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GENETIC DIVERSITY OF MAIZE (*ZEA MAYS* L.) GROWN IN LESOTHO USING MORPHOLOGICAL MAKERS

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ABSTRACT: *A large collection of maize germplasm is introduced annually to Lesotho from CYMMIT in Zimbabwe for evaluation of adaptability and yield performance. This collection is not characterized for degree of similarities and dissimilarities using morphological and other markers. The study was conducted with the objectives of (a) estimating genetic distance among maize cultivars using cluster analysis and (b) identifying morphological characters with high discriminatory power to segregate maize cultivars. The study was conducted at National University of Lesotho, Experimental farm. Randomized Complete Block Design was applied with ten treatments and three replications. Data collected using Descriptor compiled by International Board of Plant Genetic Resource Unit included number of leaves per plant, tassel colour, number of cobs, silk colour, stem colour, plant height, number of ears, ear length, cob diameter, number of kernels, kernel arrangement, kernel colour, shape of upper surface, kernel type, leaf length and tassel length. Data were subjected to GENSTAT software package to generate cluster analysis and perform principal component analysis. The results of cluster analysis revealed two big groups, of which one consisted of six cultivars and another consisted of four cultivars. Besides, there was one outlier. Two big groups were further divided into sub-groups, Three principal component analyses were used to analyze the results, which constituted 65.37% of the total variation. The first one showed variation of 26.93%, the second one showed 20.65% while the third one had 17.79%. The first principal component was constituted by ear length, tillering, maize height, total number of kernels, cross-section of cob and stem colour. The characters comprising second principal component were kernel type, kernel row arrangement, silk colour, number of ear and number of kernel row. Lastly, the character influencing separations along third principal component were number of kernel row, silk colour and number of leaves. The study was able to distinguish the cultivars.*

KEYWORD: Maize, cluster analysis, principal component analysis, Lesotho

INTRODUCTION

Maize (*Zea mays* L.) is the only cereal crop of Central American origin produced in tropical and sub-tropical regions in the world (Mushtaq *et al*. 2016; Ristic *et al*, 2013). It has a wide adaptation under varied agronomic and environmental conditions (Nikkhoy and Shiri, 2017). World-wide, maize is considered as the queen of cereal crops due to its highest yield potential among cereals. It is produced on an area of approximately 150 million hactres in nearly 160 countries consisting of a wide range of edaphic types, climatic conditions and agronomic practices (Nikkhoy and Shiri, 2017; Shazia *et al*, 2017). It constitutes 36% (782 metric ton) of the world grain production. The five largest producers of maize in the world are United State of America, Brazil, China, Mexico and India, respectively (Food and Agriculture Organization, 2016).

In Lesotho, maize is the main staple cereal crop ranking first, followed by sorghum and wheat in terms of production and area in which it is cultivated (Bureau of Statistics, 2016). It is cultivated by all farming house-holds for mainly home-consumption. Surplus is sold to generate income (Morojele and Sekoli, 2016). The ecological zones of Lesotho where maize is grown in large amount are Lowlands and Foothills. However, the other small areas where it is cultivated are mountain region and Orange river valley (Moeletsi, 2010). Even though maize is a dominant cereal crop, its productivity is still very low compared to neighbouring countries because of low yielding cultivars and composite varieties ((Bureau of Statistics, 2016). In order to improve maize productivity, Ministry of Agriculture and Food Security has forged collaboration with CYMMIT in Zimbabwe to provide maize seeds that are high yielding and can withstand conditions in Lesotho. These seeds are evaluated for adaptability and yield performance. The seeds obtained from CYMMIT are mostly hybrid crossed to exploit heterosis and available genetic variability. Nonetheless, some single strains (open pollinated varieties) are also introduced. This has resulted in many cultivars being produced and exported to Lesotho for evaluation of adaptability. The effort has broadened germplasm base from which the farmers can make choice to increase productivity. All the germplasm collection from CYMMIT has not been characterized to distinguish this wealth of maize cultivars. Morphological markers have been used successfully in the past to distinguish cultivars of many crop and forage species, and it still stands the taste of time (Rohman *et al*, 2015; Ristic *et al*. 2013). Multivariate analysis has been a powerful to use in genetic divergence of maize cultivars and other crops (Azad *et al*. 2012). It is the objective of this study therefore to (a) estimating genetic distance among maize cultivars using cluster analysis and (b) identifying morphological characters with high discriminatory power to segregate maize cultivars.

MATERIALS AND METHODS

Site description

The study was conducted at Roma Valley in the Experimental farm of the National University of Lesotho, which is situated 34 km South East of Maseru, Capital town of Lesotho. The coordinates are as follows; 29˚ 26` 48 S latitude and 27˚ 42` 29 `E longitude with the altitude of 1610 m. Roma valley is broad and fertile, and is surrounded by sand stone cliffs (Schmitz and Rooyani, 1987).
The soil type of study area consists of Berea soil and a bit of Tsiki soil. Berea soil is made from Aelion origin and seldom used as base. It has a hue as yellow as or yellower than 7.5yrs. The profile is rarely deeper than one meter and horizon development is poor, and Berea soil consists of moderate deep and moderately well drained soil (Schmitz and Rooyani, 1987). Tsiki soil is poorly drained and moderately deep, duplex soil. The ochric epipedon consists of a dark greyish brown sandy loam albic horizon. The epipedon rests on a greyish brown, gravelly sandy clay loam argillic horizon. The faces of peds in this horizon are stained with organic matter. The solum is underlain by variegated light olive brown, and fine sandy loam pedisediment. Tsiki soil occupies nearly level to gentle sloping valleys. It has formed at the base of sand stone escarpment. Sandy residuum from sand stone escarpment is, probably, the parent material for the soil (Schmitz and Rooyani, 1987).

Experimental design
Randomized Complete Block Design was used with 10 treatments and 3 replications. Treatments were maize cultivars obtained from CIMMYT in Zimbabwe through the Department of Agriculture research in Maseru. The size of main experimental plot was 50 m x 24.8 m equivalent to 1240 m^2 . The main plot was divided into 3 blocks where each block had 10 plots. Each plot had 4 rows with length of 4 m and the space between the rows was 1 m while the space between the plots was 1 m. The space between the plants was 0.3 m.

Land preparation and crop management
The land was first prepared using mould-board plough mounted to the tractor, after which disc harrow was used to level the seedbed and break the clods. Sowing was done by hands. The field was irrigated twice in a week due to drought that prevailed. Weeding was done during the growing period of the maize when observed.

Data collection
Data were collected using descriptor developed by International Plant Genetic Resource Unit (1981). The following were recorded; number of leaves per plant, tassel colour, number of cobs, silk colour, stem colour, plant height, number of ears, ear length, cob diameter, number of kernels/row, total number of kernels, kernel arrangement, kernel colour, shape of upper surface, kernel types, leaf length and tassel length

Data analysis
Data generated were subjected to GENSTAT version 16 software package to perform both Cluster analysis and principal component analysis. Cluster analysis was conducted using square Euclidean distance and complete linkage method. Principal Component Analysis was carried out based on the phenotypic correlation matrix of the adjusted means of the populations for all 17 descriptors.

RESULTS AND DISCUSSION

Cluster analysis
Dendrogram generated from cluster analysis is depicted in figure 1 below. Ten maize cultivars employed in cluster analysis were VPO 5120, QN633, CAP444NG, CAP122-60, Phb33H56, zm 521, zm 523, zm 423, CAP 9001 and QS 627. Cluster analysis revealed two big groups (A and B), of which group A consists of five cultivars (A), namely; VPO 5120, QN633, CAP444NG, CAP122-60 and Phb33H56. Group B comprised five cultivars; zm 521, zm 523, zm 423, CAP 9001 and QS 627. Besides, there was one outlier (C). Two big groups (A and B) were further divided into sub-groups, Ai and Aii. Sub-group Ai was constituted by VPO 5120, QN633 and CAP444NG while Sub-group Aii comprised of CAP122-60 and Phb33H56. Group B was also divided into two sub-group, each having two cultivars. Sub-group Bi contained zm 521 and zm 523 while sub-group Bii consisted of CAP 9001 and QS 627.

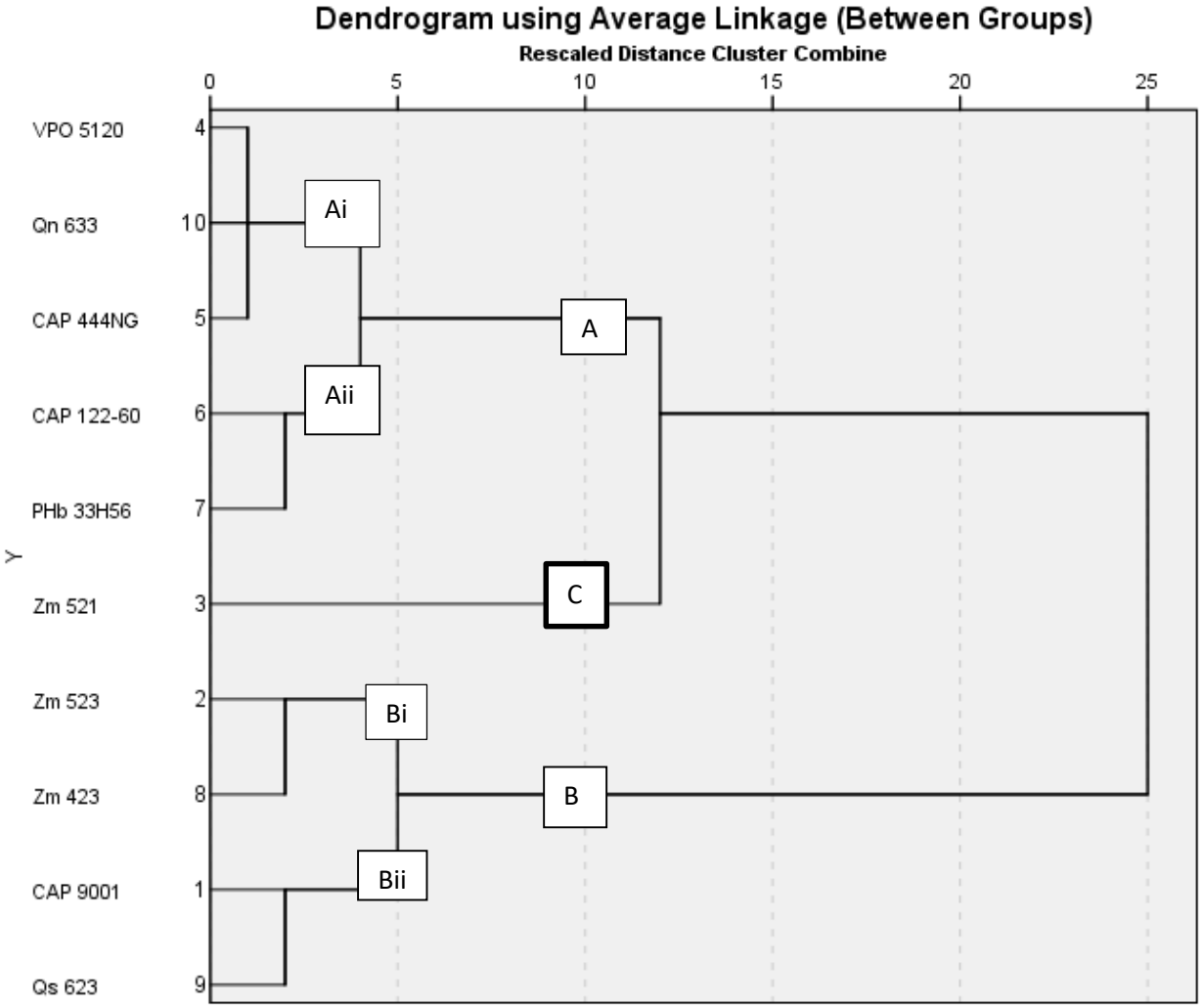

Figure1. Cluster analysis of ten maize cultivars.

Principal component analysis

Principal component analysis was used to identify characters which cause variation among maize cultivars. Three principal components were used to interpret the results, which constituted a total variation of 65.37%. The first principal component constituted a variation of 26.93%. The second one comprised 20.65% while the third principal component consisted 17.79 of the total variation. There were 18 characters used to distinguish maize cultivars. The first principal component (Table 1 and 2) were constituted by ear length (0.900), tillering (0.830), maize height (0.828), total number of kernels (0.793), cross section of cob (0.759) and stem colour (0.563). The character influencing separation along second principal component (Table 1 and Table 2) were kernel type (0.845), kernel row arrangement (0.669), silk colour (0.637), number of ear (0.595) and number of kernel row (0.591). Lastly, the character influencing separations along third principal component were number of kernel row (0.638), silk colour (0.634) and number of leaves (0.510).

Table 1. Principal components

Principal Component	Eigenvalues	
	% variance	Cumulative %
1	26.931	26.931
2	20.645	47.575
3	17.790	65.365
4	11.285	76.651
5	8.154	84.805
6	6.188	90.992
7	5.004	95.996
8	2.296	98.292
9	1.708	100.000
10	2.819E-15	100.00

Table 2; Loadings for Principal component 1, 2 and 3.

	Principal Component		
List of characters	1	2	3
Number of leaves per plant	-.039	.374	-.510•
Tassel colour	-.397	-.112	.440
Silk colour	-.129	.637•	.634•
Number of ear per plant	-.056	.595•	.375
Stem colour	-.563•	.176	.370
Height per plant	-.828•	-.075	.355
Ear length	.900•	.042	-.211
Cod diameter	.726	.328	.416
Number of kernel row	.219	.591•	.638•
Total number of kernel per plant	.793•	-.466	.355
Kernel row arrangement	.109	.669•	.410
Kernel colour	.230	.681•	-.373
Cross section of the cob	.759•	-.302	.453
Shape of the upper surface of kernel	.351	.068	.327
Kernel type	.008	.845	-.407
Leave length	.119	.579	-.419
Tassel length	-.431	.171	-.338
Tillering	.830•	.073	-.343

DISCUSSION

Cluster analysis

Cluster analysis was initially able to group maize cultivars in two big groups, which were in turn further divided into another sub-groups based on similarities in morphology among 10 cultivars

(Fig. 1). VPO 5120, Qn 633 and CAP44NG formed a sub-group implying that they shared most of the characters and probably had the same progenitors. The morphological relationships between these cultivars were very close as indicated by the cluster analysis. All of them had different origins, thus Kenya, South Africa and Zimbabwe. Again, some cultivars obtained from CYMMIT in Zimbabwe showed a higher degree of similarities in their morphology namely: Zm 523 and Zm 521, as evidenced by cluster analysis. Another cultivar (Zm 521) from the same origin was not a member of this sub-group. It differed greatly on the basis of morphology. CAP 122-60 and Phb 33H56 cultivars formed a sub-group even though they are produced by two different companies; Capstone and Pioneer, respectively. They both exhibited a higher degree of similarity in morphological features. Similarly, another two cultivars of maize called CAP 9001 and Qs 62 formed a sub-group. These two cultivars shared a common ancestor. Ristic *et al* (2013) analyzed genetic diversity in 21 maize landraces using morphological characters and generated two big groups with dendrogram, which were further sub-divided into three more sub-groups. These sub-groups further divided into very small sub-groups. He observed similarities within the members of the sub-groups from the same companies and between companies. Others were outliers. Similarly, Azad *et al*. (2012) conducted studies on genetic diversity of thirty maize inbred lines and generated a cluster analysis with groups and sub-groups at very low levels of similarities. Shazia *et al* (2017) experienced grouping of 47 maize inbred lines from Kashmir when conducting genetic diversity study. Seven sub-groups were obtained with generation of cluster analysis. Cultivars were clustered at different genetic distances. It could be concluded that since maize belong to the same genera and species, they share most of the characters which made them to be group at a certain genetic distance. Where further sub-grouping started, it showed where the difference began.

Principal analysis

In this study, three principal components were considered which contributed to separation of cultivars (Table 1). Each principal component consisted of the characters differing in discriminatory power. No one or two characters were able to discriminate one cultivar from another. Three or more characters were able to group the cultivars and additional characters managed to separate them. Ear length, tillering, height of plant, cross-section of cob and cob diameter were the most powerful characters in segregating cultivars in principal component 1 as evidenced by loadings. However, there were some other characters which made infinitesimal contributions but together with others, separation was realized. Principal component 2 also had very powerful characters that assisted in discriminating cultivars. These are kernel type, kernel colour, kernel row arrangement and silk colour. All the mentioned characters showed that they could still segregate some limited number of cultivars. There were some characters with very low loadings possessing little influence on separation of cultivars. But in combination with others, they possessed higher discriminatory power. Principal component 3 constituted by number of kernels per row, silk colour and others with low loadings contributed to segregation of cultivars. Some characters of the most recent cultivars were similar due to the hybridization among the cultivars and sharing of the same progenitors. Inbreeding was practiced to concentrate more desirable genes in hybridization. Therefore, it was that more characters would be similar. The results were consistent with findings of Rohman *et al* (2015) who used morphological characters to segregate maize inbred lines. The first three principal components constituted 76.86% of total variation, of

which 36.51 % was contributed by principal component 1, second and third principal component comprised 22.08% and 18.27%. He observed the difference in discriminatory power of characters used. Similarly, Shariz *et al* (2017) used morphological markers to distinguish 47 maize inbred lines where principal component analysis was applied used. Nikkhoy and Shiri (2017) in their genetic diversity study demonstrated the discriminatory power of principal component in segregating cultivars using morphological features.

CONCLUSION

Morphological characters were a powerful tool in identifying and separating maize cultivars, particularly where more than three characters were used. Where more characters were used, more distinctions among cultivars were obtained. Closely related cultivars were not easy to separate unless more characters were included. In this case, cultivars were sharing the same progenitors.

Recommendation

The maize cultivars introduced into Lesotho originating from elsewhere must be characterized using morphological traits to avoid fraudulence and duplication.

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