



## **Agromorphological Diversity among Popcorn (*Zea mays. everta*) Landraces Grown in Zambia**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author NB designed the study, performed the statistical analysis, wrote the protocol, literature review, and wrote the first draft of the manuscript. Authors LT and KK reviewed the manuscript. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Thirty nine (39) popcorn landraces alongside three (3) check varieties were evaluated for variability and relationships based on 15 agromorphological traits in Kabwe, Zambia during 2019/20 and 2020/21 seasons. A randomized complete block design was used with three replications in both instances. Analysis of variance revealed highly significant ( $p < 0.001$ ) differences among the popcorn landrace populations in some traits such as days to anthesis, days to silking, anthesis-silking interval, 100 seed weight, ear and plant heights. Principal component analysis also delineated these traits as the most important in contributing to the variability among the landraces alongside tassel length. The first two principal components accounted for 71.1% of total variability with PC-1 accounting for 41.7 and PC-2 with 28.4%. Genetic diversity based on discriminant analysis revealed low mean differentiation ( $D^2 = 0.12$ ) among the landrace populations. The check population 'Lion popcorn' had the largest mean genetic distance among the studied populations ( $D^2 = 0.42$ ) while ZMP 1932 was the most differentiated among the landraces ( $D^2 = 0.38$ ). Cluster analysis resulted in seven clusters with the clustering mostly based on the relative strength of the popcorn landraces in particular traits such as long A-S interval (cluster I) and high seed weight (cluster VII). Overall, two

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popcorn landraces were identified for their relatively high genetic diversity index (ZMP 1932 and ZMP 1902). These alongside the check variety 'Lion popcorn' can be used to cross with the local landraces as a way of increasing genetic diversity.

*Keywords: Popcorn landraces; agromorphological diversity; cluster analysis; principal component analysis; discriminant analysis.*

## 1. INTRODUCTION

Popcorn (*Zea mays* var. *everta* Sturt.), a specialty type of corn, is thought to be one of the oldest types of corn [1]. Also referred to as the original cereal snack food, popcorn is the finest form of flint type of maize available. The kernels of popcorn consist of hard starch grains embedded in colloidal material, which pops on heating and produces large puffed flakes [2]. This character separates popcorn from all other types of corn. Popcorn is a good low-calorie food and there are many nutritional merits in popcorn such as the presence of protein, vitamin B complex, calcium, and iron [3].

Despite the wide production and consumption of popcorn, its genetic improvement for productivity traits such as yield has lagged behind that of maize [4]. Genetic diversity research forms an essential criterion for the selection of potentially promising parents that can be utilized to generate populations that are adapted to targeted environments and from which superior progenies can be extracted during segregation [5,6]. Characterization of popcorn populations targeted for improvement can be based on agromorphological traits and/ or molecular markers [7]. Among the phenotypic, biochemical, and molecular methods [8,9] employed in the assessment of genetic diversity, the phenotypic method is a relatively inexpensive approach. A number of research efforts have utilized agromorphological traits as a singular approach or in combination with molecular markers to characterize and study the diversity of maize populations [10,11,12].

One of the main constraints to increase popcorn production in Zambia is that the yield is low. This constraint can be reduced by the development of improved varieties with high yield potential adapted to the needs of producers. The introduction of high-yielding varieties depends mostly on access to local genetic resources such as landraces. In order for local genetic resources to be of any practical use in breeding programs, their characteristics must be studied and

similarities ascertained among several genetic stocks.

This research effort was carried out in order to assess the agro-morphological diversity of forty (40) popcorn landrace populations collected from several farming locations in Zambia.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Site

The study was conducted at the Mulungushi University research farm situated at 14.4484° S, 28.4456° E in the Kabwe district of the central province of Zambia.

### 2.2 Plant Material

Thirty nine (39) popcorn landraces collected from different geographical locations in Zambia (Table 1) were used for this study.

### 2.3 Experimental Design

The experiment was laid out in a Randomized Complete Block Design with three replications.

### 2.4 Management Practices

Popcorn landraces were planted in 5m rows with 75 x 20cm spacing. Planting was preceded by land preparation and this was done by hand planting method where 3 seeds per hole was planted. A basal dressing fertilizer application at 200 kg/ha was applied at planting and this was followed by a top dressing fertilizer application of Urea at 200 Kg/ha. Weeds were controlled manually and harvesting was done by hand.

### 2.5 Data Collection

The following data was collected for each of the experiments; plant height, ear height, number of primary tassel branches, cob length, number of kernel rows, kernel color, days to anthesis, days to silking, tassel length, number of ears, 100 seed weight, anthesis-silking interval, stay green, vigour score, tassel length: tassel branches and prolificacy.

## 2.6 Data Analysis

Frequencies of plants per plot of the single qualitative scoring category was calculated. For quantitative traits. Means, standard deviations, minimum and maximum values as well as coefficient of variation (CV) was calculated. Analysis of variance (ANOVA) based on the randomized complete block model with the assumption of independent and heterogeneous error variances of environments was performed to test differences between means. Cluster, discriminant and principal component analyses were carried out using Genstat Discovery Edition.

## 3. RESULTS

### 3.1 Variability in Agromorphological Traits

Ample variability was established for kernel colour among the popcorn landraces with 51.3% of the landraces having yellow kernels, 23.1% white, 10.3% black, 7.7% mixed, 5.1% brown and 2.6% red coloured kernels. All (100%) the popcorn landraces studied had regular kernel row arrangement on the cob.

There was highly significant variability among the landraces for traits such as 100 kernel weight, anthesis-silking interval, days to silking, days to anthesis, cob length, ear length, number of kernel rows, plant height and ears per plant (Table 2). Days to anthesis and silking occurred on average  $64.7 \pm 3.9$  days and  $68.8 \pm 1.7$  days respectively giving a protandry of 4.1 days. None of the landraces had fewer days to anthesis and silking than the mean of checks. The average anthesis-silking interval was  $4.0 \pm 1.9$  days. Average plant height was  $2.3 \pm 0.3$ m while mean ear height was  $1.4 \pm 0.2$ m. The highest and lowest plant heights were recorded for ZMP 1948 ( $2.78 \pm 0.23$ m) and ZMP 1944 ( $2.0 \pm 0.15$ m) respectively whereas for ear height it was ZMP 1942 ( $1.7 \pm 0.02$ ). For anthesis-silking intervals, the overall mean was  $4.2 \pm 1.9$  days among landraces and  $2.6 \pm 1.9$  among check varieties. The highest anthesis-silking interval was recorded for ZMP 1924 ( $7.3 \pm 1.3$  days) while the shortest interval was recorded on ZMP 1904 ( $2.0 \pm 0.8$  days). The average cob length was  $14.2 \pm 3.7$ cm with ZMP 1914 having the longest cobs ( $16.6 \pm 3.7$ cm) while ZMP 1958 had the shortest cobs ( $12.3 \pm 3.7$ ). The overall mean for the number of kernel rows was  $13.6 \pm 1.1$  with landrace ZMP 1904 having the highest number of kernel rows ( $15.8 \pm 1.1$ ) while ZMP 1906 had the least number with  $12.1 \pm 1.1$  kernel rows. The

overall mean number of ears per plant was  $5.6 \pm 1.7$  (Table 2) with ZMP 1926 having the highest number of ears per plant (1.0) while ZMP 1922, ZMP 1930, ZMP 1936 and ZMP 1968 had the least number of ears per plant (0.5) (Table 2).

### 3.2 Genetic Distances and Cluster Analysis

Genetic distance based on pairwise intergroup distances (Mahalanobis  $D^2$ ) generally revealed low levels of differentiation among the popcorn landraces studied (Table 3). Genetic distance was calculated as  $GD = D^2/100$ . Genetic distances ranged from 0.01 to 0.9 with an average of 0.12. Forty-one percent of the landraces had average genetic distances of less than 0.1, an indication of low genetic diversity among the landrace populations. The closest pairs were ZMP 1908/ZMP 1960 (0.01), ZMP 1908/ZMP 1968 (0.01), ZMP 1916/ZMP 1970 (0.01), ZMP 1924/ZMP 1944 (0.01), ZMP 1914/ZMP 1942 (0.01) and ZMP 1924/ZMP 1966 (0.02) (Table 2). The greatest dissimilarities were between ZMP 1934/Lion popcorn (0.9), ZMP 1914/Lion popcorn (0.68), and ZMP 1902/Lion popcorn (0.63). Based on average genetic distances, the most distant popcorn landraces were ZMP 1932 (0.38), ZMP 1904 (0.15), ZMP 1920 (0.19) and ZMP 1940 (0.17). Lion popcorn was the most genetically distant check variety with an average genetic distance of 0.42 while EIHYB 79 was the least distant with an average genetic distance of 0.16 (Table 3).

Hierarchical Cluster analysis was carried out among the popcorn landraces based on data collected on 15 variables as specified above. The analysis was based on the Unweighted Pair Group with Arithmetic Mean (UPGMA) using Genstat Discovery Edition. This resulted in the grouping of the popcorn landraces in 7 distinct clusters as follows. Cluster 1 (long anthesis-silking interval, low prolificacy, high vigor index, early onset of anthesis) Cluster II (late onset of anthesis, and late-onset of silking). Cluster III (short anthesis-silking interval, low cob placement, low plant height, low number of tassel branches, short cob length, high number of kernel rows). Cluster IV (long tassel length, sparsely packed tassel branches, high stay-green index). Cluster V (low 100 seed weight) Cluster VI (low stay green score, high number of tassel branches, short tassel length, compact tassels, and high prolificacy). Cluster VII (Short anthesis-silking interval and cob length and high seed weight) (Fig. 1).

**Table 1. Popcorn populations used in the study**

S/N	Name	Source	S/N	Name	Source	S/N	Name	Source
1	ZMP 1902	Kabwe	15	ZMP 1926	Kapiri	29	ZMP 1952	Kabwe
2	ZMP 1904	Chisamba	16	ZMP 1928	Kapiri	30	ZMP 1954	Mansa
3	ZMP 1906	Chisamba	17	Lion Popcorn (check)	Shoprite	31	ZMP 1956	Mansa
4	ZMP 1908	Chisamba	18	ZMP 1930	Kapiri	32	ZMP 1958	Mansa
5	EIHYB 79 (Check)	ZARI	19	ZMP 1932	Kapiri	33	ZMP 1960	Mansa
6	ZM 7323	ZARI	20	ZMP 1934	Kabwe	34	ZMP 1962	Mansa
7	ZMP 1910	Chisamba	21	ZMP 1936	Kabwe	35	ZMP 1964	Mansa
8	ZMP 1912	Chisamba	22	ZMP 1938	Kabwe	36	Premium Instant (check)	Shoprite
9	ZMP 1914	Kabwe	23	ZMP 1940	Kabwe	37	ZMP 1966	Mansa
10	ZMP 1916	Kabwe	24	ZMP 1942	Kabwe	38	ZMP 1968	Chipata
11	ZMP 1918	Kabwe	25	ZMP 1944	Kabwe	39	ZMP 1970	Mumbwa
12	ZMP 1920	Kabwe	26	ZMP 1946	Kabwe	40	ZMP 1972	Serenje I
13	ZMP 1922	Chisamba	27	ZMP 1948	Kabwe	41	ZMP 1974	Serenje II
14	ZMP 1924	Chisamba	28	ZMP 1950	Kabwe	42	ZMP 1976	Lunte

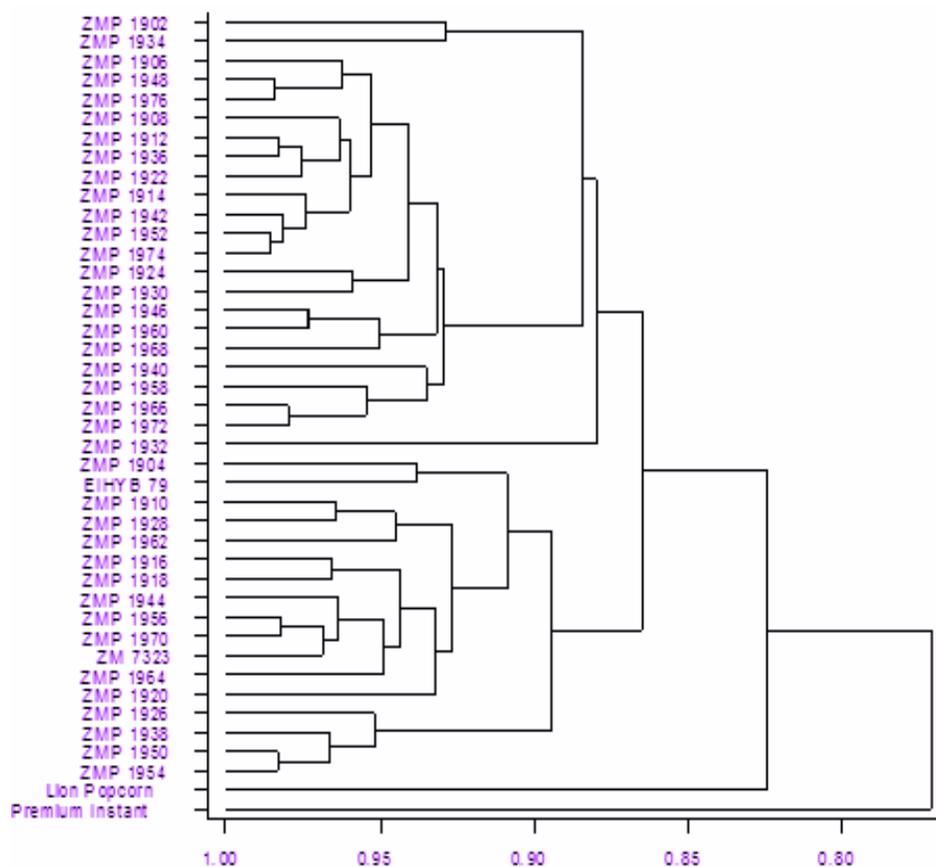
**Table 2. Variability in agromorphological traits among popcorn landrace populations**

No	Trait	Overall Mean±SD	Min	Max	CV (%)	Mean Square
1	100 seed weight	17.5±3.4	11.3	28.8	4.2	22.29***
2	ASI (days)	4.0±1.9	1	10	39.6	4.46***
3	Anthesis (Days)	64.7±3.8	54	71	3.2	26.3***
4	Silking (Days)	68.8±4.5	58	76	3.6	35.13***
5	Cob length (cm)	14.2± 1.5	10.7	18.5	9.5	2.78ns
6	Cob number	14.8±3.7	8	25	22.2	15.64ns
7	Ear height (m)	1.4±0.2	0.7	1.9	7.5	0.088***
8	Number of Kernel Rows	13.6± 1.1	12	16	7	1.63*
9	Plant height (m)	2.3± 0.3	1.5	2.8	3.7	0.118***
10	Ears per plant	0.7±0.1	0.32	1.1	20.1	0.033*
11	Stay Green	5.6± 1.7	1	8	26.7	3.5ns
12	TH/TB	2.3± 0.5	1	4	20.7	0.34ns
13	Number of tassel branches	21.4± 5.1	10.2	45	23.6	25.8ns
14	Tassel length	45.9± 3.8	34.6	54.2	7.5	16.37ns
15	Vigour score	2.7±0.7	1	4	20.8	0.69*
16	Popping Expansion	318.3±146.4	160	950	50.0	2253***

\*significant at  $p=0.05$ , \*\*\* significant at  $p=0.001$

**Table 3. Genetic distances for individual popcorn landrace populations**

Popcorn Landrace	Max	Mean	Popcorn Landrace	Max	Mean
Lion Popcorn	0.9	0.42	ZMP 1944	0.42	0.1
ZMP 1932	0.66	0.38	ZMP 1948	0.54	0.1
ZMP 1902	0.63	0.21	ZMP 1962	0.34	0.1
Premium Instant	0.58	0.2	ZMP 1964	0.54	0.1
ZMP 1920	0.81	0.19	ZMP 1974	0.57	0.1
ZMP 1940	0.44	0.17	ZMP 1908	0.46	0.09
EIHYB 79	0.43	0.16	ZMP 1922	0.54	0.09
ZMP 1904	0.63	0.15	ZMP 1928	0.37	0.09
ZMP 1910	0.67	0.14	ZMP 1966	0.49	0.09
ZMP 1914	0.68	0.14	ZMP 1972	0.45	0.09
ZMP 1934	0.54	0.14	ZMP 1946	0.42	0.08
ZMP 1918	0.65	0.13	ZMP 1950	0.4	0.08
ZMP 1930	0.61	0.13	ZMP 1954	0.36	0.08
ZMP 1924	0.63	0.12	ZMP 1958	0.33	0.08
ZMP 1942	0.61	0.12	ZMP 1960	0.4	0.08
ZMP 1906	0.46	0.11	ZMP 1968	0.41	0.08
ZMP 1916	0.58	0.11	ZMP 1976	0.48	0.08
ZMP 1936	0.51	0.11	ZMP 1912	0.42	0.07
ZMP 1970	0.57	0.11	ZMP 1938	0.44	0.07
ZM 7323	0.51	0.11	ZMP 1952	0.43	0.07
ZMP 1926	0.41	0.1	ZMP 1956	0.34	0.07



**Fig. 1. Dendrogram showing the clustering of popcorn landraces grown in Zambia**

### 3.3 Principal Component Analysis

Principal component analysis revealed that only three principal components had eigenvectors greater than 1 and only two of these were significant. The first two principal components counted for 53.36% of the variation. The first principal component accounted for 39.8% of the variation and delineated the important traits as anthesis-silking interval, days to anthesis, days to silking, ear height, plant height, and number of tassel branches. The second principal component accounted for 22.5% of the variation and identified important traits as number of cobs, cob length, stay green, ratio of tassel length: tassel branches and vigour score. (Fig. 2).

### 4. DISCUSSION

This study examined the variability and relationships among selected popcorn landraces grown in Zambia based on their agromorphological characters. It was important to study the variability among the popcorn landraces so that the information generated can be used to facilitate the sustainable exploitation of this genetic resource in breeding programs. Based on coefficients of variability for the respective traits, it was established that the anthesis-silking interval contributed the highest amount of variability observed among the popcorn landraces (Table 4). Other traits that had high variability include 100 seed weight, days to anthesis, days to silking, ear height and

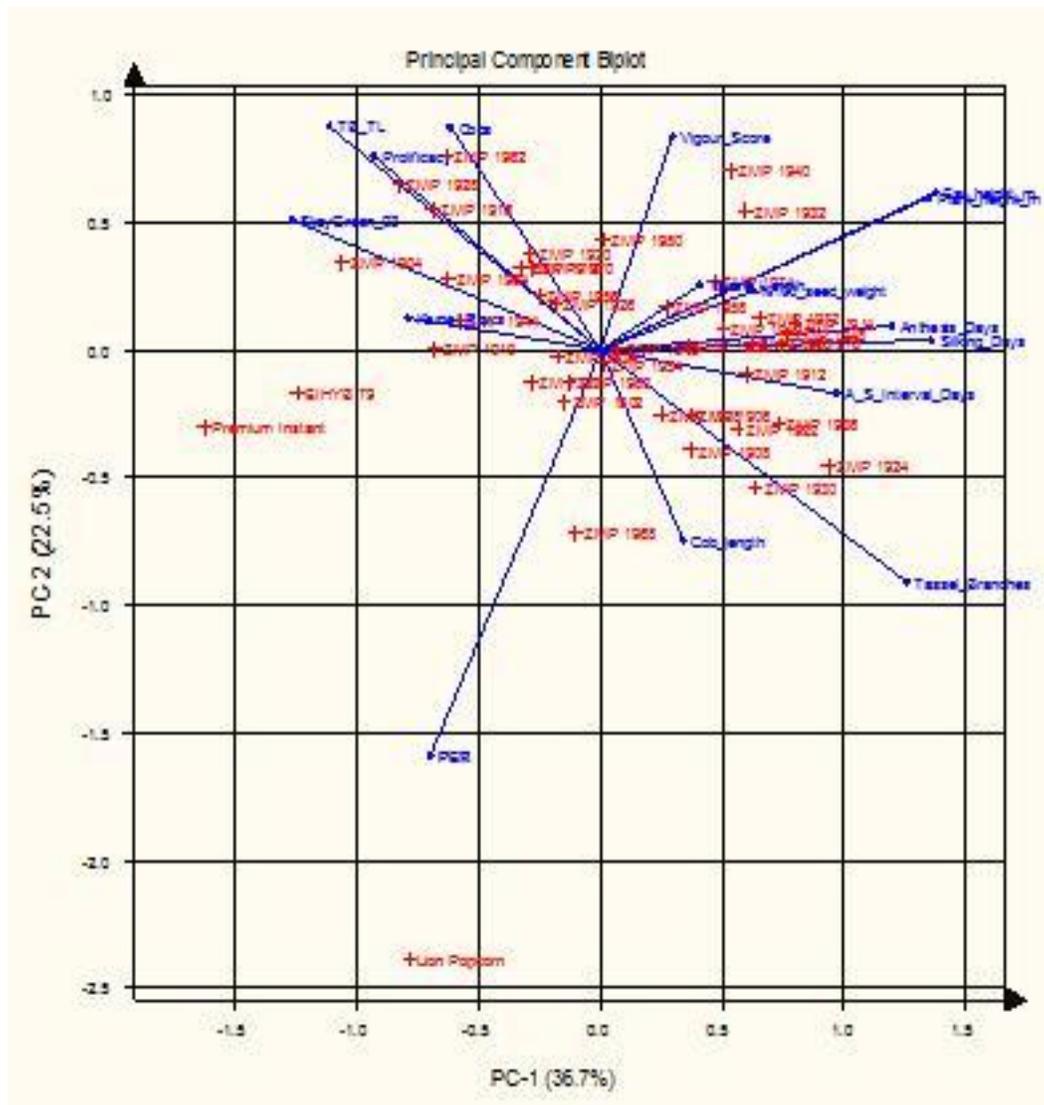
plant height. Twumasi et al. [13] and Tardieu et al. [14] also found high variability for anthesis-silking interval, 100 seed weight, days to silking, ear height, days to anthesis and plant height among tropical maize landraces. Anthesis-silking interval is used as indirect selection criteria for drought tolerance in maize and popcorn improvement with 2-4 days being considered the most acceptable interval [15,16]. In this regard, ZMP 1916, ZMP 1922, ZMP 1984, ZMP 1960 and ZM 7323 could be considered as good potential sources of drought tolerance due to their relatively shorter mean anthesis-silking interval.

Landraces are normally the result of considerable evolutionary, anthropogenic and genetic events hence they are expected to harbour considerable genetic divergence. In this study, we established relatively low mean genetic distance (0.12) among the popcorn landraces collected from farming communities in Zambia. The mean genetic distance observed in our study was much lower than the mean genetic distance (>0.7) established by Twumasi et al. [13] among maize land races of tropical origin (Table 3). This can be attributed to having very few popcorn landraces which have been shared widely among the farmers as they exchange seeds.

Cluster analysis and principal component analyses were mostly unable to distinguish popcorn landrace populations on the basis of geographic origin with landraces collected from

**Table 4. Latent vectors (loadings)**

Trait	1	2	3	4
A_S_Interval_Days	0.25	-0.04	-0.05	0.13
Anthesis_Days	0.31	0.11	0.09	0.09
Cob_length	0.10	-0.13	-0.35	-0.54
Cobs	-0.17	0.22	0.48	-0.41
Ear_height_m	0.34	0.28	0.09	-0.01
Kernel_Rows	-0.21	-0.03	0.00	0.13
PEV	-0.14	-0.46	-0.18	-0.28
Plant_height_m	0.33	0.31	-0.03	0.00
Prolificacy	-0.25	0.17	0.42	-0.26
Silking_Days	0.35	0.09	0.06	0.14
StayGreen_93	-0.33	0.23	-0.29	0.12
TB_TL	-0.30	0.36	-0.25	0.15
Tassel_Branches	0.34	-0.30	0.04	-0.23
Tassel_Length	0.10	0.29	-0.50	-0.23
Vigour_Score	0.05	0.37	-0.06	-0.43
Eigenvalues	5.96	2.04	1.96	1.14
Individual Percentage	39.76	13.60	13.00	7.60
Cumulative Percentage	39.76	53.36	66.36	73.96



**Fig. 2. Principal component biplot for popcorn landraces grown in Zambia**

the same location being dispersed in different clusters. Hartings et al. [17] and Sharmah et al. [18] had similar findings which established a lack of relationship between geographic origin and clustering. Other studies such as Hossain et al [19] found a very close relationship between maize landrace origin and clustering. Moreover, a few land races such as ZMP 1942/ ZMP 1952 (Kabwe), ZMP 1916/ZMP 1918 (Kafulamase) from similar geographical locations were grouped in the same cluster (Fig. 1). Among the three check varieties used in this study, only one (EIHYB 79) clustered with the landraces while two check varieties (Lion and premium Instant popcorn) could not be clustered in any category (Fig. 1). This entails that these two genotypes

were highly differentiated from the rest of the populations under consideration and could thus be very good potential parents in crossing programmes aimed at increasing genetic diversity of the popcorn populations. Furthermore, we did not establish a general relationship between kernel colour and clustering with several landraces of the same colour being grouped in the diverse clusters. However, ZMP 1910 (Chisamba)/ZMP 1928 (Kapiri) and ZMP 1956 (Mansa)/ZMP 1970 (Mumbwa) were landraces of the same kernel color collected from different locations and closely linked in cluster analysis. This could be due to the movement of seeds across geographical locations as farmers exchange and share seeds as evidenced by the

fact that the relative genetic distances between the respective pairs of landraces were generally low. Okumus p [20] also found a close relationship between kernel colour and relative genetic distances among orange and yellow coloured maize landraces.

Principal component analysis identified anthesis-silking interval, days to anthesis, days to silking, ear height, plant height and number of tassel branches as the most critical traits determining variability among the popcorn landraces as these accounted for almost 40% of the total variation. Belaria et al.; [21], also established that traits related to maturity duration and plant height were major contributors to variability among maize populations.

## 5. CONCLUSION

We conclude that popcorn landraces collected from different locations in Zambia had considerable phenotypic variability. However, the mean genetic divergence among the landraces was low. Therefore, any breeding efforts on these populations would also require some investment in increased genetic diversity through hybridization with more divergent genotypes. A number of potential candidates for parent selection as well as population improvement were identified such as those with short anthesis-silking intervals, early maturing and high biomass yield. The findings established by this research effort will also be very useful in popcorn germplasm management and utilization.

## COMPETING INTEREST

Authors have declared that no competing interests exist.

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