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## Variability in Nutritional Traits in *Tetrapleura tetraptera* (Schum and Thonn.) Taub. From Cross River State, Nigeria

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**Abstract:** *Tetrapleura tetraptera* (Schum. and Thonn.) is highly valued for its nutritional and medicinal attributes. However, the plant grows wild in the forest and is getting gradually wiped out with no substantial conservation or breeding efforts made. For any meaningful progress to be made in this direction, the nature of variation in this crop must be ascertained. In this study, dry pods of twenty accessions of *Tetrapleura tetraptera* collected from six local government areas in Cross River State were evaluated for variation in nutrient and phytochemical composition. Genetic component analysis showed that these attributes had more genetic than non-genetic factors contributing to the variability which indicates considerable scope for selection among the studied accessions. High estimates of broad sense heritability with relatively high genetic advance were obtained for saponin, riboflavin and niacin contents, indicating that these attributes were under the control of additive gene effect. Therefore, the plant can be improved with respect to these attributes through direct selection. Principal Component Analysis showed that all the attributes examined contributed maximally to the observed diversity among these accessions. Four clusters emerged with the following accessions: AKA3 from cluster1, IKM1 from cluster3, AKP3 from cluster2, AKP5 from cluster3 and BOK1 from cluster 4 identified as superior based on multiple trait performance suggesting that selection of superior accessions may be carried out for more than one trait. This technique will broaden the genetic base of *T. tetraptera* germplasm for domestication and varietal development.

**Key words:** Nutrients, phytochemicals, *Tetrapleura tetraptera*, clusters PCA

### INTRODUCTION

*Tetrapleura tetraptera* (Schum and Thonn), commonly known as aidan tree, belongs to the family fabaceae. It is highly valued in Nigeria and beyond for its nutritional and medicinal properties. The fruits and seeds add good aroma and flavor to food, (Essien *et al.*, 1994; Okwu, 2003; Aladesanmi, 2007; Omokhua and Ukoimah, 2008), eliminate the pungent odour and inhibit fungal growth in cassava fufu (Okwu, 2004). The fruit also prevents post partum contraction in nursing mothers and is used for the management of convulsions, leprosy, inflammation and rheumatism (Enwere, 1998). The leaves are essential for the treatment of epilepsy (Akah and Nwabie, 1993) and possess strong molluscicidal activity (Adewunmi *et al.*, 1991). The aqueous fruit extract has also been shown to possess hypoglycaemic properties (Ojewole and Adewunmi, 2004).

Unfortunately, its continuous availability is threatened by extinction as a result of over exploitation. This calls for intensification of conservation and breeding efforts on this crop. To do this effectively, information on variability and heritability estimates in key attributes must be known. In this study, twenty accessions of *T. tetraptera*

from Cross River State, Nigeria were screened for variation in composition and concentration of key nutrients and phytochemicals. Heritability estimates of these attributes in *T. tetraptera* are reported for the first time.

### MATERIALS AND METHODS

Mature pods of twenty accessions of *Tetrapleura tetraptera* were collected from different locations in Akamkpa, Akpabuyo, Bakassi, Boki, Ikom and Odukpani, Local Government Areas in Cross River State, Nigeria (Table 1). They were washed manually with distilled water and sundried for three days. The dried fruits were pulverized into fine powder using a grinder (Bxinator: BLG-400), then sieved through mesh sieves (1 mm) and stored in air tight bottles. These were then used for the various analyses.

**Proximate analysis:** Moisture, ash, crude protein and crude fat were determined using the methods of (Onwunka, 2005; Udo *et al.*, 2009), nitrogen free extract (NFE) referred to as soluble carbohydrate was obtained by subtracting all the other components (except fat and dry matter) from 100%.  $NFE = 100 - (\text{ash} + \text{crude\% fibre} + \text{crude protein} + \text{moisture\%})$ .

Table 1: Accessions of *Tetrapleura tetraptera* and their collection sites

Accession	Location	L.G.A.	Latitude	Longitude	Altitude(M)
AKA1	Akor	Akamkpa	05°22/57N	008°16/44E	89
AKA2	Oban	Akamkpa	05°36/45N	008°50/41E	102
AKA3	Aningeje	Akamkpa	05°24/53N	008°13/48E	98
AKP1	Ikot Ekpo	Akpabuyo	04°33/45N	008°23/34E	97
AKP2	Ekpene-Efioeyo	Akpabuyo	04°27/41N	008°21/38E	92
AKP3	Efak Inang	Akpabuyo	04°24/38N	008°18/31E	86
AKP4	Ikot Edem Ndarake	Akpabuyo	04°27/41N	008°21/38E	92
AKP5	Ikot Edem Ita	Akpabuyo	04°39/22N	008°33/44E	97
AKP6	Ikot Ewa	Akpabuyo	04°34/46N	008°26/37E	94
AKP7	Ekpri Ikang	Bakassi	04°12/13N	008°04/59E	63
AKP8	Esighi	Bakassi	04°13/18N	008°09/62E	48
AKP9	Ikot offiong Ambai	Akpabuyo	04°33/48N	008°29/37E	79
AKP10	Ikot Nakanda	Akpabuyo	05°06/32N	008°11/48E	77
AKP11	Etomkpe Inameti	Akpabuyo	04°11/34N	008°14/38E	93
BOK1	Isorbendegekem	Boki	06°26/40N	008°48/41E	118
BOK2	Afi-forest	Boki	06°23/59N	008°45/04E	109
IKM1	Etara community	Ikom	05°46/60N	008°31/60E	121
IKM2	Akparabong	Ikom	06°01/60N	008°45/00E	109
ODU1	Ikot Ukpa	Odukpani	05°07/33N	008°21/19E	60
ODU2	Ikot Eyo-Okon	Odukpani	04°46/00N	007°57/00E	49

**Phytochemical analysis:** Alkaloids, flavonoids, phenols, saponins, sterol and tannins were estimated by the methods described by Harbone (1973) and Edeoga *et al.* (2006). Hydrogen cyanide was estimated using the alkaline extraction method described by Onwunka (2005).

**Vitamin and mineral analysis:** Vitamins A and E contents were estimated by the methods described by association of vitamin chemists (Pearson, 1976); vitamin C was estimated by the method described by Kirk and Sawyer (1998), B-complex vitamins (thiamine, riboflavin and niacin) were estimated by spectrophotometric method described by James (1995). Phosphorus, Calcium, Magnesium, Potassium and Sodium were estimated as described by AOAC (2000) while Iron and Zinc were estimated as described by James (1995).

**Data analysis:** Data obtained were subjected to analysis of variance using Genstat Discovery Edition 4 (Genstat, 2007) software. Genotypic, phenotypic and error variances were estimated using the formulae of Wricke and Weber (1986) and Prasad *et al.* (2010):

$$VG = \text{MSG} - \text{MSE}/r, VP = \text{MSG}/r, VE = \text{MSE}/r$$

where, MSG, MSE and r, are the mean squares of genotypes, mean squares of error and number of replications, respectively. The phenotypic (PCV) and genotypic (GCV) coefficients of variations were estimated by the methods of Burton (1952), Johnson *et al.* (1955) and Kumar *et al.* (1985) as follows:

$$\text{PCV} = \sqrt{\text{VP}/X} * 100, \text{GCV} = \sqrt{\text{VP}/X} * 100$$

where, VP, VG and X are phenotypic variance, genotypic variance and grand mean respectively, for each attribute under consideration. Broad sense heritability ( $h^2Bs$ ) expressed as the ratio of VG to the VP was estimated on genotypic mean basis as described by Allard (1991). Genetic advance (GA) was estimated by the method of Fehr (1987) as  $GA = K (Sp) h^2Bs$ , where K is a constant (2.06 at 5% selection pressure), Sp is the phenotypic standard deviation  $\sqrt{\text{VP}}$ ,  $h^2Bs$  is the broad sense heritability. GA was calculated as a percentage of the mean.

Factor analysis based on Principal Component Analysis (PCA) was performed using Genstat Discovery Edition 4 (Genstat, 2007) software to characterize the accessions in relation to the most discriminating traits (Johnson, 1998). Hierarchical cluster analysis using Ward's method with squared Euclidean distance (HCA) was performed using SPSS version 15.0 for windows.

## RESULTS AND DISCUSSION

**Genetic component analysis:** Estimates of phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in the broad sense and genetic advance for proximate composition, phytochemicals, vitamins and minerals are shown in Table 2. PCV ranged from 0.012 to 0.115 in the proximate attributes, 0.008 to 0.118 in phytochemicals, 0.004 to 0.023 in vitamins and 0.000 to 0.045 in minerals. GCV ranged from 0.027 to 0.115 in proximate components, 0.008 to 0.023 in vitamins and 0.000 to

Table 2: Phenotypic Coefficient of Variation (PCV), Genotypic Coefficient of Variation (GCV), Broad sense heritability (h<sup>2</sup>Bs), Genetic Advance (GA%), for proximate, phytochemicals, vitamins and minerals in 20 accessions of *T. tetraptera* from Cross River state

Attributes	Mean	PCV	GCV	PCV-GCV	h <sup>2</sup> Bs	GA%
<b>Proximate</b>						
Moisture	9.080	0.055	0.055	0.000	99.944	1.251
Ash	3.932	0.027	0.027	0.000	99.934	1.392
Fibre	3.267	0.012	0.012	0.000	99.975	0.755
Fat	18.328	0.066	0.066	0.000	99.983	0.743
Protein	6.790	0.036	0.036	0.000	99.939	1.096
CHO	58.589	0.115	0.115	0.000	99.971	0.404
<b>Phytochemicals</b>						
Tannin	0.408	0.026	0.026	0.000	99.797	13.035
Sterol	0.091	0.061	0.060	0.001	97.424	0.122
Alkaloid	2.151	0.031	0.031	0.000	99.918	2.925
Phenol	0.075	0.118	0.113	0.005	92.252	0.224
Flavonoid	2.481	0.008	0.008	0.000	99.987	0.702
Saponin	0.654	0.072	0.071	0.000	99.263	22.544
HCN	4.416	0.013	0.013	0.000	99.975	0.625
<b>Vitamins</b>						
A	3.360	0.005	0.005	0.00	99.992	0.2045
Thiamin	-	-	-	-	-	-
Riboflavin	0.023	0.004	0.004	0.000	99.151	37.687
Niacin	0.081	0.016	0.016	0.000	99.509	39.557
C	1.005	0.017	0.017	0.000	99.877	3.385
E	2.871	0.023	0.023	0.000	99.938	1.624
<b>Minerals</b>						
Calcium	149.231	0.000	0.000	0.000	99.903	0.000
Iron	1.753	0.045	0.045	0.000	99.646	5.241
Potassium	251.744	0.016	0.016	0.000	99.998	1.339
Magnesium	87.623	0.009	0.009	0.000	99.999	2.002
Sodium	-	-	-	-	-	-
Phosphorus	37.890	0.017	0.017	0.000	99.993	0.089
Zinc	0.800	0.041	0.041	0.000	99.352	10.467

Not significant

0.045 in minerals. Broad sense heritability ranged from 92.252 to 99.999 while Genetic advance ranged from 0.000 to 39.557. PCV were generally slightly higher than GCV, indicating that these attributes had, to some degree, interacted slightly with the environment. Correspondingly, Estimates of broad sense heritability were very high for all the attributes. Therefore, selection for these attributes is likely to be dependable and effective. This is similar to earlier reports in the same family (Fabaceae) by Makeen *et al.* (2007) in *Vigna radiata*, Nwofia *et al.* (2006) and Idahosa *et al.* (2010) in *Vigna unguiculata*, Nechifor *et al.* (2011) in *Phaseolus vulgaris*.

Heritability in conjunction with genetic advance is more effective and reliable in predicting the results and effectiveness of selection (Johnson *et al.* 1955). High estimate of broad sense heritability (h<sup>2</sup>B) with relatively high genetic advance obtained for saponin, riboflavin and niacin (Table 2), indicates that these attributes are under the control of additive gene effects and therefore the plant can be improved with respect to these attributes through direct selection. However, relatively high estimates of broad sense heritability with low genetic advance were obtained for other attributes in this study, indicating the presence of non-additive gene action. Thus, direct selection for such attributes may not be effective. In such situations, recombination breeding

Table 3: Eigenvector and eigenvalues for principal components based on proximate composition in 20 accessions of *T. tetraptera* from cross river state

Attributes	PC1	PC2	PC3
Ash	0.121	-0.269	0.747
Carbohydrate	-0.651	0.258	0.115
Fat	0.683	-0.113	-0.052
Fibre	0.224	0.565	-0.045
Moisture	-0.087	-0.421	-0.638
Protein	-0.195	-0.593	0.131
Eigenvalue	2.030	1.815	1.260
Percentage variation	33.8	30.2	21.0
C.P.V.	33.8	64.1	85.1

C.P.V. : Cumulative percentage variation

may give better response for improvement (Subi and Idris, 2013). Result from the present study is in line with earlier reports on nutrient analyses and yield by Rohman *et al.* (2003) in mung bean, Idahosa (2010) in *Vigna unguiculata*, Nechifor *et al.* (2011) in *Phaseolus vulgaris*, Nwofia *et al.* (2012) in *Cucurbita* spp. and Nwofia and Adikibe (2012) in *Ocimum gratissimum*.

**Principal component analysis:** Principal component analysis (PCA) showed that the first three Principal components (PCs) were important with eigenvalues greater than 1.00 and explained 85.1% of the total variation in the proximate composition of the fruits of the

20 accessions of *T. tetraptera* (Table 3).  $PC_1$  had an eigenvalue of 2.030 and accounted for 33.9% of the total variation. Fat and carbohydrate were important contributing variables here.  $PC_2$  had high loading for crude protein and crude fibre contents with an eigenvalue of 1.815 and percentage variation of 30.2%.  $PC_3$  had an eigenvalue of 1.260 contributing 21.0% of the variation and had ash and moisture as the main contributing variables.

The PCA of phytochemical attributes are shown in Table 4. The first four PCs were important and accounted for 77.7% of the total variation observed.  $PC_1$  had eigenvalue of 1.595 and accounted for 22.8% of the total variation. Two attributes namely phenol and saponin were important contributing variables here.  $PC_2$  had high loading for flavonoid and tannin with eigenvalue of 1.573 and percentage variation of 22.5%.  $PC_3$  had eigenvalue of 1.280 and this accounted for 18.3% of the variation with hydrogen cyanide being the major contributing variable.  $PC_4$  had high loading for alkaloid with eigenvalue of 0.994 and percentage variation of 14.2%.  $PC_5$  had eigenvalues less than 1.00 and thus regarded as making no significant contribution to the total variation.

The PCA for vitamins and minerals are shown in Table 5. The first five PCs accounted for 79.6% of the total variation observed in the vitamins and mineral

composition.  $PC_1$  had eigenvalue of 3.337 and accounted for 25.7% of the total variation. Zinc, magnesium and vitamin E were the important contributing variables in  $PC_1$ .  $PC_2$  had high loading for phosphorus, sodium and vitamin C with an eigenvalue of 2.826 and percentage variation of 21.7%.  $PC_3$  had eigenvalue of 1.877 and this contributed for 14.4% of the variation.  $PC_3$  had high loading for vitamin A and vitamin C.  $PC_4$  had high loading for calcium with an eigenvalue of 1.335 and contributed 10.3% of the variation.  $PC_5$  had eigenvalue of 0.979 and contributed 7.5% to the total variation with thiamin being the major contributing variable.

The principal component analyses indicate that all the nutrient and phytochemical attributes contributed maximally towards the observed diversity among the accessions. This is in line with earlier reports by Nwofia and Adikibe (2012) in *Ocimum gratissimum* and Nwofia *et al.* (2012) in *Cucurbita* spp.

**Cluster analysis:** Cluster analysis based on Squared Euclidean Distance using Ward's method gave 4 clusters for 19 nutrient and 7 phytochemical attributes (Fig. 1). Clusters 1 and 3 contained 8 accessions each while clusters 2 and 4 had 3 and 1 accession, respectively. Cluster 1 had the following accessions:

Table 4: Eigenvector and eigenvalues for principal components based on seven phytochemical traits in 20 accessions of *T. tetraptera* from cross river state

Attributes	PC1	PC2	PC3	PC4	PC5
Alkaloid	-0.376	-0.062	0.368	0.645	0.382
Flavonoid	-0.255	0.618	0.089	0.266	-0.607
Hydrogen cyanide	-0.088	-0.159	-0.681	0.097	0.647
Phenol	0.618	0.140	-0.245	0.349	-0.119
Saponin	0.455	0.526	0.077	0.116	0.327
Sterol	-0.270	0.504	-0.069	-0.542	0.265
Tannin	0.351	-0.970	0.567	-0.274	0.483
Eigenvalue	1.595	1.573	1.280	0.994	0.740
variation (%)	22.8	22.5	18.3	14.2	10.6
Cumulative variation (%)	22.8	45.2	63.5	77.7	88.3

Table 5: Eigenvector and eigenvalues for principal components based on seven vitamin and mineral traits in 20 accessions of *T. tetraptera* cross river state

Attributes	PC1	PC2	PC3	PC4	PC5
Vit. A	-0.182	-0.141	-0.621	0.040	0.058
Thiamin	-0.020	-0.144	-0.209	-0.323	0.768
Riboflavin	-0.393	-0.170	0.052	0.276	-0.238
Niacin	-0.118	-0.414	0.151	0.391	0.116
C	0.145	0.169	0.451	-0.354	0.101
E	-0.408	-0.106	-0.248	-0.242	-0.042
Calcium	0.089	-0.135	-0.274	-0.460	-0.526
Iron	-0.328	0.321	0.122	-0.291	-0.085
Potassium	0.366	0.272	-0.286	0.280	0.038
Magnesium	-0.411	0.301	-0.012	-0.093	0.087
Sodium	0.022	0.441	-0.208	0.154	0.108
Phosphorus	-0.094	0.481	-0.142	0.217	-0.068
Zinc	-0.428	0.080	0.211	0.164	0.125
Eigenvalue	3.337	2.826	1.877	1.335	0.979
variation (%)	25.7	21.7	14.4	10.3	7.5
Cumulative variation (%)	25.7	47.4	61.8	72.1	79.6

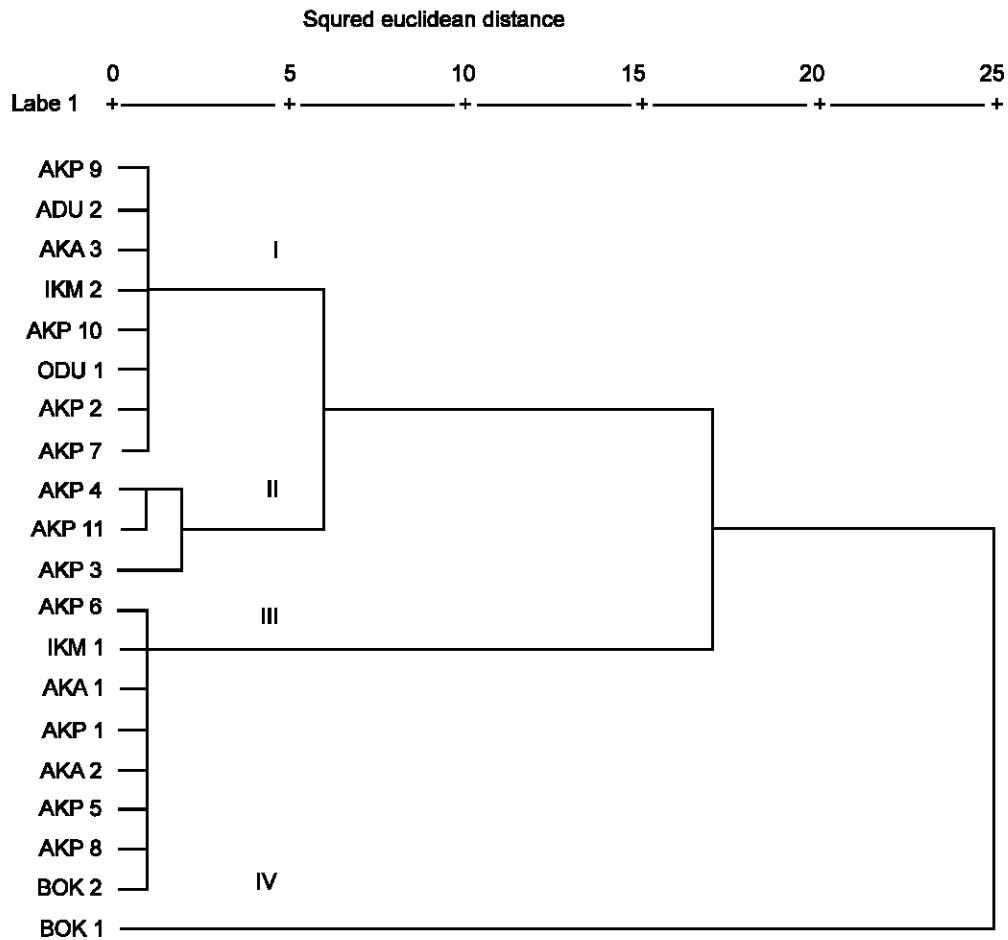


Fig. 1: Cluster pattern for nutrient and phytochemical attributes in 20 accessions of *T. tetraptera* generated from Hierarchical Cluster Analysis using Ward's method

AKP9, ODU2, AKA3, IKM2, AKP10, ODU1, AKP2 and AKP7. Members of cluster1 had low tannin content, moderate fat and calcium contents, while sterol, thiamin, potassium and sodium contents were high. Cluster 2 was made up of AKP4, AKP11 and AKP3. The unique features among members of cluster 2 included low alkaloid and thiamine contents, high ash, saponin and vitamin A contents. Accessions AKP6, IKM1, AKA1, AKP1, AKA2, AKP5, AKP8 and BOK2, were grouped into cluster 3 and were rich in crude fiber, carbohydrate, tannins, but low in total fat and calcium. BOK1 was the only accession in cluster 4 and was characterized by high fat, alkaloid and niacin, but low zinc, phosphorus, magnesium, Vitamin A, hydrogen cyanide, saponin and carbohydrate. This shows that the studied accessions can actually be subdivided into four groups and the unique attributes observed among accessions in each cluster could be exploited for varietal development and improvement.

Based on the information from analysis of variance and mean separation coupled with clustering pattern, the following accessions have been identified for use in breeding programmes:

- AKA3 from cluster 1 with high zinc concentration (0.85 mg/100 g), high riboflavin content (1.81 mg/100 g) and high alkaloid content (2.59%)
- IKM1 from cluster 3 with high saponin content (0.75%), low tannin content (0.26%), high alkaloid content (2.31%).
- AKP3 from cluster 2 with high saponin content (0.8%), high zinc content (0.84 mg/100 g), high magnesium content (94.13 mg/100 g) and relatively low tannin level (0.31%)
- AKP5 from cluster 3 with high niacin content (0.11%) and high alkaloid content (2.58%)
- BOK1 from cluster 4 with relatively high vitamin C (1.06 mg/100 g) and iron contents (1.81 mg/100 g) and relatively high alkaloid content (2.76%)

Table 6: Variation among clusters for nutrient and phytochemical attributes in 20 accessions *T. tetraptera* from cross river state

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Frequency	8	3	8	1
Attributes	X	X	X	X
Moisture content	9.32	8.86	8.86	10.19
Ash	3.86	4.08	3.94	3.94
Crude fibre	3.12	3.14	3.34	3.15
Total fat	19.04	18.49	16.95	24.71
Crude protein	6.72	6.77	6.81	6.85
Carbohydrate	57.93	58.67	60.11	51.17
Tannins	0.35	0.37	0.46	0.41
Sterol	0.95	0.11	0.08	0.09
Alkaloids	2.25	1.81	2.17	2.76
Phenols	0.06	0.10	0.08	0.08
Flavonoids	2.52	2.89	2.27	2.95
Saponins	0.63	0.74	0.67	0.06
Hydrogen cyanide	4.37	4.63	4.47	0.04
Vit. A	3.34	3.51	3.33	2.73
Thiamine	0.53	0.04	0.43	0.11
Riboflavin	0.02	0.03	0.03	0.02
Niacin	0.07	0.10	0.20	0.50
Vit. C	1.00	0.88	1.06	1.06
Vit. E	2.74	2.91	2.99	2.68
Calcium	156.85	174.87	134.98	176.40
Iron	1.77	1.72	1.75	1.81
Potassium	253.53	246.86	249.43	248.77
Magnesium	89.48	87.68	88.11	80.18
Sodium	11.63	9.88	11.04	10.17
Phosphorus	38.48	36.08	38.57	34.9
Zinc	0.81	0.82	0.81	0.69

**Conclusion:** High estimates of broad sense heritability with high genetic advance were obtained for saponin, riboflavin and niacin, indicating the presence of additive gene action and hence the possibility of direct selection for these traits. IKM1, AKA3, AKP3, AKP5 and BOK1, were identified as superior genotypes based on multiple trait performance.

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