2.69	14.57	–30.03	3.30	2.77
Tpt32	16.57	–29.17	0.49	2.47	12.84	–30.19	0.90	3.12
Tpt33	15.58	–29.22	2.37	2.60	13.20	–29.83	2.78	3.11
Tpt4	14.03	–29.26	2.73	2.85	14.11	–30.32	1.86	2.85
Tpt42	15.31	–29.61	1.81	2.62	13.78	–30.22	2.61	2.91
Tpt43	15.34	–29.63	1.44	2.62	13.11	–30.46	1.97	3.09
Tpt48	14.88	–29.70	2.31	2.69	14.18	–30.60	1.60	2.82
Tpt51	14.31	–29.38	2.59	2.80	13.44	–30.36	1.36	2.98
Tpt53	17.83	–30.17	1.59	2.26	12.70	–30.59	1.50	3.18
Tpt6	15.55	–28.79	2.68	2.58	13.49	–30.15	2.84	2.97
C.V	12.47	–1.97	60.75	11.91	9.61	–1.06	54.39	9.7
F value	1.21^{ns}	2.75**	1.54^{ns}	1.08^{ns}	1.74*	2.03*	1.69*	1.77*

Note: RCN; Root Carbon Nitrogen; Rɗ^{13}Ccorr: Root ɗ^{13}Carbon correlation; Rɗ^{15}N: Root ɗ^{15}N; RN: Root Nitrogen; SCN; Shoot Carbon Nitrogen; Sɗ^{13}Ccorr: Shoot ɗ^{13} Carbon correlation; Sɗ^{15}N: Shoot ɗ^{15}N; SN: Shoot Nitrogen ***Significant probability level at $P \leq .0001$.

Discussion

The pod length, dry pod weight, total seed weight, total number of seeds, number of seeds per pod, and 100-seed fresh weight exhibited high genotypic (s^2g) and phenotypic (s^2p) variances. The total number of seeds per accession, total seed weight, terminal leaf length, terminal leaf width, pod length, dry pod weight, and number of seeds per pod also exhibited a GCV and PCV. The outcome from the current study was like previous work in winged bean accessions (Afridatul and Syukur 2021; Kant and Nandan 2018; Mohamadali et al. 2010; Prasanth et al. 2016; Rajeshwar et al. 2009). Furthermore, PCV values were observed to be higher than GCV for all the traits evaluated. Based on this classification, the PCV value was low for flower width; medium for terminal leaf length, leaf rachis, seed length, seed thickness, seed width, and 100-seed fresh weight. It was high for terminal leaf length, terminal leaf width, pod length, dry pod weight, and number of seeds per pod, total seed weight, and total number of seeds.

The GCV values were low for terminal petiole length, leaf rachis, terminal leaf length, flower width, seed length, seed thickness, and 100-seed fresh weight; no trait was in the medium range. Values were high for dry pod weight, number of seeds per pod, pod length, total seed weight, and total number of seeds. The high GCV indicates the choice of trait improvements that may be possible through selection. In a study on winged beans by Rajeshwar et al. (2009) high genetic advance was observed for almost all the characters under study. The results obtained herein are in line with Prasanth et al. (2016) who recorded substantial differences among accessions of different genotypes evaluated for variability.

In the accessions studied, the PCV value was low for flower width; medium for terminal leaf length, leaf rachis and seed parameters (seed length, seed thickness, seed width, and 100-seed fresh weight). It was high for terminal leaf length, terminal leaf width, dry pod weight, number of seeds per pod, pod length, total seed weight, and total number of seeds. The broad-sense heritability ranged from 24.13% for 100-seed fresh weight to 50.98% for dry pod weight. Singh (2001) believed that if the heritability of a character is very high, 80% or more, selection for such a character would be done with ease. This is because there would be a close correspondence between the accession and the phenotype due to the relatively small contribution of the environment to the phenotype. However, for characters with low heritability, 40% or less, selection may be considered difficult because of environment. Based on these criteria, the heritability values were moderate (31-50) for terminal petiole length, leaf rachis, dry pod weight, seeds per pod, and seed length, and low at less than 30 for terminal leaf length, terminal leaf width, flower width, and seed thickness.

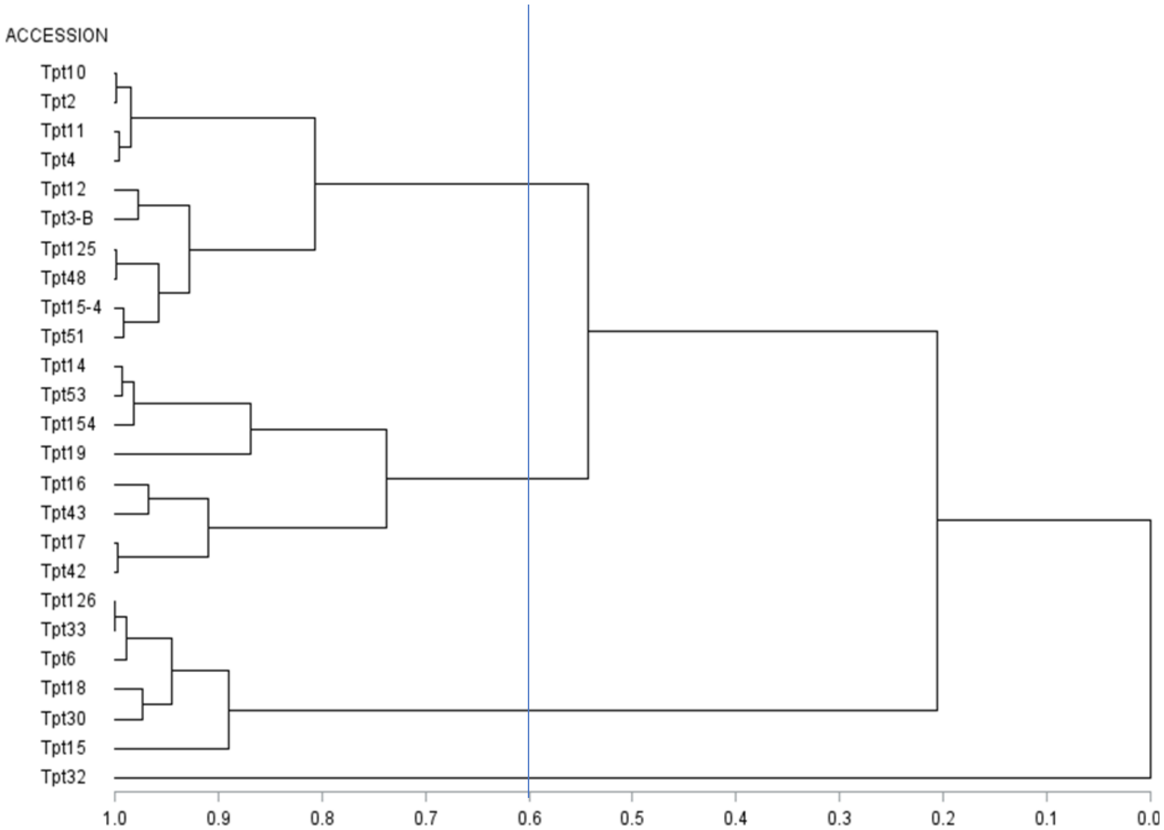

Figure 2. Dendrogram showing the hierarchical structure of winged bean shoots and roots.

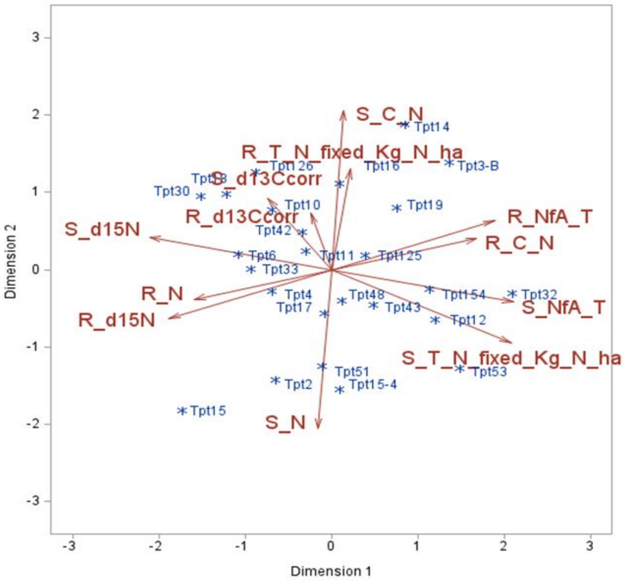

Figure 3. Mean performance of characters and stability of 25 accessions of winged bean as measured by principal components.

This study revealed that the nodulation ability of winged beans were high compared with other legumes previously reported (Abaidoo et al. 2017; Waluyo and Lie 2016). Consequently, an increase in N could be attributed to the high number of nodules as the bacteria in the nodules could be freely fixing N (Boddey et al. 2017; Kumar et al. 2016; Tampakaki et al. 2017). Therefore, high N content may position winged bean as the desired crop for the future providing the needed protein and other nutritional values to the ever-growing global population (Martin 2017).

N fixation by winged beans could be comparable to that of other legumes capable of producing ureide (such as cowpea, desmodium and soybean to mention but a few) (Yoneyama 2017). Furthermore, due to its capacity to fix N, some studies (Anugroho et al. 2010; Hikam et al. 1991; Hikam et al. 1992; Rahman et al. 2014b) have demonstrated that winged beans could contribute significantly to the amount of N available for plant growth and development either when intercropped or planted solely.

The amount of N fixed (Kg/ha) in the shoots varied among the accessions with Tpt32 fixing 25.76 Kg/ha, followed by Tpt15-4 at 24.23 Kg/ha. The accession fixing the least was Tpt30 that measured 9.02 Kg/ha with considerably lower amounts fixed in the roots. Similar results involving other legumes were obtained by other researchers further elucidating the potential role of N fixation in modern agriculture (Abaidoo et al. 2017; Chalk 2016; Foyer et al. 2016; Kermah et al. 2018; Kumar et al. 2016; Li et al. 2017; Singh et al. 2016). Therefore, winged beans can be described as a good N fixer and could serve as a good constituent of sustainable agricultural practices.

Water use efficiency by plants has been reported to decrease with water in the soil and increase with the soil water decline (Fang et al. 2010; Hartman and Danin 2010; Lawson and Pike 2017; Mapope and Dakora 2016). In this present study, the values obtained show these accessions could be stable in their expression of water use. The extent of C in plants is compared to photosynthetic activity (Belane et al. 2011). The values of Carbon concentrates for winged bean was not more than 40 % which was in agreement with previous reports for other legumes (Mohale et al. 2014; Polania et al. 2016).

A higher C content indicates presence of large amount of plant biomass (Mohale et al. 2014). The lower C content in plants could be moderated by N nutrition. The C and N ratio may undoubtedly be a viable tool to estimate N condition (Mohale et al. 2014). The non-legume grass used as a reference plant had over 24 gg^{-1} while accessions of winged bean exhibited C and N ratios below 24 gg^{-1}. This finding is similar to outcomes from previous researches (Murray et al. 2017). In all cases, the C and N ratio value increased with decreasing δ^{15}N values. These outcomes support the opinion that photosynthetic activities in winged beans could have been mediated by N nutrition, as accessions producing low C and N-fixed ratios yielded high N (Adams et al. 2016; Ulm et al. 2017; Yoneyama 2017). Future research is needed in all areas of germplasm improvement and may focus on studies where winged bean is intercropped with different legumes or cereals to measure how significant its contribution to food and farming systems in different agroecological zones. The study investigated the variability of growth, seed yield, nodulation, and N fixation in accessions of winged beans. Results indicated there are great opportunities for the selection of promising accessions from current germplasm collections for improvement programs for seed yield and other characters. The understanding of the genetic variability of winged beans could be carefully exploited to provide higher grain yield as well as other economic and important traits. Winged beans can be improved based on the genetic variability observed in the selected accessions from the germplasm collections to boost food security and conservation of the plant genetic materials. Winged bean accessions could fix atmospheric Nitrogen and nodulate well which may help reduce dependence on microbial inoculants and chemical N fertilizer application.

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