Genetic variation of Zambian maize inbred lines commonly used in breeding

Chanda, R.¹, Mungoma, C.², Osiru, D.S.O.¹, Mwala, M.³ & Mwansa, K.⁴ ¹Department of Crop Science, Makerere University, P.O. Box 7062, Kampala, Uganda ²Seed Control and Certification Institute, P.O. Box 350199, Chilanga, Zambia ³Department of Crop Science, University of Zambia, P.O. Box 32379, Lusaka, Zambia ⁴Zambia Agriculture Research Institute, Mount Makulu Research Station, P/B 7, Chilanga, Zambia Corresponding author: edchazm@yahoo.com Abstract In sub-Saharan Africa (SSA), breeders have the challenge of developing high yielding maize hybrids that are adapted to farmers' conditions. This however requires knowledge on the breeding value of elite maize germplasm. Ten (10) maize inbred lines were grown under low nitrogen and optimal conditions at Golden valley and Chilanga in Zambia. The randomized complete block was used, with 2 row plots, 5.0m long and replicated twice. The inbreds were planted at a spacing of 0.50m, with two plants left per station and 0.90m between rows. Preliminary results for data collected under optimal conditions at Golden Valley indicates that variation exists among the elite germplasm tested. This offers opportunity for maize breeding in Zambia to maximize heterosis. However, the variation will be validated with testcross performance. Key words: Heterosis, maize breeding, Zambia Résumé En Afrique sub-saharienne, les sélectionneurs ont le défi de développer des hybrides de maïs de haut rendement, qui sont adaptés aux conditions des agriculteurs. Toutefois, ceci exige la connaissance de la valeur génétique du germoplasme de maïs d'élite. Dix (10) souches pures de maïs ont poussé sous un faible taux d'azote et dans les conditions optimales à Golden Valley et à Chilanga en Zambie. Le bloc complet randomisé a été employé, avec des parcelles formées de deux rangées, ayant 5 mètres de long et deux fois reproduites. Les espèces pures ont été plantées à un espacement de 0.50m, avec deux plantes laissées par station et un espacement de 0.9m entre les rangées. Les résultats préliminaires pour des données collectées dans des conditions optimales à Golden Valley indiquent que la variation existe parmi le germoplasme d'élite examiné. Ceci procure l'occasion à la sélection du maïs en Zambie afin de maximiser la fertilité de l'espèce hybride. Cependant, la

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variation sera validée avec la performance de la technique de croisement génétique.

Mots clés: Hétérosis, reproduction du maïs, Zambie

In Zambia, maize is the main source of energy and the major cash crop. The crop is mostly grown by resource poor farmers in marginal areas. This has resulted in maize being produced under biotic and abiotic stress which has eventually resulted in perpetual food insecurity. The situation is further worsened by rapid population growth and climate change. Climate change predictions indicate that most parts of Africa will experience higher temperatures and changing rainfall levels, resulting in crop yield depression (Yesuf *et al.*, 2008). The negative impact of climate change in Africa has been estimated to be highest in maize, causing yield loss of 22% in maize and losses of 22, 17, 17, 18, and 8% for maize, sorghum, millet, groundnut, and cassava, respectively, by mid century (Schlenker and Lobell, 2010).

There is therefore need to develop maize hybrids that are high yielding and tolerant to abiotic and biotic stresses, as a key component of yield – enhancing adaptation strategies. Since adaptation measures have a positive effect on crop yields, the adoption of yield-related adaptation strategies increases the agricultural productivity of poor farm households (Yesuf *et al.*, 2008), who are the main focus of agricultural development. Thus, knowledge on the performance per se of elite germplasm is key to the development of such hybrids, especially where parent selection is based on *a priori* as opposed to *a posteriori* choice (Singh *et al.*, 2008). The aim of this study was to determine the genetic variation of maize inbred lines commonly used in breeding hybrids in Zambia by using multivariate techniques.

Inbred-hybrid breeding depends on the generation of inbred lines, such that when crossed to form a hybrid, heterosis is fully exploited. Thus, parental selection is an important step that would result in the development of high yielding and stress tolerant maize hybrids. Parental selection can be on *a priori* or *a posterior* basis (Singh *et al.*, 2008). According to Dias *et al.* (2004), the *a priori* choice is based on the association between divergence and heterosis. This perhaps explains the emphasis on the global review of the existing maize heterotic groups and heterotic patterns by Reif *et al.* (2005). Successes have been recorded by an *a priori* parental selection (Dias *et al.*, 2004).

Background

Literature Summary

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	However, morphological descriptors are key for <i>a priori</i> choice, though they can be reinforced by data from molecular markers (Dias <i>et al.</i> , 2004). The evaluation of inbred lines based on morphological characteristics has been the prevalent method because it provides unique genotype identification (Vladislava <i>et al.</i> , 2006) and thus is the hallmark of Plant Variety Protection under the Union for the Protection of New Plant Varieties (UPOV) convention.
	In assessing the genetic variability of genotypes based on morphological traits, cluster analysis and other multivariate analysis are often employed (Aydýn <i>et al.</i> , 2007; Aliu <i>et al.</i> , 2009). Indeed, when Babic <i>et al.</i> (2008) evaluated 45 inbredlines using 30 phenotypic traits, they found that Wards method (Incremental sum of square) was better than other grouping methods used, namely between group linkage, within group linkage and nearest neighbour. In addition, morphological data can further be augmented by testcross data and molecular data (Aydin <i>et al.</i> , 2007; Dias <i>et al.</i> , 2008; Aliu <i>et al.</i> , 2009).
Study Description	Ten maize inbredlines commonly used in the Zambian maize breeding programme were grown at Golden Valley. Each plot consisted of two rows, 5m long and interspaced at 0.90m. Two plants were left to grow at each station, that were spaced 0.50m, to achieve a plant population of 44,000 plants. The plots were basal dressed with compound D fertilizer (10N:20P:10K) at 350 kg/ha and latter topdressed with Urea (46%N) at 250 kg/ ha.
	The Quantitative variables considered in the multivariate analyses were:
	 (a) phonological beginning (BEGTASEL), 50% (50TASSEL) and end of tasselling (ENDTASEL) beginning (BEGANTH), 50% (50ANTH) and end of anthesis (ENDANTH) Tasseling silking interval (TAI) physiological maturity (MATURITY) anthesis period (ANTHPERI)
	 (b) morphological number of tassel branches (TBNo) average tassel length (AVGTLNG) leaf length and leaf width (LfL and LfW) in cm

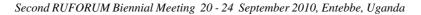
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	 leaf area (LFA) in cm² ear length (EL) and ear diameter (ED) in cm number of grains per row (KperROW) number of rows per ear (ERNo) plant and ear height (PH and EH) in cm number of leaves per plant (LfNo) (c) grain grain yield (GY) in kg/ha 100-grain weight (HSW) in grammes 					
Statistical Analysis	The univariate analysis of variance was used to evaluate differences between inbred lines and the analysis of genetic diversity was performed using GenStat discovery Edition 3 programme (Buysse <i>et al.</i> , 2007). The phenotypic traits that were considered for cluster analysis are hundred seed weight, number of leaves, leaf length, leaf width, plaant height, ear height, number of grain rows, number of kernels per row, average length of tassels, number of tassel branches, days to maturity, ear length and ear diameter. These traits are required by UPOV for Plant Breeders Rights.					
Research Application	Genotypes were significantly different ($p \le 0.05$) in 40% of the 25 traits recorded (Table 1). Furthermore, the results suggest the use of a tassel index to understand tassel size, as the genotypes did not differ in the number of branches, yet highly significant ($p=0.001$) differences for average tassel length were observed. This has implications on the time to peak pollen production, a critical component for synchronisation in seed production (Chanda, 2007).					
	Results on the simple correlation among characters are presented in Table 2. Grain yield was found to be higly positively correlated ($p \le 0.05$) to number of tassel branches (TB No) and ear height (EH). Plant height (PH) and leaf width (LfW) were also correlated to grain yield though not significantly. This implies that for breeding purposes to improve the inbred lines, emphasise should be given to these traits. The number of kernels per row and number of grain rows were negatively correlated ($r = -0.52$). In addition, ear length (EL) was strongly negatively correlated to number of grain rows. This indicates that breeding for ear traits could aid the developing of superior inbred lines.					
	In seed production involving single crosses, seed yield is an issue. The results suggest that this can be overcome by					

Table 1.	viean valu	105 101	18 50		to inbream	lles.				
Genotype	BEG TASE	LT	EL	END TASEL	TAS PERIO	BEG- ANTH	50 ANTH	ENI ANT		ANTH PERI
EN1	53			65	12	60	65	67		7
EN2	49			59	10	56	61	62		6
EN3	59			68	9	64	68	72		8
EN4	53			63	10	62	67	66		5
EN5	56			67	10	64	68	70		7
EN6	56			66	11	64	67	70		6
EN7	53			63	10	63	64	68		5
EN8	51			59	8	57	62	63		6
EN9	56			66	10	65	68	69		5
EN10	54			63	9	62	68	66		4
Mean	53.9)	5	64.0	10.0	61.5	65.8	67.4	4	5.9
Probability	0.07	75	11	0.040	0.613	0.000	0.067	7 0.03	38	0.925
CV%	6.3			5.1	17.1	3.2	4.4	4.9		56.8
Genotype	TAI	TBNc	٩VG	TALNG	Maturity	PH(cm)	EH(cm)	ED(cm)	GLS	ELCM
EN1	-6	19	3	7	104	178	60	13	1	14
EN2	-8	17	3	9	103	184	68	13	2	16
EN3	-4	17	3	7	106	203	86	13	2	15
EN4	-10	23	4	2	107	229	104	14	1	15
EN5	-7	18	3	9	106	204	79	14	1	17
EN6	-7	19	4	7	103	226	97	14	3	16
EN7	-7	14	4	4	104	202	74	13	1	14
EN8	-8	12	3	4	107	131	41	14	2	14
EN9	-6	13	4	6	103	225	93	13	1	17
EN10	-10	15	3	7	109	198	79	14	2	18
Mean	-7.3	16.¢	4	0.1	105.2	198.0	78.1	13.6	1.7	15.5
Probability	0.001	0.39	0	.001	0.872	0.002	0.001	0.475	0.098	0.398
CV%	20	33.5	7	.7	5.0	11.1	17.7	5.4	39.6	14.2
Genotype	Lf L (cm	n) Lf	m)	L FA(cn	n ²) ERNo	KperRO	W LfNo	HSW(g) GY	r (kg/ha)
EN1	69			410	13	28	12	24.9		1849
EN2	61			312	12	27	12	24.7		1422
EN3	72			441	13	27	13	24.5		2589
EN4	74			438	13	30	15	28.5		2667
EN5	68			403	13	31	14	29.7		1850
EN6	71			392	14	27	13	22.9		1450
EN7	71			385	13	31	12	21.8		1733
EN8	70			385	12	25	13	32.5		242
EN9	73			426	11	33	13	32.3		1299
EN10	69			424	10	32	12	30.9		2194
Mean	69.9			401.7		29.1				1729.3
Probability	0.144	4	83	0.062						0.049
CV%	6.6			10.6	10.4	10.8	9.7	10.3		41.4

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 Table 1. Mean values for
 ts scored on 10 inbredlines.

GY kg/ha 0.67 0.22 0.63 0.64* 0.07 0.63 0.53 0.53 0.54 0.23 0.53 0.53 0.53 0.53 0.54 0.53 0.53 0.53 0.54 0.53 0.54 0.53 0.54 0.53 0.54 0.53 0.54 0.53 0.51 0.03 0.11 0.33 0.23 0.03 0.11 0.33 0.33 0.33 0.33 0.33 0.33 0.33 0.33 0.31 0.33 0.31 0.33 0.31 0.33 0.31 0.33			TB No	GLS	Avg Tasel Length (cm)	Maturity	ity PH cm	cm	EH cm	EL cm	ED cm	LfL cm	LfW cm	LFA	
		GY kg/ha	0.67 ^a	-0.22	0.06	0.2		63	0.64 ^a	0.07	0.05	0.29	0.59	0.52	I
		HSW LfNo	-0.34	-0.07	-0.27	0.5		22 32	-0.15 0.44	0.47 -0.03	0.09	0.19 0.43	0.33	0.29	
		KNo per ROW	-0.10	-0.60	0.45	0.0		54	0.43	0.50	-0.14	0.23	0.33	0.32	
IFA 0.20 -0.10 0.05 0.49 0.39 0.45 0.09 0.17 0.88* 0.90* LfWcm 0.20 -0.09 -0.22 0.39 0.27 0.30 0.25 0.36 0.90* LfLcm 0.13 -0.06 0.13 0.26 0.49 0.36 0.35 0.36 <		ERNo	0.55	0.03	0.12	-0.2(13	0.17	-0.63 ^a	0.40	0.27	-0.07	0.10	
		LFA	0.20	-0.10	0.05	0.49		39	0.45	0.09	0.17	0.88 °	0.90°		
LfLcm 0.13 -0.06 0.33 0.26 0.42 0.49 -0.10 0.27 ED cm 0.49 0.36 0.18 0.25 0.23 0.31 0.09 0.37 EL cm -0.23 0.12 0.17 0.21 0.36 0.36 0.36 EL cm -0.23 0.12 0.17 0.21 0.36 0.36 0.36 PH cm 0.52 -0.10 0.97* -0.10 0.97* 0.36 <t< td=""><th></th><td>LfW cm</td><td>0.20</td><td>-0.09</td><td>-0.22</td><td>0.59</td><td></td><td>27</td><td>0.30</td><td>0.25</td><td>0.05</td><td>0.58</td><td></td><td></td><td></td></t<>		LfW cm	0.20	-0.09	-0.22	0.59		27	0.30	0.25	0.05	0.58			
EDcm 0.49 0.36 0.18 0.25 0.23 0.31 0.09 ELcm -0.23 0.12 0.17 0.21 0.36 0.36 0.36 EHcm 0.52 -0.15 0.70* -0.10 0.97* 0.36 0.36 PHcm 0.48 -0.27 0.77* -0.10 0.97* -0.36 Maturity 0.04 0.24 -0.62 -0.23 -0.36 -0.36 AvgTaselLengthcm 0.12 -0.22 -0.13 -0.23 -0.36 -0.36 AvgTaselLengthcm 0.12 -0.22 -0.77* -0.23 -0.36 -0.36 AvgTaselLengthcm 0.12 -0.22 -0.37 -0.33 -0.33 -0.36 GLS -0.13 ERNo KNo per ROW -0.18 -0.30 -0.30 GY kop per ROW -0.53 0.30 -0.30 -0.30 -0.30 -0.30 KNo per ROW -0.52 -0.30 -0.30 -0.30 -0.30		LfL cm	0.13	-0.06	0.33	0.20		42	0.49	-0.10	0.27				
-0.23 0.12 0.17 0.21 0.36 0.36 ty 0.52 -0.15 0.70* -0.10 0.97° 0.36 0.48 -0.27 0.77* -0.10 0.97° 0.97° 0.48 -0.27 0.77* -0.10 0.97° 0.12 0.24 -0.23 0.97° 0.12 -0.22 0.77* -0.23 0.12 -0.22 0.77* -0.23 0.12 -0.22 0.77* -0.23 0.12 -0.22 0.77* -0.23 1 0.12 -0.22 0.97° 1 0.12 0.22 -0.30 1 0.19 0.29 0.30 0.33 0.08 0.30 0.30 1 <row< td=""> -0.52 0.30 0.30</row<>		ED cm	0.49	0.36	0.18	0.2:		23	0.31	0.09					
Hcm 0.52 -0.15 0.70° -0.10 0.97° PH cm 0.48 -0.27 0.77° -0.23 Maturity 0.04 0.24 -0.62 AvgTaseLLength cm 0.12 -0.23 -0.13 AvgTaseLLength cm 0.12 -0.22 -0.62 GLS -0.13 -0.22 -0.62 FRNo 0.12 -0.23 -0.63 GT set Length cm 0.12 -0.23 -0.63 GY g/maturity 0.13 -0.23 -0.33 GY kg/ha 0.19 0.29 0.30 HSW -0.58 0.30 0.30 KNo per ROW -0.51 0.30 -0.30		EL cm	-0.23	0.12	0.17	0.2		36	0.36						
PH cm 0.48 -0.27 0.77b -0.23 Maturity 0.04 0.24 -0.62 -0.13 AvgTaseLLength cm 0.12 -0.22 -0.13 -0.13 AvgTaseLLength cm 0.12 -0.22 -0.13 -0.13 GLS -0.13 -0.22 -0.23 -0.13 GY kg/ha 0.19 0.29 0.32 -0.30 GY kg/ha 0.19 0.29 0.30 -0.30 KNo per ROW -0.53 -0.30 -0.30 -0.30		EH cm	0.52	-0.15	0.70^{a}	-0.1(_	∍ <i>L</i> t							(
Maturity 0.04 0.24 -0.62 AvgTaselLength cm 0.12 -0.22 GLS -0.13 -0.22 ERNo 0.19 0.29 0.30 GY kg/ha 0.19 0.29 0.30 LfNo 0.33 0.030 0.30 KNo per ROW -0.52 -0.30		PH cm	0.48	-0.27	0.77^{b}	-0.2	3								Cha
AvgTaselLength cm 0.12 -0.22 GLS -0.13 -0.22 ERNo KNo per ROW LfNo HSWT GY kg/ha 0.19 0.29 0.30 LfNo 0.33 0.030 -0.30 KNo per ROW -0.52 -0.30 -0.30		Maturity	0.04	0.24	-0.62										ınd
FRNo KNo per ROW LfNo HSWT g/ha 0.19 0.29 0.32 -0.30 -0.58 0.29 0.30 -0.30 per ROW -0.52 0.30 -0.30	18/	AvgTasel Length cm GLS	0.12 -0.13	-0.22											a, R. et
z/ha 0.19 0.29 0.32 -0.58 0.29 0.30 0.33 0.08 per ROW -0.52			ERNo	KNo per l	MO	,fNo	HSWT								al.
		GY kg/ha HSW LfNo KNo per ROW	0.19 -0.58 0.33 -0.52	0.29 0.29 0.08	0 0	.32 .30	-0.30								



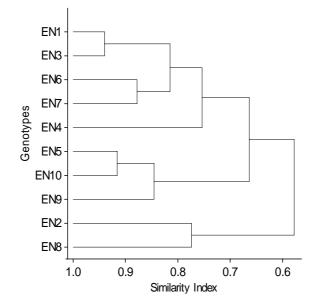


Figure 1. Cluster of 10 inbredlines based on 15 phenotypic traits using the Euclidean similarity distance and the Furthest Neighbour grouping.

Table 3. Between and within groups similarity matrix.

1	87.3					
2	79.8	-				
3	79.6	78.4	87.4			
4	81.0	57.8	72.3	-		
5	75.9	64.8	74.1	77.4	-	
	1	2	3	4	5	

increasing the number of tassel branches without necessarily increasing the average length (Tables 1 and 2). In the inbreds studied, grey leaf spot negatively impacted on the number of grains per row (r = -0.60).

The genotypes were grouped differently (Fig. 1). Five groups were identified, with the biggest group having 4 genotypes while genotypes EN4, EN8 and EN2 were grouped alone. The similarity between and within groups is shown in Table 3.

Recommendations Evaluation of inbredlines for agronomic performance is important for assessing the breeding value and selection of parents for hybrid breeding. The information is also important for plant variety protection under the UPOV system. However, the groups generated from phenotypic data should be fine tuned by molecular and testcross data. This will be followed up in this study.

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Acknowledgement	This study is funded by RUFORUM as part of the PhD of the first author. We also thank RUFORUM for facilitating presentation of this paper during the Biennial Conference in Entebbe, Uganda.
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