International Journal of Agriculture and Forestry 2015, 5(4): 211-216
DOI: 10.5923/j.ijaf.20150504.01

Variability in Nutritional Traits of *Piper nigrum* Accessions from Nigeria

Nwofia Godson E.*, Chilekwe Kelechukwu

Department of Agronomy, Micheal Okpara University of Agriculture, Umudike, Nigeria

Abstract There is limited information on variability of nutritional traits in leafy vegetables and almost none on *Piper nigrum* accessions from Nigeria, hence the objective of this study was to determine the genetic variability that exists in the nutritional traits of the leaves and fruits of these accessions. The leaves and fruits of nine accessions of *Piper nigrum* collected from four states of Nigeria were analyzed for proximate, mineral, vitamins and phytochemical compositions. All the determinants were done in triplicates. Analysis of variance was used to partition the variability into the components due to genetic and non-genetic factors. Phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), estimates of broad sense heritability (h^2B) and genetic advance (GA) as well as principal component analysis (PCA) were also done. The result showed that most of the variability observed in the nutritional traits of *P. nigrum* has more genetic than non-genetic basis and indicates that considerable scope for selection exists among the accessions for most of the traits. High heritability and genetic advance was observed for ash, crude fibre, iron, hydrogen cyanide and riboflavin in the leaves while in the fruits iron, hydrogen cyanide and riboflavin had high heritability and genetic advance indicating that these traits are controlled mainly by additive genes. The PCA showed that the first five PCs were important and explained 80% and 79.4% of the total variation in both the leaves and fruits of these accessions. The results obtained here showed that the nutritional traits can be improved upon by selection as most of them are controlled by additive gene effects.

Keywords Variability, Genotypic coefficient of variability, Heritability, Principal components analysis, Nutritional traits, *Piper nigrum*

1. Introduction

Piper nigrum (Black pepper) a member of the family piperaceae is a perennial woody climber. It is native to India, Indonesia, Malaysia, South America and West Indies but is now widely cultivated in the tropical regions. Black pepper is used worldwide cuisines [1]. The spicy taste is mainly due to the presence of piperine, a pungent alkaloid [2] that enhances the bio availability of various structurally and theurapeutically diverse drugs [3]. It also contains safrol, pinere, sabinere, limorene, canyophylene and linaonol compounds. The leaves and fruits are used in indigenous system of medicine in many rural areas of the world. Due to its multidimensional effect on various systems of the body, it has been described as antipyretic and diuretic, aphrodisiac, immune-stimulant, antioxidant, hepatoprotective, digestive, rubefacient counter irritant, antiseptic and anti spasmodic ([4-6]).

Knowledge of genetic variability in populations is a pre-requisite for selection of superior genotypes over the existing cultivars [7]. The application of genetic variation can be utilized in future improvement programmes. Crop improvement depends not only on the magnitude of phenotypic variability but also on the extent to which the characters are heritable. Hence, it is important to partition the observed variation into its heritable and non-heritable components ([8-11]). Information on variability of nutritional traits on leafy vegetables are scanty in literature and for *P. nigrum* it is almost non-existent. Hence, the objective of this study was to determine the genetic variability in the nutritional traits of some Nigerian accessions of *Piper nigrum* as well as determine the heritability and genetic advance of the traits.

2. Materials and Methods

Leaves and fruits of nine accessions of *Piper nigrum* were collected from local markets in four states of Nigeria (Table 1) and used in this study. The accessions were identified at the Department of Botany and voucher specimen deposited. Proximate, mineral, vitamins and phytochemicals compositions were carried out on the leaves and fruits.

* Corresponding author:
enwofia@yahoo.co.uk (Nwofia Godson E.)
Published online at http://journal.sapub.org/ijaf
Copyright © 2015 Scientific & Academic Publishing. All Rights Reserved

Table 1. Accessions list showing place of collection and the Agro ecology

Accession	State of Collection	Agro ecology
Ahoada	Rivers	Humid Rain forest
Egbema	Imo	Humid Rain forest
Elele	Rivers	Humid Rain forest
Ndioro	Abia	Humid Rain forest
Omoku	Rivers	Humid Rain forest
Owerri	Imo	Humid Rain forest
Port harcourt	Rivers	Humid Rain forest
Umuahia	Abia	Humid Rain forest
Yenogoa	Bayelsa	Humid Rain forest

2.1. Proximate Analysis

Moisture content was determined by the method of [12] and [13]. Crude protein (N x 6.25) was determined by the kjedahl method. The ash, crude fat and crude fibre was determined according to the [14]. The carbohydrate content was obtained by the difference as the nitrogen free extract. Energy value was determined using the Atwater formula: Energy value (K cal/ 100g) = (4 x % carbohydrate) + (9 x % crude fat) + (4 x % crude protein).

2.2. Mineral Analysis

The mineral content of the leaves and fruits were determined by the dry ash extraction method described by [13] and [15]. Calcium and magnesium was determined by the versanate EDTA complexiметric titration method while the Iron and Zinc was determined using Atomic absorption spectrophotometer as described by [16] and [17].

2.3. Vitamin Analysis

Vitamin C was determined by the method outlined by [15] while the B-vitamins was determined by the spectrophotometric method described by [18].

2.4. Phytochemical Analysis

Alkaloid was determined by the alkaline precipitation gravimetric method as described by [19]. Tannins and oxalate contents were determined using Folins-Deins spectrophotometric method as described by [20]. Flavonoids were determined using acidification and ethyl acetate extraction as described by [19] Hydrogen cyanide was estimated using the alkaline titration method as described by [14]. All the determinants were done in triplicates.

2.5. Data Analysis

Analysis of variance was performed on all the data using Genstat discovery edition 3 [21] The analysis of variance was used to partition the gross variability into the components due to genetic and non-genetic environmental factors and to estimate their magnitude. Genotypic, phenotypic and error variances were estimated using the formulae of [22], [13].

$$VG = \frac{MSG - MSE}{r}$$

$$VP = \frac{MSG}{r}$$

$$VE = \frac{MSE}{r}$$

Where; MSG, MSE and r are the mean squares genotypes, mean squares error and number of replications, respectively. The phenotypic (PCV) and genotypic (GCV) coefficients of variations were estimated by the methods of [23] and [24] and [25] as follows:

$$PCV = \frac{\sqrt{VP}}{X} \times 100$$

$$GCV = \frac{\sqrt{VG}}{X} \times 100$$

Where; VP, VG and x are phenotypic and genotypic variances and grand mean respectively for each attribute under consideration. Broad sense heritability (h^2B) was expressed as the ratio of VG to the VP and was estimated on genotypic mean basis as described by [26]. Genetic advance was estimated by the method of [27] as GA = k (sp)h^2B where k is a constant (2.06 at 5% selection pressure), sp is the phenotypic standard deviation √VP, h^2B is the broad sense heritability. GA was calculated as a percentage of the mean. Factor analysis based on principal component analysis (PCA) was performed according to [25] to examine the inter trait relationships among these nutritional traits.

3. Results and Discussion

The analysis of variance showed that the nutritional traits of the leaves and fruits of the Nigerian accessions of *Piper nigrum* were highly significant (P<0.001) except vitamin C in the leaves that was not significant. This suggests the possibility of improving these traits through genotypic selection (Table 2 and 3). Estimates of the variance components (Table 4 and 5) showed that the phenotypic and genotypic variances were close to each other. The error variances were lower than the genotypic variances for all traits. These low error variances indicate that the genotypic component was the major contributor to the total variance for these nutritional traits. It can be concluded that most of the variability observed in the nutritional traits of *P. nigrum* has more of a genetic than non-genetic basis. This indicates that there is considerable scope for selection among the accessions for the traits. Similar results have been reported by [8], [9], [28]) and [11]).

The phenotypic coefficient of variation (PCV) was generally higher or equal to the genotypic coefficient of variation (GCV) for all the traits. The closeness of the PCV and GCV indicates that there was little influence of the environment in the expression of these traits in the leaves and

fruits (Tables 6 and 7). Heritability is as a predictive instrument used in exercising the reliability of phenotypic value. Hence, it helps the breeder to make selection for traits with high heritability estimates. The genetic advance is a useful indicator of the progress that can be expected as a result of exercising selection on the population [28]. Heritability was high for all the nutritional traits indicating that environmental influence had low influence on the expression of these traits. Heritability along with genetic advance is more useful than heritability alone in predicting the resultant effect of selecting good individual genotypes as it suggests the presence of additive gene effects. High heritability and genetic advance was observed for ash, crude fibre, iron, hydrogen cyanide, tannin and riboflavin in the leaves while in the fruits, high heritability and genetic advance was observed for iron, hydrogen cyanide and riboflavin indicating that heritability for these traits is mainly due to additive gene effects. [28].

Table 2. Mean Squares and variance ratios obtained in analysis of variance of nutritional traits of the leaves of Nigerian accessions of *Piper nigrum*

	Mean Squares		
Attributes	**Genotype**	**Error**	**Variance ratio**
Ash	2.2087454	0.0005731	3853.71**
Carbohydrate	6.0282	0.3164	19.05***
Fat	0.0415759	0.0001968	211.30***
Fibre	3.3206148	0.0002926	11348.94***
Moisture content	0.9173537	0.0005023	1826.25***
Protein	1.919567	0.002019	950.54***
Energy value	159.291	5.237	30.42***
Calcium	45.40089	0.04367	1039.59***
Iron	0.2759417	0.0007069	393.33***
Magnesium	51.19537	0.08793	582.23***
Zinc	0.231370	0.000805	28.69***
Alkaloid	0.0121417	0.0003778	32.14***
Flavonoid	0.0055370	0.0002454	22.57***
Hydrogen cyanide	0.0469704	0.0002662	176.45***
Oxalate	0.0040370	0.0001731	23.32***
Tannin	0.042325	0.00009028	468.83***
Thiamine	0.011425	0.0001444	79.10***
Riboflavin	0.0109417	0.0001194	91.60***
Niacin	0.0100704	0.0002329	43.24***
Vitamin C	1886.00	1521.00	1.24^{ns}

Ns=not significant,
*=significant at 5%,
**= significant at 1%,
***= significant at 0.1%

Table 3. Mean Squares and variance ratios obtained in analysis of variance of nutritional traits of the fruits of fruits of Nigerian accession of *Piper nigrum*

	Mean Squares		
Attributes	**Genotype**	**Error**	**Variance ratio**
Ash	0.5595667	0.0007875	710.56***
Carbohydrate	2.42373	0.05047	48.03***
Fat	1.7013981	0.009148	1859.83***
Fibre	0.2578009	0.0009870	261.19***
Moisture content	2.58769	0.04764	54.31***
Protein	0.3874093	0.0003398	1140.06***
Energy value	140.9990	0.8628	163.41***
Calcium	28.7225	0.4129	69.56***
Iron	0.2902815	0.0002579	1125.69***
Magnesium	41.4647	0.3120	132.89***
Zinc	0.0213259	0.0001231	173.17***
Alkaloid	0.0300565	0.0001704	176.42***
Flavonoid	0.0105667	0.0001028	102.81***
Hydrogen cyanide	0.0935370	0.00009537	98.08***
Oxalate	0.0059565	0.0001037	57.44***
Tannin	0.2810343	0.0001718	1636.21***
Thiamine	0.0086417	0.0001500	57.61***
Riboflavin	0.0125231	0.0002023	61.90***
Niacin	0.0068093	0.0001565	43.51***
Vitamin C	9.090642	0.001761	5161.88***

Ns=not significant
*=significant at 5%
**= significant at 1%,
***=significant at 0.1%

Table 4. Phenotypic (VP), genotypic (VG) and error variances (VE) for Nutritional traits of the leaves of Nigerian accessions of *Piper nigrum*

Attributes	**VP**	**VG**	**VE**
Ash	0.73625	0.73606	0.00010
Carbohydrates	2.00940	1.09039	0.10500
Fat	0.01386	0.01379	0.00007
Fibre	1.10687	1.10677	0.00010
Moisture content	0.30579	0.30562	0.00167
Protein	0.63986	0.63918	0.00673
Energy value	53.09700	51.35133	1.74567
Calcium	15.13336	15.11881	0.01456
Iron	0.09198	0.09175	0.00024
Magnesium	17.06512	17.03581	0.02931
Zinc	0.00771	0.00744	0.00027
Alkaloid	0.00405	0.00392	0.00013
Flavonoid	0.00185	0.00176	0.00008
Hydrogen cyanide	0.01566	0.01557	0.00009
Oxalate	0.00135	0.00129	0.00006
Tannin	0.01411	0.01408	0.00003
Thiamine	0.00381	0.00376	0.00005
Riboflamin	0.00365	0.00361	0.00004
Niacin	0.00336	0.00328	0.00008
Vitamin c	628.66670	121.66670	507.00000

Table 5. Phenotypic (VP), genotypic (VG) and error variances (VE) for Nutritional traits of the fruits of Nigerian accessions of *Piper nigrum*

Attributes	VP	VG	VE
Ash	0.18652	0.18626	0.00026
Carbohydrates	0.80791	0.79109	0.01682
Fat	0.56713	0.56683	0.00031
Fibre	0.08593	0.08561	0.00033
Moisture content	0.86256	0.84668	0.01588
Protein	0.12914	0.12902	0.00011
Energy value	46.99967	46.71207	0.28760
Calcium	9.57417	9.43653	0.13763
Iron	0.09676	0.09668	0.00009
Magnesium	13.82157	13.71757	0.10400
Zinc	0.00711	0.00707	0.00004
Alkaloid	0.01002	0.00996	0.00006
Flavonoid	0.00352	0.00349	0.00003
Hydrogen cyanide	0.00312	0.00309	0.00003
Oxalate	0.00199	0.00195	0.00003
Tannin	0.09368	0.09362	0.00006
Thiamine	0.00288	0.00283	0.00005
Riboflamin	0.00417	0.00411	0.00007
Niacin	0.00227	0.00222	0.00005
Vitamin c	3.03021	3.02963	0.00059

Table 6. Phenotypic coefficient of variability (PCV), genotypic coefficient of variability (GCV), broad sense heritability (h^2B) and Genetic advance (GA) of nutritional traits of the leaves of Nigerian accessions of *Piper nigrum*

Attributes	MEAN	PCV	GCV	H2b	GA
Ash	11.79	7.28	7.28	99.97	14.99
Carbohydrates	43.98	3.22	3.14	94.75	6.29
Fat	3.81	3.09	3.08	99.53	6.34
Fibre	12.26	8.58	8.58	99.99	17.68
Moisture content	9.14	6.05	6.05	99.95	12.46
Protein	19.14	4.18	4.18	99.89	8.6
Energy value	286.12	2.54	2.5	96.71	5.06
Calcium	266.16	1.46	1.46	99.9	3.01
Iron	3.14	9.67	9.66	99.74	19.87
Magnesium	318.72	1.29	1.29	99.83	2.67
Zinc	1.803	4.87	4.79	96.52	9.68
Alkaloid	0.86	7.42	7.3	96.89	13.21
Flavonoid	0.69	6.19	6.05	95.57	9.55
Hydrogen cyanide	1.13	11.04	11.01	99.43	19.42
Oxalate	0.47	7.79	7.63	95.71	13.12
Tannin	1.69	7.04	7.03	99.79	25.8
Thiamine	1.22	5.04	5.01	98.74	10.26
Riboflamin	0.71	8.55	8.49	98.91	17.41
Niacin	0.85	6.85	6.77	97.69	13.78
Vitamin c	225	11.14	4.9	19.35	4.44

Table 7. Phenotypic coefficient of variability (PCV), genotypic coefficient of variability (GCV), broad sense heritability (h^2B) and Genetic advance (GA) of nutritional traits of the leaves of Nigerian accessions of *Piper nigrum*

Attributes	MEAN	PCV	GCV	H2b	GA
Ash	7.09	6.09	6.08	99.86	12.52
Carbohydrates	52.79	1.7	1.68	97.92	3.43
Fat	14.06	5.36	5.35	99.95	11.03
Fibre	5.12	5.72	5.71	99.62	11.74
Moisture content	12.19	7.62	7.54	98.16	15.39
Protein	8.78	4.09	4.09	99.91	8.43
Energy value	372.83	1.84	1.83	99.39	3.76
Calcium	183.36	1.69	1.68	98.56	3.43
Iron	2.83	11.01	11	99.91	22.66
Magnessium	242.28	1.53	1.53	99.25	3.14
Zinc	1.59	5.31	5.29	99.42	10.87
Alkaloid	1.55	6.45	6.43	96.89	14.8
Flavonoid	1.27	4.68	4.66	95.57	12.19
Hydrogen cyanide	0.59	9.52	9.48	99.43	22.61
Oxalate	0.69	6.48	6.43	95.71	15.38
Tannin	2.44	12.53	12.53	99.79	14.47
Thiamine	0.83	6.44	6.38	98.26	13.04
Riboflamin	0.56	11.62	11.53	98.38	23.56
Niacin	0.7	6.79	6.71	97.7	13.66
Vitamin c	29.67	5.87	5.82	99.98	12.09

The principal component analysis of the nutritional traits of the leaves is shown in Table 8. PCA showed that the first five PCs were important and explained 80% of the total variation in the accessions. PC1 had an eigen value of 5.35 and accounted for 28.10% of the total variation and this represents an equivalent of three variables and indicated that ash, moisture content and thiamine were important contributing variables in distinguishing these accessions. PC2 had an eigen value of 3.137 contributing 16.50% of the total variation and had calcium, alkaloid, fibre and protein as the main contributing factors. PC3 had an eigen value of 3.00 and contributed 15.80% of the variation and had carbohydrate, crude fat, flavonoid and magnesium as the main contributing factors. PC4 with an eigen value of 2.03 contributed 10.70% of the total variation and had iron as the main contributing factor. PC5 with an eigen value of 1.69 and contributed only 8.70% of the total variation and majorly calcium, alkaloid, fibre, moisture content and protein as the contributing factors. PCA analysis of the fruits nutritional traits showed the initial five PCs are important and explained 79.40% of the total variation (Table 9). PC1 with an eigen value of 4.49 and accounted for 23.60% of the variation and this represents an equivalent of four variables and indicated

that alkaloids, calcium, moisture content and thiamine were important contributing variables in distinguishing the accessions. PC2 had an eigen value of 3.29 contributing 17.30% of the variation and had hydrogen cyanide, iron, magnesium and zinc as the main contributing factors. PC3 with eigen value of 2.97 and contributed 15.70% of the variation and had carbohydrate, flavonoid, protein and riboflavin as the main contributing traits. PC4 with an eigen value of 2.45 contributed 12.90% and had crude fat, crude fibre, tannin and vitamin C as the main contributing traits. PC5 had an eigen value of 1.89 and contributed only 9-90% of the total variation and had ash and oxalate as the main contributing traits. PCA is one of the most frequently used multivariate techniques [29] and has been used to examine association among traits [30] and [31]. PCA revealed that PC1 in the leaves explained 28.10% of the total variation and showed that these accessions were highly variable for ash, moisture content and thiamine while in the fruits, PC1 explained 23.60% of the total variation indicating that alkaloids, calcium, moisture content and thiamine in these accessions were the main distinguishing traits.

Table 8. Eigen vector for principal components of nutritional traits of the leaves of Nigerian accessions of *Piper nigrum*

Attributes	PC1	PC2	PC3	PC4	PC5
Ash	0.322	-0.002	0.144	0.349	-0.178
Calcium	-0.139	-0.404	-0.039	-0.117	0.432
Carbohydrates	-0.294	0.204	-0.321	0.046	-0.108
Fat	-0.270	-0.034	0.357	0.122	0.050
Alkaloid	-0.066	0.347	0.294	-0.004	-0.420
Fibre	0.011	-0.456	0.199	-0.067	-0.348
Flavonoid	-0.201	0.298	0.320	-0.004	0.101
Hydrogen cyanide	-0.232	-0.292	-0.197	0.158	-0.148
Iron	0.255	-0.146	0.085	-0.392	-0.046
Magnesium	0.233	0.027	0.377	0.036	-0.022
Moisture content	0.345	-0.065	-0.016	0.124	0.383
Oxalate	0.156	-0.220	0.223	-0.159	-0.029
Protein	-0.126	0.326	0.081	-0.391	0.334
Tannin	-0.258	-0.112	-0.260	-0.043	-0.273
Thiamine	-0.353	0.002	0.170	-0.004	0.110
Riboflanin	-0.126	-0.077	0.271	-0.125	-0.119
Niacin	0.207	0.094	-0.231	-0.421	-0.266
Vitamin c	-0.150	-0.147	0.123	0.379	0.055
Zinc	0.260	0.248	-0.188	0.362	0.034
Eigenvalue	5.348	3.137	3.001	2.028	1.693
Percentage variation	28.100	16.500	15.800	10.700	8.700
Cummulative	28.100	44.700	60.500	71.100	80.000

Table 9. Eigen vector for principal components of nutritional traits of the leaves of Nigerian accessions of *Piper nigrum*

Attributes	PC1	PC2	PC3	PC4	PC5
Alkaloid	-0.349	0.168	-0.152	0.331	0.035
Ash	0.054	-0.227	0.275	0.221	-0.434
Calcium	-0.384	-0.111	-0.209	-0.037	0.039
Carbohydrates	-0.260	-0.140	-0.343	0.208	0.183
Fat	-0.244	-0.012	0.295	0.393	-0.113
Fibre	0.262	-0.255	-0.180	0.349	-0.073
Flavonoid	0.006	-0.222	-0.360	0.030	-0.051
Hydrogen cyanide	0.085	-0.338	-0.016	-0.070	0.103
Iron	-0.263	-0.404	0.051	0.012	-0.081
Magnesium	-0.196	0.317	0.091	-0.145	-0.350
Moisture content	0.307	0.314	-0.123	-0.160	0.080
Oxalate	0.078	0.115	-0.206	0.061	-0.435
Protein	0.097	0.117	0.441	0.244	0.193
Tannin	-0.055	0.275	0.146	0.436	0.271
Thiamine	-0.409	0.089	-0.020	-0.123	-0.146
Riboflanin	-0.227	-0.256	0.359	0.086	0.097
Niacin	-0.216	0.061	0.134	0.088	0.285
Vitamin c	-0.022	0.058	0.127	0.416	-0.441
Zinc	-0.197	0.339	-0.208	0.108	0.055
Eigenvalue	4.492	3.289	2.974	2.449	1.886
Percentage variation	23.600	17.300	15.700	12.900	9.900
Cummulative	23.600	41.000	56.600	69.500	79.400

4. Conclusions

The results obtained in this study showed that variability exists in the leaves and fruits of Nigerian accessions of *Piper nigrum*. The variability obtained was more genetic than environmental. The genotypic effect was further evidenced by the high heritabilities and genetic advance observed for ash, crude fibre, iron, hydrogen cyanide, tannin and riboflavin in the leaves and iron hydrogen cyanide and riboflavin in the fruits indicating that these traits are controlled by additive genes which shows that selection can be effective for their improvement.

REFERENCES

[1] Srinivasan, K. 2007. Black pepper and its pungent principle-piperine: A review of diverse physiological effects. Critical Rev. Food Nut. 47:735-748.

[2] Tripathi, A.K., D.C. Jain and S. Kumar, 1995. Secondary metabolites and their biological and medical activities of <I>*Piper*</I> species plants. J. Med. Arom. Plant Sci., 18: 302-321

[3] Khajuria, A., N. Thusu and U. Zutshi, 2002. Piperine modulates permeabilitycharacteristics of intestine by inducing alterations in membrane dynamics: Influence on brush border membrane fluidity, ultra-structure and enzyme kinetics. Phytomedicine, 9(3): 224-231.

[4] Stohr, J.R., P.G. Xiao and R. Baur, 2001. Constituents of Chinese <I>*Piper*</I> species and their inhibitory activity on prostaglandin and leukotriene biosynthesis <I>*In vitro*</I>. *J.* Ethnopharmacol*.,* 75*:*133-139.

[5] Sawangjaroen, N., K. Sawangjaroen and P. Poonpanang,. 2004. Effects of *Piper longum* fruit, *Piper sarmentosum* root and *Quercus infectoria* nut gall on caecal amoebiasis in mice. J. Ethnopharm. 91:357-360.

[6] Trivedi, M.N., A. Khemani, U.D. Vachhani, C.P. Shah and D.D. Santani, 2011. Pharmacognostic, phytochemical analysis and anti-microbial activity of two <I>*Piper*</I> species. Int. J. Comp. Pharm., 7(5):1-4.

[7] Abdulfiyaz, R., K.T. Ramya, Chikkalingaih, B.C. Ajal, C. Gireesh and R.S. Kulkami, 2011. Genetic variability, correlation and path coefficient analysis in rice (<I>Oryza sativa</I> L) under alkaline soil condition. Electron. J. Plant Breed., 2: 531-537.

[8] Baye, T. 2002. Genotypic and phenotypic variability in Vernonia galamensis nvar Ethiopia germplasm collected from eastern Ethiopia. J. of Agric. Sci. (Camb). 139:161-168.

[9] Nwofia, G.E., E.E. Ene-Obong and P.I. Okocha,. 2006. Genotypic and phenotypic variability in cowpea grown in a humid environment in Nigeria.Trop.Sci.46(1):82-86.

[10] Kashiani, P., G. Saleh, N.A.P. Abdullah and S.N. Abdullah,. 2010. Variation and genetic studies on selected sweet corn inbred lines. Asian J. crop sci. 2:78-84.

[11] Nwofia, G. E. and P. Ojimelukwe 2012. Variability in proximate, mineral and vitamin contents of *Carica papaya* leaves, fruit pulp and seeds. Int. J. med. And Arom. Plants. 2(1): 90-96.

[12] Prasad, S.R., R. Prakash, C.M. Sharma and M.F. Haque,. 1981. Genotypic and Phenotypic variability in quantitative characters in Oat. Indian J. Agric. Sci. 51:480-482.

[13] James, C.S., 1995. Analytical Chemistry of Foods. Chapman and Hall, New York, USA.

[14] AOAC,. 1990. Official Method of Analysis.. 15th Edn.,. Association of Official Analytical Chemists,. Benjamin Franklin Station, Washington DC., USA.

[15] Kirk, R.S. and R. Sawyer, 1998. Pearson's Composition and Composition Analysis of Foods. 9th Edn., Churchill Livingstone, Edinburgh, Uk., pp: 615-616.

[16] Pearson, D., 1976. The Chemical Analysis of Foods. 7th Edn., Churchill Livingstone, Edinburgh, UK., pp: 488-496.

[17] Carpenter, S. and P. Hendricks, 2003. Pearspon Laboratory Techniques in Food Analysis. Butterworth and Co., Uk., pp: 10-17.

[18] Okwu, D.E.2004. The phytochemicals and vitamins contents of indigenous spices of South Eastern, Nigeria. J. Sust. Agric. Environ. 6:30-34.

[19] Harborne, I.B.,. 1973. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.. 2nd Edn.,. Chapman and Hall,. New York,. pp: 88-185.

[20] Hang, W. and H.J. Lautszch, 1983. Comparatively methods for the rapid determination of oxalate and phytate in cereal products. J. sci. food and Agric. 34:1423-1426.

[21] Genstat 2007. Genstat for window, Discovery. 3rd edn, Lawes Agricultural trust, Rothamsted experimental station, U.K.

[22] Wricke, G. and W.E. Weber,. 1986. Quantitative Genetics and Selection in Plant Breeding Walter de Gruyter and Co.,. Berlin. .

[23] Burton, G.W., 1952. Quantitative inheritance in grasses. Proceedings of the 6th International Grassland Congress, August 17-23, 1952, Pennsylvania State College, USA., pp: 277-283.

[24] Johnson, D. E. 1998. Applied multivariate methods for data analysis. Duxbery press, New York.

[25] Kumar, A., S.C. Misra, Y. P. Singh nad B.P.S. Chanhan 1985. Variability and correlation studies in Triticale. J. of Mah. Agric. Univ. 10: 273-275.

[26] Allard, R.W.,. 1991. Principles of plant breeding. 2nd edn. New York; Wiley and Sons.

[27] Fehr, W.R., 1987. Principles of cultivar development. Vol. 1., New York, Mac Millian.

[28] Sood, S., N. Kumar, K.S. Chandel and P. Sharma, 2011. Determination of genetic variation for morphological and yield traits in bell pepper (*Capsicum annum* var *grossum*). *Indian Journal of Agricultural Sciences* 81(7): 590-594.

[29] Crossa, J. 1990. Statistical analysis of multi-location trials. Adv. Agron. 44:55-85.

[30] Ntawuruhunga, P. 2000. Evaluation of cassava (*Manihot esculenta* Crantz) genotypes for adaptation for different altitudes. PhD thesis, Makerere, University, Uganda.

[31] Akimbo, O., M. Labuschagne and M. Fregene, 2012. Increased storage protein from interspecific F₁ hybrids between cassava <I>*Manihot esculenta*</I> Crantz and its wild progenitor (<I>*M. esculenta*</I> ssp. <I>Flabellifolia</I>). Euphytica, 185: 303-311.