Assessment of diversity among tropical and subtropical maize inbreds based on morphological traitsand carotenoid content

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ABSTRACT

The available maize germplasm contains sufficient genetic variability for pro-vitamin A content which could be utilized for development of pro-vitamin enriched varieties aimed at reducing vitamin A deficiency among millions of resource-limited Africans.Fifty-onemaize inbred lines from Nigeria and CIMMYT (Zimbabwe) comprising of thirty-sevenyellow and fourteen white endosperm types were evaluated in replicated trials at the Institute of Agricultural Research and Training, Moor Plantation, Ikenne and the Teaching and Research farm, University of Ilorin. The objective was to assess the extent of diversity among the inbreds using morphological traitsand carotenoid content. The results showed wide variability (P =.05) among the inbred lines for grain yield and other traits as well as total carotenoids. Differences in weather factors in the two locations significantly affected the expression of the traits investigated especially grain yield which recorded the highest deficit in Ilorin due to prolonged drought-stress that occurred at flowering period. Although many of the inbred lines exhibited differential performances for grain yield across the two locations, three inbred lines- BD74-49, BD74-68 and BD74-81 showed consistency in their performance for this trait across the two locations. Significant variation in kernel carotenoid content was also obtained among the 40 maize genotypes investigated. Total carotenoid ranged from0.03µg/g in the white kernel inbred TZEI 65 to 56.52µg/g in a light orange coloured inbred line BD74-89. The Unweighted Pair Group Method with Arithmetic Averages (UPGMA) dendrogram constructed from the morphological traitsclustered the 51 inbred lines into two groups based essentially on their geographical origin, while the dendrogram generated based on total carotenoid content clustered the 40 inbred lines into two major groups with clusters of at least three inbred lines in a sub-cluster. Clustering patterns revealed that lines with high carotenoid content were found in different sub-clusters indicating the feasibility of developing high carotenoid maize varieties from the currently available gene pool.

Key words: Zea mays L., Pro-vitamin A, morphological traits, carotenoid content, inbred lines.

1. INTRODUCTION

Carotenoids which are natural plant pigments in the endosperm and fruits of crop plants are important source of vitamin A and antioxidants that are essential for human consumption. Dietary carotenoids are essential precursors of vitamin A and retinoid compounds needed in animal morphogenesis [3], which plays an important role in the proper functioning of the eye, growth of bone, reproduction, cell division, and cell differentiation. It also regulates the immune system, which helps preventinfections by making white blood cells that destroy harmful bacteria and viruses. Maize (*Zea mays* L.) kernel contains tremendous variation in content and composition of several coloured pigments collectively known as carotenoidsand is knownfor its genetic diversity of carotenoid content and profiles [18]. Consequently, it is one of the potential crops for bio-fortification aimed at increasing the bio-available micronutrient content of food cropsthrough genetic selection through plant breeding. [21]as well as [32] hypothesized thatexploitationof the natural genetic diversity of maize for carotenoidsthrough bio-fortification will increase pro-vitamin A concentration in maize endosperm, which will be beneficial for public health. Secondly, the process also holds promise for sustainable and cost-effective food-based solutions to combatmicronutrient deficiencies in Africa where maize is a major security and staple cropfor more than 300 million people [27].However, pro-vitamin A usually constitutes only10 to 20% of the total carotenoids in maize kernel, and the study conducted by [22] revealed that thecommonly cultivated and consumed yellow maize cultivars have less than 2µg/gdry weight pro-vitamin A.

Diversity study is important in assessing the extent, pattern and association among breeding populations. Genetic diversity assessment has also been used in the identification of parental lines for evolving superior genotypes that could be utilized in crosses and in the establishment of heterotic groups that could yield superior hybrids in maize.Genetic diversity analysis in crop improvement and morphological characterization of genotypes is considered as the first step [13]. Therefore, morphological data play key role in management of genetic resources [28]. identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection and introgressing desirable genes from diverse germplasm into the available genetic base [30].

In recent years, molecular breeding techniques including SSR-based markers [29, 31] have been utilized in the assessment of genetic diversity among breeding populations for different maize attributes including carotenoid contents. The importance of developing pro-vitamin A enriched maize variety underscores the necessity to have information on the extent of diversity among inbred parents with respect to pro-vitamin contents. Such varieties could be used in the development of yellow endosperm maize varieties (Open Pollinated Varieties and hybrids) which possess genes for adaptation and superior agronomic performance and thus represent a good genetic base to breed tropical maize for high levels of pro-vitamin A carotenoids [25]. Intwo separate studies, [1] as well as [2] assessed the nature and extent of the genetic diversity among tropically adapted yellow endosperm maize inbred lines at the International Institute of Tropical Agriculture (IITA) maize breeding programme. In the first study, [1] noted the diversity of carotenoid contents among 38 tropically adapted yellow endosperm maize inbred lines while the second report [2]contained information on the nature of variability for different carotenoid contents among the newly developed 122 inbred lines and grouped into three major groups based on carotenoid contents. In the study reported herein, 51 maize inbred linescollected from two major geographical regions (Nigeria and Zimbabwe), were evaluated in two locations using 12

morphological traits (including grain yield) and carotenoid content. The objectives were to (i) assess the extent of genetic diversity among white and yellow endosperm maize inbred lines, (ii) identify lines with high carotenoids content, and (iii) delineate the genotypes for future breeding purposes.

2. MATERIALS AND METHODS

2.1 Source of Genetic Materials

Fifty-one maize inbred lines were received from three different sources viz., CIMMYT (Zimbabwe), International Institute of Tropical Agriculture (IITA), Ibadan and Institute of Agricultural Research and Training (IAR&T), Ibadan were used for this study. The details of the inbred lines including their grain colour are presented in Table 1.

2.2 Description of Experimental Sites

Ikenne (61 m above sea level, Latitude 6^{o} 52'N and Longitude 3^{o} 42' E) is in the rain forest ecology with annual rainfall and temperature ranges of 1000-1200 mm and 22°C – 32.2°Crespectively. Ilorin (370 m above sea leveland Latitude 8^{o} 30' N and, Longitude 4^{o} 32'E)is in the southern guinea savannah ecology withannual rainfallof1000mm and temperature range of 24°C – 35°C. However, while rainfall distribution at both locations is bimodal, mid-season drought is likely to occur at any stage of crop growth. This is a regular feature of weather pattern at Ilorin which makes the zone a drought-prone ecology.

2.3 Field Evaluation

The inbred lines were evaluated at the IAR&T sub-station, Ikenne during the growing season of 2012 and at the Teaching and Research Farm, University of Ilorin between July-September of 2013.Seeds were sown at each location using a randomized complete block design with three replicates. Each plot consisted of single row with spacing of 75cm between the rows and plant to plant spacing of 25cm, giving a plant population of 53,333 plants/ha. Pre-emergence herbicide (Atrazine 223 g/L + Metolachlor 277 g/L) was applied at planting at both sites followed by supplementary hand weeding as required. The recommended fertilizer dose and other cultural practices in each zone were adopted.

2.4 Morphological study

Data were recorded from five random plants in a plot on twelve traits namely; days to mid-anthesis and silking, anthesis-silking interval (ASI), plant and ear heights (cm), leaf length and width (cm), ear length and diameter (cm), number of rows per ear, number of kernels per row and grain yield (kg/plot). Days to mid-anthesis and silking were estimated as the number of days from planting to when 50% of the plants in a plot have shed pollen and had silk emergence respectively. ASI was estimated as the difference between the mid-anthesis and silking. Plant height was measured from the soil surface to the tip of the central axis while ear height was recorded in centimeters from the distanceof the ground to the base of the uppermost developed ear. Grain yield was obtained by weighing dehuskedears in each plot in kg/plot and later converted to tons/ha after adjusting the moisture level to 12% and assuming a shelling percentage of 80%.

2.5 Data Analyses

Data collected were subjected to analyses of variance (ANOVA) on individual location prior to combine locations analysis. The quantitative traits were also subjectedto cluster analysis.

Sequential Agglomerative HierarchicalNon-overlapping (SAHN) clustering was performed on similaritymatrices utilizing the Unweighted Pair Group Method with ArithmeticAverages (UPGMA) method. Data analysis was done usingNTSYSpcsoftware,version 2.02i [24]

2.6 Carotenoid analysis

Forty of the inbred lines were further analyzed for their carotenoid contents. Thirty (30) seeds each of the forty (40) samples were ground to a very fine powder using grinding mill.Approximately 0.5g of the ground corn was weighed in to a beaker. About 20 ml of cold acetone was added to the ground flour and allowed to stand for one (1) hour. The mixture was filtered and 10ml of distilled water was added to the filtrate. The filtrate was then transferred to a separating funnel and 5ml petroleum ether was added slowly to the filtrate, allowing it to flow into it by the side of the funnel. To avoid the formation of an emulsion,the lower filtrate was collected carefully. Optical density (OD) was recorded immediately at 450 nm using dual bean spectrophotometer against petroleum ether as blank. Kernel carotenoids were quantified usingLambert-Beer equation.

Spectrophotometry was used to determine optical density (OD) values of each sample at 450nm and subsequently, using the Lambert Beer equation, carotenoid content of each sample was determined as follows. The data on kernel carotenoids were analyzed in a completely randomized design (CRD) to test the statistical validity of variance among different genotypes for maize kernel carotenoids content. The total carotenoid content of maize kernel was determined spectrophotometrically using "The Beer-Lambert equation";

$E = \varepsilon \times c \times d$
Where,
E = extinction (photometer reading)
ε = molar extinction coefficient
c = concentration
d = distance = 1, to determine concentration
$c = E/\varepsilon$
Lutein, Zeaxanthin and beta-carotene are the major carotenoids in maize. Therefore, an average value for ε and for the molecular mass was used.

ε lutein = 122,688l/mol cm; M = 568 g/mol
εzeaxanthin = 133,480 l/mol cm; M = 568 g/mol
ε β-carotene = 134,000 l/mol cm; M = 537 g/mol
ε average = 130,056 l/mol cm;
M average = 557.7g/mol.

Hence, $c = OD/130056 \times 557.7 \times 1000/0.5$

Where
C = concentration of total carotenoids content (µg/g) in a given sample on dry weight basis;
OD = optical density taken at 450nm wavelength using a spectrophotometer;
W = weight of sample (0.5g).
Each sample was analyzed in three replicates and the data was subjected to statistical analyses using completely randomized design (CRD).

Table 1: Maize inbred lines used for genetic diversity study

S/N	Genotype	Pedigree	Source	Grain Colour
1	Eruwa-3-1	Unknown	IAR&T	White
2	Ijebu-Ode W-2	Unknown	IAR&T	White
3	Ikole 1-W	Unknown	IAR&T	White
4	Kishi 4-1	Unknown	IAR&T	White
5	Isara 1-W	Unknown	IAR&T	White
6	Bdg-1	Unknown	IAR&T	White
7	BodijaShgari	Unknown	IAR&T	White
8	TFE 20ABI-1	Unknown	IAR&T	Yellow
9	TZM 228Y	Unknown	IAR&T	Yellow
10	TZM-1327-A	Unknown	IAR&T	Yellow
11	Isara-1-Y	Unknown	IAR&T	Yellow
12	Ijebu-Igbo-Y	Unknown	IAR&T	Yellow
13	Eruwa Local5-1	Unknown	IAR&T	Yellow
14	TZEI 135	TZE-Y Pop STR Co S6 Inbred 17-2-3	IITA IB	Yellow
15	TZEI 157	TZE-Y Pop STR Co S6 Inbred 102-1-2	IITA IB	Yellow
16	TZEI 17	TZE Comp5-Y C6 S6 Inbred 35	IITA IB	Yellow
17	TZEI 10	TZE-Y Pop STR Co S6 Inbred 152	IITA IB	Yellow
18	TZEI 16	TZE Comp5-Y C6 S6 Inbred 31	IITA IB	Yellow
19	TZEEI 74	TZEF-Y SR BC1 x 9450 STR S6 Inb 5A	IITA IB	Yellow
20	TZEEI 72	TZEF-Y SR BC1 x 9450 STR S6 Inb 2C	IITA IB	Yellow
21	TZEEI 68	TZEE-Y SR BC1 x 9450 STR S6 Inb 11	IITA IB	Yellow
22	TZEEI 64	TZEE-Y SR BC1 x 9450 STR S6 Inb 8A	IITA IB	Yellow
23	TZEEI 82	TZEF-Y POP STR COS6 Inb44	IITA IB	Yellow
24	TZEEI 75	TZEF-Y SR BC1 x 9450 STR S6 Inb 7B	IITA IB	Yellow
25	TZEEI 73	TZEF-Y SR BC1 x 9450 STR S6 Inb 3A	IITA IB	Yellow
26	TZEEI 59	TZEE-Y SR BC1 x 9450 STR S6 Inb 3A	IITA IB	Yellow
27	TZEEI 76	TZEF-Y SR BC1 x 9450 STR S6 Inb 8B	IITA IB	Yellow
28	TZEI 65	TZE-W Pop STR Co S6 Inbred 141-1-2	IITA IB	White
29	TZEI 165	TZE Comp5-Y C6 S6 Inbred 8A	IITA IB	Yellow
30	TZEI 146	TZE-Y Pop STR Co S7 Inbred 49-3-3	IITA IB	Yellow
31	TZEI 129	TZE-Y Pop STR Co S6 Inbred 16-1-3	IITA IB	Yellow
32	BD74-43	CML12-B	CYMMIT	Yellow
33	BD74-44	CML130-B	CYMMIT	Yellow
34	BD74-48	CML161-B	CYMMIT	Yellow
35	BD74-49	CML165-B	CYMMIT	Yellow
36	BD74-68	CML451-B	CYMMIT	Yellow
27	BD74-81	CLYN242-B	CYMMIT	Yellow
38	BD74-88	CLYN243-B	CYMMIT	Yellow
39	BD74-89	CLYN244-B	CYMMIT	Yellow
40	BD74-90	CLYN226-B	CYMMIT	Yellow
41	BD74-91	CLYN246-B	CYMMIT	Yellow
42	BD74-92	DTPYC9-F114-2-4-1-1-B	CYMMIT	Yellow
43	BD74-107	CLRCY031-B	CYMMIT	Yellow
44	BD74-109	CLQRCYQ14-B	CYMMIT	Yellow
45	BD74-112	CLYN208-B	CYMMIT	Yellow
46	BD74-155	CLYN247-B	CYMMIT	White
47	BD74-156	(DTPYC9-F46-1-2-1-2-B)-B	CYMMIT	White
48	BD74-157	(DTPYC9-F38-4-3-1-1-B)-B	CYMMIT	White
49	BD74-158	(DTPYC9-F15-3-4-1-1-B)-B	CYMMIT	White
50	BD74-159	(DTPYC9-F143-5-4-1-2-B)-B	CYMMIT	White
51	BD74-160	(DTPYC9-F13-2-3-1-2-B)-B	CYMMIT	White

3. RESULTS AND DISCUSSION

3.1 Genotypic performance for grain yield and related characters

The maize inbreds differed significantly (P = .01) in the expression of the characters evaluated from one location to the other except kernel rows/ear (Table 2), indicating differences in the environmental factors at each location. Means for all the characters were higher at Ikenne compared to Ilorin with mean plant and ear heights >100cm and accompanied by a difference of 1.97 t/ha in grain yield. Majority of the inbred lines also yielded less than 1.0 t/ha at Ilorin which may be due to prolonged drought stress towards the flowering period which supports earlier reports of differential performances of maize genotypes occasioned by drought and air temperature stresses [17, 9, 14, 23, 10, 8]. The genotypes also differed significantly (P= .01) for all the characters with the magnitude of the mean squares due to genotype being larger than the genotype by locationinteraction mean squares for all the characters except for anthesis-silking-interval and number of rows/kernels. The genotype by location interaction effects also differed significantly (P = .01) for all the characters which suggests differences in the genotypes response to the prevailing environmental conditions in both locations. Consequently, the differences in weather factors at the two locations contributed largely to the observed inconsistency in the inbred lines performance especially for grain yield.

The ranges in the means among the inbred lines were very large for most of the traits studied except for the flowering traits, leaf width and ear diameter (Table 3). For example, differences between the highest and poorest yielding inbred was > 3 t/ha while, plant and ear heights were the most variable among the inbred lines with a difference of > 200cm between the tallest and shortest genotype and > 100cm between the highest and lower ear placements.

Mean performances of the maize inbredsfor characters across the two locations showed less variation for flowering traits (Table 3). The inbred lines were highly variable for the characters studied with different genotypes exhibiting superiority for each of the characters. For example, inbred line BD74-49 was the highest yielding genotype with a superiority of 1.32 t/ha over TZEEI 76 which was the poorest yielding inbred. Another inbred line (BD74-157) had the largest ear diameter while inbred BD74-49 exhibited superiority of 38.98% over TZEI 68 for ear length. The highest number of kernel rows per ear was recorded in genotype BD74-157 while inbred line BD74-49 had maximum number of kernels per row. Inbred BD74-158 wasthe tallest and also had the highest ear placementwhile inbred TZEI 17 was the shortest with the corresponding lowest ear placement. Values obtained for leaf parameters showed that inbred BD74-92 had the longest leaf while inbred BD74-48 had the broadest leaf. The inbred lines also changed rank for grain yield from one location to another with Inbreds BD74-49, and BD74-89, Kishi 4-1, BD74-68 and BD74-159 in that order being the top five yielding genotypes at Ikenne while inbreds BD7-49, BD7-81, Eruwa 3-1, BD7-68 and BD74-107 were the five top yielding hybrids at Ilorin. Therefore, the alteration in ranking of the inbred lines in their performances for grain yield and other traits observed, must have been due to response to the factors of environment in each location which justifies the use of the multi-trait selection method to identify the best genotypes for further varietal (OPVs and Hybrid) development programme.

3.2 Cluster analysis of genotypes based on morphological traits and grain yield

The dendrogram from the data generated based on the 12 attributes and revealed by UPGMA, clustered the inbred populations into two major groups with similarity coefficient of 1.40(Figure 1).The first major group comprised 21 inbred lines and all were of Nigerian origin (i.e. IITA and

IAR&T) while the second major group comprising 30 inbred lines were all from CYMMIT except Eruwa 3-1 which is of Nigerian origin. The genotypes in the first major group were further sub-divided into four sub-clusters of 10, 4, 2 and 5 genotypes respectively. The second major group wasalso further sub-divided into eight sub-clusters. Sub-clusters I and II in the second group comprisedtwo and four genotypes respectively with a similarity coefficient of 0.68 while Sub-clusters III, IV, V and VI respectively comprised five, two, two, and four genotypes. Sub-clusters VII and VIII had seven and two genotypes respectively.

3.3 Estimation oftotal carotenoid in genotypes

The quantitative estimation of the total carotenoid of the 40 inbred lines selected for the investigation revealed significant differences ($p< 0.01$) which indicated that they were genetically different for this character (Table 4), which is the basic need to take up any breeding programme for improvement of any trait. For example, the mean value for kernel carotenoids content varied from minimum of 0.03µg/g dry weight in inbred line TZEI 65 to as high as 56.52µg/g dry weight in inbred BD74-89 with population mean of 22.37µg/g. [26] in their own study also reported wide range variability (6.5 - 67.3µg/g) for carotenoid contents in maize. Several studies have earlier reported carotenoid content ranging from 0.94 µg/g to 66.0µg/g in different maize populations which also was dependent on the population being investigated, region where the study was conducted and stage of improvement of the population in maize [6, 7, 12, 11, 20, 5, 13].

Carotenoid biosynthesis occurs during seed development [16] and the accumulation of carotenoidsimpact a yellow-orange colour to the endosperm and are an easily scored phenotype. Consequently, the higher mean values for total carotenoid associated with coloured endosperm in the inbred lines is an indication of considerable high concentration of total carotenoids [15]. When coloured endosperm was taken into consideration, the range of kernel carotenoids content varied from a minimum of 7.7µg/g to a maximum of 56.52µg/g dry weight in maize inbred BD74-89. [4] hypothesized that the dominant *Yellow1* (*Y1*) allele in maize controls the first rate-limiting step in the carotenoid biosynthesis by coding for the enzyme phytoene synthase, while the homozygous recessive allelic state (*y1y1*) results in almost no synthesis of carotenoids in the endosperm. In the present study, white kernel inbred lines (for example, TZEI 65: 0.03 µg/g) having *y1* allele in the endosperm produced almost no carotenoid in the endosperm. Presence of similar negligible amount of total carotenoids (1.14µg/g) in the white maize endosperm (*y1y1y1*) was also reported by [4]. The significant range for the total carotenoids (7.71 - 56.52µg/g) in the yellow/orange inbred lines could be due to the control of carotenoid biosynthesis by other genes, besides the *Y1*.

The inbred lines with kernels of different shades of yellow comprised genotypes with relatively high and low total kernel carotenoids. Thus, the impact of total carotenoid content could not be ascertained in this study. [11, 19] also observed poor association between these two parameters in their own studies. However, inbred lines- BD74-89, TZEEI 82, Bodija-Shagari-Y, PVA SYN 13 (9), TZEEI 75, TZEI 146, TZEI 165 and Eruwa Local 5-1-Y had high kernel carotenoid content and can be advanced for breeding programmes geared towards improved carotenoid content in future maize varieties (OPVs and hybrids) of maize.

3.4 Cluster Analysis of the Genotypes based on Carotenoid Content

The dendrogram of the data generated from total carotenoid content separated the 40 maize inbred lines into two major clusters (Figure 2) with the larger cluster (Cluster I) having31 genotypes and Cluster II with nine (9) genotypes. However, the clustering of the inbred lines for carotenoid content was not based on geographical origin. Cluster I was further subdivided into five sub-clusters of 11, 6, 2, 7 and 5 genotypes respectively while cluster II was also sub-divided into two sub-clusters of 3 inbred lines each. The least genetic similarity was observed in genotypes TZEI 65, TZEEI 82 and BD74-89. Due to wider level of diversity based on carotenoid content, nine (9) genotypes were clearly separated and grouped in a distinct cluster.

4. Conclusion

Hybrid maize breeding programmes depended on selection of diverse inbred lines in order to utilize hybrid vigour as a means of improving crop productivity. Consequently, understanding the geneticdiversity among important breeding materials is generallyconsidered a critical first step to achieve success inbreeding programmes. The selection process of good performing and stable genotypes is usually complicated by the intangible forces of environment resulting in the phenomenon of genotype by environment (G x E) interaction.Consequently, Prolonged drought stress which coincided with the grain filling period at Ilorin significantly reduced grain yield in that location compared to Ikenne. The difference between the two locations was 1.97 t/ha representing >80 percent yield advantage. However, three inbred lines – BD74-49, BD74-68 and BD74-81 still showed consistency in their performance for this grain yield across the two locations, indicating their stability of performance for grain yield.

The carotenoid analysis using spectrophotometer results show that nine (9) inbred lines(BD74-89, Bodija-Shagari, Eruwa Local 5-1, TZEI 146, TZEI 165, TZEEI 82, TZEEI 75, PVA SYN 13(9) and TZEI 64) had the highest carotenoid content. Inbred line – BD74-89 was identified as one of the potential sources of carotenoids and hopefully pro-vitamin A as it exhibited the highest carotenoid content among the 40 inbred lines. The 31 inbred lines included in group 1 of the dendrogram had low concentration of carotenoid except for TZEI 165 and Eruwa 5-1-Y while 9 inbred lines included in group II had increased concentration of carotenoid.

Table 2:Performance of maize inbredsat Ikenne and Ilorin and combined analysis of variance for morphological traits

Location	Days to 50% Anthesis (days)	Days to 50% Silking (days)	Anthesis Silking Interval (days)	Plant height (cm)	Ear height (cm)	Leaf length (cm)	Leaf width (cm)	Ear diameter (cm)	Ear length (cm)	No. of rows per ear (number)	No. of Kernel per row (number)	Grain yield (t/ha)
Ikenne	56.51	59.33	2.96	196.87	105.68	60.97	6.14	4.24	17.32	12.10	18.81	2.20
Ilorin	57.10	60.45	3.41	94.70	36.68	50.22	5.86	1.44	11.05	11.17	18.81	0.23
Mean	56.81	59.99	3.20	145.78	71.08	55.58	6.0	2.84	14.18	11.63	18.81	1.21
Range	7.00	8.00	4.60	123.00	93.00	64.00	6.50	7.48	21.50	10.00	36.00	3.57
SE±	0.68	0.69	0.30	10.74	8.86	5.21	0.61	0.75	2.63	1.85	4.95	0.25
F – Test												
Replication	1.95	3.26	0.42	132.46	26.80	45.31	0.76	0.95	38.45	26.00	124.51	0.16
Location (L)	28.26**	64.97**	10.62**	789509.13**	366234.08**	8767.04**	6.29**	600.34**	3006.74**	66.82**	0.00	295.75**
Genotype (G)	5.99**	6.40**	0.39**	7479.06**	4703.57**	788.46**	9.37**	1.61**	19.71**	5.23**	75.64**	0.54**
G x L	2.88**	2.87**	0.56**	795.51**	1139.83**	99.94**	0.84**	0.51**	9.98**	5.84**	29.18**	0.29**
Pooled Error	0.46	0.48	0.09	115.28	78.57	27.16	0.32	0.56	6.94	3.42	24.50	0.06
%CV	1.20	1.15	9.60	7.36	12.46	9.37	10.23	26.44	18.58	15.90	26.31	21.21

**, Significant F-Test at 0.01 level of probability

Table 3:Agronomic, performance of maize inbreds at Ikenne and Ilorin combined

Genotype	Days to anthesis (days)	Days to silking (days)	ASI (days)	Plant height (cm)	Ear height (cm)	Leaf length (cm)	Leaf width (cm)	Ear diameter (cm)	Ear length (cm)	No. of row per ear (no)	No. kernel per row (no)	Grain yield (t/ha)
BDG-1	57	60	3	113.50	46.27	49.98	4.97	2.65	13.03	11.67	17.67	1.00
BodijaShagari	58	62	3	125.88	54.88	50.70	5.62	2.71	13.83	10.50	17.83	1.25
BD74-109	58	61	4	179.00	104.00	64.03	6.35	2.98	15.00	11.83	16.67	1.35
BD74-107	57	60	3	192.17	109.50	71.02	7.47	3.19	17.02	12.00	17.00	1.53
BD74-112	57	61	4	182.17	110.33	56.62	6.23	3.19	15.95	12.67	18.00	1.37
BD74-90	57	61	4	162.50	91.17	56.62	4.95	2.79	13.18	12.00	18.17	1.42
BD74-81	56	59	3	185.33	102.83	70.67	8.10	3.71	17.62	12.50	27.17	1.55
BD74-88	58	61	4	179.50	98.17	62.55	6.78	3.39	15.13	10.67	14.33	1.26
BD74-89	57	60	3	183.50	97.83	74.02	7.68	3.05	14.70	11.83	19.83	1.61
BD74-91	58	61	3	192.17	103.50	71.28	7.83	3.29	17.75	12.67	24.33	1.45
BD74-155	59	62	3	195.67	112.00	67.65	7.02	3.24	17.17	12.67	18.00	1.39
BD74-43	57	60	3	189.17	101.50	69.80	7.83	2.95	12.88	11.17	18.33	1.55
BD74-44	56	59	3	167.50	93.67	62.98	6.90	3.23	14.23	12.83	21.83	1.45
BD74-48	58	61	3	198.33	106.00	72.05	8.38	3.41	16.88	12.00	22.33	1.54
BD74-49	58	61	4	200.83	107.50	76.88	8.10	3.97	19.55	13.17	29.33	2.04
BD74-68	56	59	3	186.67	101.33	74.95	8.05	3.48	16.65	12.67	23.83	1.70
BD74-157	59	63	4	197.67	111.67	71.75	6.82	3.78	14.45	14.67	24.00	1.39
BD74-158	58	61	4	206.67	113.75	64.67	7.40	3.09	16.98	11.83	23.67	1.37
BD74-159	59	62	3	193.50	105.91	64.00	7.33	3.43	14.77	11.67	20.67	1.51
BD74-160	59	62	3	188.67	106.50	65.97	7.02	3.20	14.52	12.33	22.50	1.43
BD74-92	57	60	3	180.17	107.00	77.93	8.05	3.14	15.07	11.67	16.17	1.47
BD74-156	59	62	3	177.50	102.17	60.83	6.78	3.66	15.75	11.17	19.83	1.49
Eruwa-3-1	57	60	3	145.67	60.01	60.83	6.53	3.62	16.65	14.50	25.33	1.37
Eruwa Local5-1	58	61	3	104.43	43.97	51.27	5.27	2.57	13.45	11.00	18.00	1.25
Ijebu-Igbo-Y	57	60	3	142.50	68.73	55.80	5.27	2.48	13.55	11.67	15.33	1.22
Ijebu-Ode-W-2	57	60	3	141.08	71.73	49.50	6.20	3.07	12.10	11.33	16.00	1.32
Ikole-1-W	56	60	3	131.33	63.75	53.48	5.90	3.46	12.55	11.00	15.83	1.47
Isara-1-Y	58	61	3	114.85	57.08	48.93	5.52	3.07	13.15	11.33	25.33	1.40

Isara-I-W	56	60	4	110.11	48.08	45.92	6.75	3.46	13.40	11.17	15.83	1.24
Kishi-4-1	57	60	3	129.48	56.67	51.87	5.60	2.54	14.22	11.33	15.33	1.56
TFE 20ABI-1	57	60	3	139.81	56.43	58.53	5.63	2.67	13.20	11.50	18.67	0.94
TZEEI 129	55	58	3	115.17	43.25	46.12	4.48	2.56	14.40	11.00	19.00	0.79
TZEEI 146	55	59	4	113.25	47.27	51.67	4.67	2.44	12.88	10.83	16.83	1.03
TZEEI 165	56	58	3	110.08	43.83	44.43	4.43	2.56	11.93	11.00	17.00	0.93
TZEEI 59	56	59	4	116.67	45.97	41.27	4.80	1.96	13.28	11.83	18.00	0.90
TZEEI 65	56	59	3	109.17	43.17	43.47	5.72	2.18	12.25	11.33	18.00	0.87
TZEEI 76	56	59	3	116.33	46.50	43.45	5.30	2.19	13.67	11.17	16.67	0.72
TZEI 10	56	59	3	123.67	46.50	44.67	5.10	2.27	14.07	11.17	18.33	0.84
TZEI 135	56	59	3	114.83	44.50	40.33	4.73	2.77	11.77	12.50	20.33	0.98
TZEI 157	56	60	4	109.50	43.17	50.50	4.30	2.34	13.38	11.33	16.83	0.82
TZEI 16	57	60	4	119.17	44.00	46.83	5.53	2.14	12.70	11.33	16.67	0.75
TZEI 17	56	59	3	100.83	36.83	44.00	4.17	2.54	12.67	10.67	19.17	0.89
TZEI 64	56	59	3	110.00	42.83	46.83	5.03	1.96	13.52	11.00	13.33	0.99
TZEI 68	56	59	3	106.00	39.62	36.83	4.87	2.17	11.93	10.83	15.83	0.86
TZEI 172	55	58	3	108.67	44.00	42.58	5.17	2.03	13.42	10.00	17.00	0.93
TZEI 173	57	60	3	118.00	50.67	43.90	5.33	2.65	12.97	12.00	17.67	0.81
TZEI 174	56	59	4	119.50	49.33	38.67	4.05	2.29	11.83	9.83	12.50	1.00
TZEI 175	56	59	3	109.17	40.17	48.60	4.08	2.74	14.40	10.67	15.67	0.93
TZEI 182	57	60	4	115.00	47.67	46.00	4.77	2.34	12.45	11.17	14.83	0.94
TZM-1327-A	57	61	4	126.98	53.17	54.00	5.68	2.56	12.30	11.33	19.17	1.02
TZM 228-Y	56	60	4	135.76	58.91	47.67	5.63	2.39	13.17	11.67	17.33	1.19
Mean	**56.90**	**60.08**	**3.31**	**145.79**	**71.08**	**55.59**	**6.00**	**2.86**	**14.19**	**11.64**	**18.77**	**1.22**
SE+	**0.15**	**0.16**	**0.06**	**4.94**	**3.92**	**1.61**	**0.17**	**0.07**	**0.25**	**0.13**	**0.50**	**0.04**

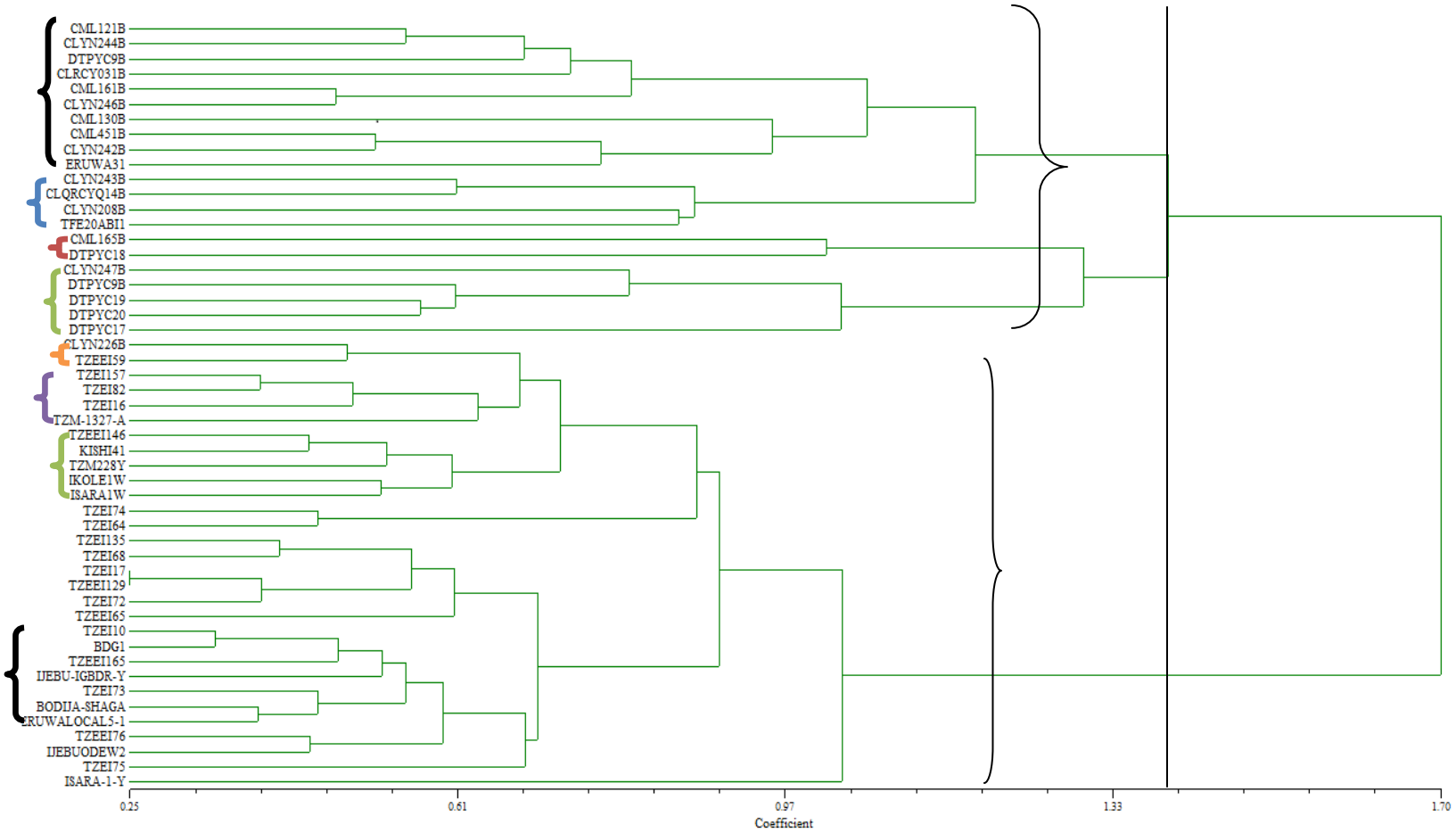

Figure 1: Dendrogram of 51 maize inbred lines revealed by UPGMA cluster analysis based on 12 different morphological and yield traits.

Table 4: Kernel Colour and Total Carotenoids in 40 maize inbred lines

S/N	Genotype	Kernel Colour	Total Carotenoids (µg/g) dry weight
1	Bodija Shagari	Orange	33.93
2	BD74-107	Yellow	21.97
3	BD74-90	Yellow	24.46
4	BD74-81	Orange	10.07
5	BD74-88	Orange	26.42
6	BD74-89	Light orange	56.52
7	BD74-91	Orange	25.81
8	BD74-43	Orange	14.43
9	BD74-44	Light orange	16.97
10	BD74-48	Orange	14.56
11	BD74-49	Orange	19.31
12	BD74-68	Orange	7.71
13	BD74-92	Yellow	13.91
14	Eruwa Local 5-1	Orange	31.38
15	Ijebu-Igbo-Y	Yellow	19.04
16	Ijebu-Ode-Y	Yellow	16.32
17	Isara-1-Y	Light orange	25.77
18	TZEI 129	Yellow	16.37
19	TZEI 146	Yellow	33.46
20	TZEI 165	Yellow	31.23
21	TZEEI 59	Yellow	19.58
22	TZEI 65	White	0.03
23	TZEEI 76	Light Orange	12.84
24	TZEI 135	Light orange	15.20
25	TZEI 157	Light orange	27.70
26	TZEI 16	Light orange	19.52
27	TZEI 17	Light orange	15.95
28	TZEEI 68	Yellow	26.42
29	TZEEI 72	Yellow	22.90
30	TZEEI 73	Orange	29.39
31	TZEEI 74	Orange	9.78
32	TZEEI 75	Yellow	30.16
33	TZEEI 82	Yellow	43.97
34	TZEI 64	Light orange	29.57
35	TZEI 181	Yellow	20.58
36	TZEI 128	Light orange	16.98
37	TZEI 67	Light orange	25.86
38	TZM 228-Y	Yellow	16.53
39	PVA SYN 13 (9)	Light orange	34.64
40	PVA SYN 16 (9)	Light orange	18.07
Mean			22.37
SE ±			1.64
%CV			7.35

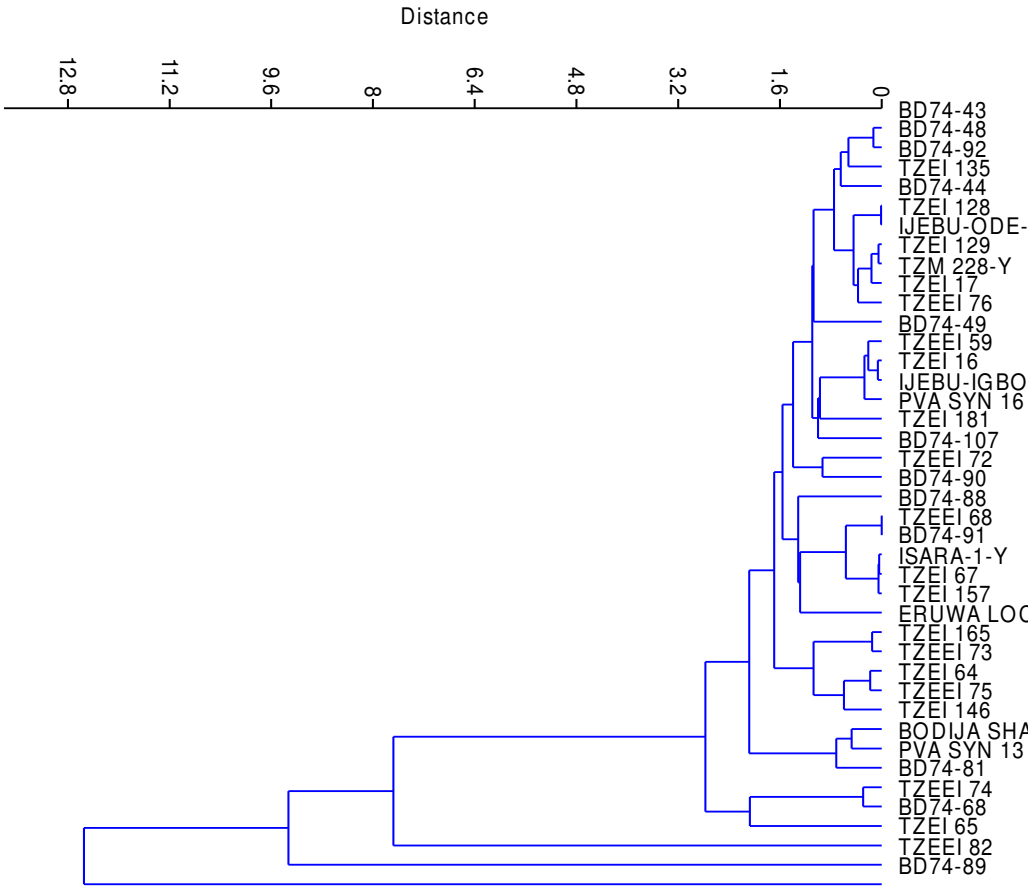

Figure 2: Dendrogram of total carotenoids of 40 tropical and temperate yellow maize inbred lines.

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