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Full Length Research

Evaluation of genetic variability and interrelationships among M_3 and M_4 maize inbred lines in Kenya

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ABSTRACT: The present study was conducted at Jomo Kenyatta University of Agriculture and Technology, Juja to assess the agronomic performance of mutant maize lines of 39 filial generation 3 (M_3) and filial generation 4 (M_4) maize lines and a check variety among agro-morphological traits with their association with grain yield. Data on various agro-morphological characters were recorded using morphological descriptors for maize and analyzed using Genstat Release 14.1. Data was also subjected to XLSTAT 2014 and DARwin 6.0.12 software for principal and cluster analyses. Results obtained differed significantly in herbicide tolerance days for both M_3 and M_4 ($p \leq 0.01$). However, plant height, maturity days, flag leaf length and width, grains ear^{-1}, ear length, ear diameter and grain yield plant^{-1} differed significantly in M_3 and M_4 lines ($p \leq 0.05$). Grain yield plant^{-1} showed a strong significant positive correlation with anthesis days, plant height, grains ear^{-1} and ear diameter and length but negatively correlated with days to pollen shedding, tasseling, maturity and tolerance in M_3 while flag leaf width, harvestable and total ears plant^{-1} showed positive and significant correlation but negatively correlated with tolerance days in M_4. Principal component analysis showed variations among mutated maize lines in M_3 and M_4 with first seven principal components (PC) indicating that the first six PCs explained 78.69% and first six PCs contributing 71.28% respectively of the total variation. Cluster analysis showed three clusters and seven sub-clusters indicating differences in morphological diversity among the M_3 inbred lines and two clusters with cluster one of hybrid 513 and three sub-clusters in cluster two of hybrid 520. Plant height, flowering days and ear length were crucial phenological traits determining grain yield among herbicide tolerant lines showing significant variability that could be considered in hybridization and development of herbicide tolerant hybrid genotypes in future maize breeding programmes.

Keywords: Cluster analysis, morphological traits, principal component analysis, variations, *Zea mays.*

INTRODUCTION

Maize is the most important staple food for Kenya ranking globally third in production after wheat and rice respectively with maize having the highest grain yield potential per hectare than other cereals (FAO, IFAD, UNICEF, WFP, and WHO, 2018). Maize is a versatile high producing crop with a wider adaptability thus named queen of cereals. Maize is not only an important food crop for human consumption, but also a basic element of animal feed and raw material for manufacturing of many industrial products such as corn starch, maltodextrins, corn oil, corn syrup and products of fermentation and distilleries. Recently used in the production of biofuel (Skoufogianni et al., 2019).

Grain yield in maize is the most imperative trait related to other morphological, physiological and agronomic traits. Improving these traits increases maize genotypes production. Despite this, declining yields as result of both biotic and abiotic stress factors is experienced. Effective breeding strategies of high yielding varieties is a sure option ensuring sustainable food security in the country as total maize production does not meet increased consumption (Nelimor et al., 2020). Combining ability analysis provides an opportunity to a plant breeder to select genotypes on the basis of strong correlations among grain yield contributing traits as reported by Ali et al. (2015), Nyaga et al. (2020) and Yu et al. (2020)

The characterization of morphological variability is useful tool for identification of accessions with desirable characteristics like earliness, herbicide tolerance, drought tolerance, saline tolerance, disease resistance, or improved ear trait. The characterization and grouping of germplasm helps the breeders to avoid duplication in sampling populations (Franco-duran et al., 2019) The variation in morphological traits allows the breeder to select inbred lines for development and production of superiorquality. Inbred lines with similar plant height, ear height can be used for development of synthetic varieties. Cluster analysis is a convenient method for organizing data sets so that information can be retrieved more efficiently and be easily understood without the need for complicated mathematical techniques. Cluster analysis is frequently used to classify maize (*Zea mays* L.) accessions and can be used by breeders and geneticists to identify subsets of accessions which have potential utility for specific breeding or genetic purposes (Shrestha, 2016).

Different researches have been conducted to determine the correlation of grain yield with other agronomic traits. As grain yield in maize is quantitative in nature and polygenically controlled, effective yield improvement and simultaneous improvement in yield components are imperative (Akinyele et al., 2019; Bello and Olaoye, 2012). Multivariate methods summarizes information, eliminates “noise” from the data sets and reveals the structure of the data sets (Kose et al., 2018). In addition, multivariate methods can also be used for determining yield stability and identifying genotypic groups possessing desirable traits (Baraki et al., 2020). Cluster analysis can identify differences among genotypes for the breeder via classification of genotypes (Iqbal et al., 2018).

In Kenya, several maize varieties are grown in various agro-ecological zones with diverse production potentials in yield performance and adaptation towards biotic and abiotic stresses. Some of highland varieties includes Hybrid Seed Maize (H6213, H624, H620, H626, H629, H513 etc.); Pioneer (30G19, 3812W); DK (90-89, 777, 8031 and 8033); PAN (691, 4M-19, 8M-93); KS 614; SY 594 among others (Naseem et al., 2018); Schroeder et al., 2013).

MATERIALS AND METHODS

Study area description and experimental material

The study was conducted in Juja, Jomo Kenyatta University of Agriculture and Technology Farm, from October, 2014 to September, 2015 under rainfed conditions and supplementary irrigation provided when necessary. Juja is located 36 km North-East of Nairobi along the Thika-Nairobi highway. It lies between latitudes 3°35" and 1°45" South of the Equator and longitudes of 36º35" and 37º25" East (GoK, 1997). Juja is located in the upper midland zone 4 which is semi-humid to semi-arid at 1520 meters above sea level with a mean annual temperature of 20ºC and mean maximum temperature of 30ºC. The area receives low rainfall of 856 mm/annum recorded over ten years with a bimodal distribution and has three types of soil which are shallow clay soils over trachytic tuff with very shallow sandy clay soils over murram and deep clay (Vertisols) soils (Batjes, 2006) and previously under cowpea.

Two hybrid seed varieties comprising of H513 and H520 mutagenized with ethylmethyl sulfonate and one check variety were utilized in this research.

Treatments and experimental design

Experimental materials comprised of 39 M_3 lines arising from M_2 and one check variety. The forty maize lines of H513 and H520 were evaluated in a randomized complete block design (RCBD) with three replications. Ten M_3 plants were grown at inter row spacing of 75 cm and intra-row spacing of 30 cm to enhance a population of about 26,666 plants ha^{-1}. At maturity, M_3 seeds from each cob were harvested and threshed separately and divided into two portions.

A random sample of fifty seeds from the 1st batch of seeds from each cob was drilled in single row of 1 meter long. Ten days after emergence, the seedlings were sprayed with x1 glyphosate (200g/20 litres). Seven to fourteen days after spraying the susceptible, the tolerant lines were identified.

During flowering, all plants in a row were self-pollinated. After maturity, seeds of each progeny rows were harvested in bulk to yield generation two and spray four ($M_{2:4}$). From the 88 maize lines, 39 lines and one check variety survived and yielded the second batch. Then ten seeds were randomly selected from the second batch of the survived tolerant lines. The retrieved seeds were grown in progeny single rows 5 m long at inter- intra row spacing of 75 cm x 50 cm in RCBD with three replications for morphological characterization. Besides cultural, agronomic practices were followed as per the standard recommendations and need based crop management measures were followed to maintain healthy crop.

Data collection

Data on agronomical characters were recorded on each of the five pre-tagged plants from the middle of each row and tagged at various phenological stages with growth parameters included days to tasseling, silking, anthesis, pollen shedding and physiological maturity and plant height and yield and yield component parameters included grain yield plant^{-1}, grains ear^{-1}, ear diameter and ear length as shown in Table 1.

Table 1. Agronomical characters.

Traits	Denotation	Definition
Plant height	PH	Measured in cm with a metric tape as the distance from ground level to the tip of the maize plant excluding the tassel at harvest stage.
Days to silking	DTS	Obtained from date of sowing to when silks have emerged on 50% of the plants.
Days to tasseling	DTT	Achieved from date of sowing to when 50% of the plants have tasseled.
Days to pollen shed	DPS	Counting the number of days from sowing to pollen shedding when anthers dehiscenced.
Days to anthesis	DA	Recorded as number of days from sowing to silking.
Total ears plant^{-1}	TEP	Achieved by counting the actual number of ears on each tagged plant.
Harvestable ears plant^{-1}	HEP	Recorded by counting the number of ears with kernels from each tagged plant.
Days to maturity	DM	Recorded by counting the number of days from emergence to when the tagged plants attained physiological maturity.
Ear length	EL	Measured in cm the length from the base to the tip of dehusked ear of the tagged plants.
Ear diameter	ED	Taken in cm as the average diameter at the middle of the cob using outside caliper and ruler of dehusked cob.
Grains ear^{-1}	NGE	Obtained by the following equation: NGE= NRE×AGR**.**
Flag leaf length	FLL	It was measured in cm using a caliper and ruler from the start of the sheath to leaf apex.
Flag leaf width	FLW	Measured in cm using a caliper ruler as the diameter at the middle of the leaf.
Grain yield plant^{-1}	GY	Recorded in grams using electronic balance as the grain yield from each tagged plant.
Tolerance days	TLD	Counted from the fourth day after spraying plants with herbicide to eventual death or constant number of plant(s) survival in a line.

Data analysis

Data on the various agro-morphological were analyzed for ANOVA using the Genstat 14th (Genstat Release 14.1) at 5% level of significance to determine significance of variation among inbred lines. Besides, principal component analysis (PCA) was conducted after standardization to mean of zero and variance of one (Payne et al., 2011). Cluster analysis was done using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis on DARwin software version 6.0.12 grouping the inbred lines to avoid duplication in breeding programme.

RESULTS

Variation in agronomical traits

Analysis of Variance showed significant differences among the evaluated M_3 and M_4 maize lines for most of the traits recorded such as plant height, grains ear^{-1}, days to silking, tasseling, pollen shed, anthesis and maturity, ear length and diameter, flag leaf length and width, grain yield plant^{-1} and tolerance days (Tables 2 and 3).

Herbicide tolerance days, days to silking, flag leaf width and grain yield plant^{-1} were highly significant ($p<0.01$) while ear diameter, plant height, number of grain ear^{-1}, ear length and grains ear^{-1}, flag leaf length (cm) and days to physiological maturity were significant ($p<0.05$) in M_3. In M_4 generation, tolerance days, ear length showed highly significant ($p<0.01$) differences among the maize lines. Plant height, ear diameter, days to tasseling, days to silking, flag leaf length and width, grain yield plant^{-1} and grain ear^{-1} were significant ($p< 0.05$) among the 37 maize lines.

Correlation among agronomic traits recorded in M_3 and M_4 maize lines

Pearson's Correlation analysis results of studied traits among 39 M_3 and 37 M_4 maize lines are presented in Tables 4 and 5. Results for the M_3 generation indicated that grain yield plant^{-1} was positive and significantly correlated with plant height ($r = 0.55^{**}$), grain ear^{-1} (r=

Table 2. Analysis of variance for yield and some yield components of 40 M_3 evaluated maize lines.

Traits	DF	Sum squares (SS)	Mean squares (MS)	P-value	%CV
Plant height	39	73378	1881	0.013	14.0
Silking days	39	2286.05	58.62	<0.001	5.0
Tassling days	39	1538.35	39.44	0.001	4.5
Pollen shedding days	39	3735.50	95.78	0.001	6.6
Total ears plant^{-1}	39	45.887	1.1753	NS	30.9
Harvestable ears plant^{-1}	39	4.0735	0.1044	NS	23.6
Anthesis days	39	3354.66	86.02	<0.001	6.2
Ear length	39	341.725	8.762	0.046	12.2
Ear diameter	39	8.2148	0.2106	0.019	8.8
Grains ear^{-1}	39	384759	9866	0.017	18.4
Flag leaf length	39	3149.65	80.76	0.020	19.1
Flag leaf width	39	1.592	0.796	0.004	20.7
Days to maturity	39	7076.2	181.4	0.050	7.3
Grain yield plant^{-1}	39	54267.2	1391.5	0.006	32.5
Tolerance days	39	1617.99	41.49	<0.001	11.1

Note: DF- degree of freedom Maize lines minus 1, NS- non significant at 5% level of significance.

Table 3. Analysis of variance for yield and yield components of 37 M_4 evaluated maize lines.

Character	DF	Sum squares (SS)	Mean squares (MS)	P-value	%CV
Plant height(cm)	36	33613.3	933.7	0.02	8.3
Days to silking	36	953.61	26.48	0.05	4.1
Days to tasseling	36	1721.91	47.83	0.048	5.7
Days to pollen shed	36	553.51	15.38	0.042	3.7
Total ears plant	36	7.5858	0.2107	0.805	25.9
Harvestable ears plant	36	2.123	0.059	0.777	22.3
Ear diameter(cm)	36	11.74	0.3261	0.018	9.9
Ear length(cm)	36	354.338	9.843	0.006	11
Grains ear^{-1}	36	245688	6825	0.028	14.3
Flag leaf length(cm)	36	1738.74	48.3	0.015	14.1
Flag leaf width(cm)	36	39.382	1.094	0.027	13.8
Days to anthesis	36	553.51	15.38	0.042	3.7
Days to maturity	36	5368.62	149.13	0.05	6.2
Grain yield plant^{-1}	36	37790.9	1049.7	0.040	9.0
Tolerance days	36	1543.76	42.88	<0.001	12.0

0.58**), anthesis days (r= 0.24**), ear diameter (r= 0.68**) and ear diameter (r= 0.53**) but significant (p<0.05) and negatively correlated with herbicide tolerance days (r= -0.38*), maturity days (r=-0.47**), pollen shedding days (r= -0.67**), tasseling days (r= -0.67**) and silking days (r= -0.76**) (Table 6).

In M_4, the grain yield ear exhibited positive and significant correlation with harvestable ears plant^{-1} (r= 0.28**), total ears plant^{-1} and flag leaf width (r= 0.28*) tolerance days (r= 0.27*). Moreover, the trait exhibited negative and significant correlation with grains ear^{-1} (r= -0.30*).

Tolerance days showed significant and positive correlation with days to pollen shedding (r= 0.32**) and negatively and significantly correlated with grain yield plant^{-1} (r=-0.27*) and anthesis days (r=-0.27*).

Tolerance days in M_3 showed positive and significant correlated with anthesis days (r= 0.24**), days to pollen shed (r= 0.50**), tasseling days (r= 0.52**) and silking days (r= 0.48**). However, the trait showed negative and significant correlation with ear diameter (r= -0.40**), grain yield plant (r=-0.38**), grain ear (r=-0.38*) and plant height (r=-0.42**).

In M_4 generation, tolerance days exhibited positive and significant correlation with days to pollen shed (r= 0.32*). The trait also showed negative and significant correlation with anthesis days (r=-0.27*) and grain yield plant^{-1} (r=-0.27*).

Table 4. Correlation coefficients of agronomic components among the assessed 39 M_3 maize lines in JKUAT in 2014

Trait	DA	DPS	DTS	DTT	ED	EL	FLL	FLW	GY	HEP	NGE	DM	PH	TEP	TLD
DA	1.00														
DPS	0.24**	1.00													
DTS	0.24**	0.84**	1.00												
DTT	0.24*	0.77**	0.92**	1.00											
ED	0.24*	-0.42**	-0.55**	-0.42**	1.00										
EL	0.24^{ns}	-0.26	-0.34*	-0.38*	0.34*	1.00									
FLL	0.24^{ns}	-0.29	-0.08	-0.23	-0.05	0.38*	1.00								
FLW	0.24**	-0.62**	-0.53**	-0.62**	0.20	0.44**	0.76**	1.00							
GY	0.24**	-0.67**	-0.76**	-0.67**	0.68**	0.53**	0.04	0.07	1.00						
HEP	0.24^{ns}	-0.10	-0.28	-0.20	0.61**	0.44**	-0.13	0.12	0.35*	1.00					
NGE	0.24**	-0.53**	-0.51**	-0.57**	0.20	0.69**	0.34*	0.53**	0.58**	0.10	1.00				
DM	0.24**	0.51**	0.57**	0.57**	-0.19	-0.28	-0.29	-0.47**	-0.47**	-0.11	-0.47**	1.00			
PH	0.24**	-0.50**	-0.66**	-0.73**	0.46**	0.53**	0.36*	0.55**	0.55**	0.49**	0.44**	-0.40**	1.00		
TEP	0.24^{ns}	0.16	-0.09	-0.01	0.17	0.34*	-0.08	-0.02	0.24	0.41**	0.10	-0.09	0.28	1.00	
TLD	0.24**	0.50**	0.52**	0.48**	-0.40**	-0.13	0.03	-0.26	-0.38*	-0.17	-0.31*	0.12	-0.42**	0.12	1.00

Table 5. Correlation coefficients of agronomic components among the assessed 37 M_4 maize lines in JKUAT 2015.

Trait	DA	DDT	DPS	DTS	ED	EL	FLL	FLW	GY	HEP	NGE	DM	PH	TEP	TLD
DA	1.00														
DDT	0.09	1.00													
DPS	-0.27*	0.12	1.00												
DTS	0.30*	-0.15	-0.02	1.00											
ED	0.14	-0.66**	-0.23	0.14	1.00										
EL	0.12	-0.02	-0.25	0.04	0.06	1.00									
FLL	-0.02	0.13	-0.07	-0.14	-0.15	0.13	1.00								
FLW	0.05	0.12	0.26	-0.10	-0.15	-0.08	0.33*	1.00							
GY	-0.12	0.01	0.10	-0.07	0.16	-0.03	-0.02	0.29*	1.00						
HEP	-0.11	0.23	-0.09	-0.20	-0.17	-0.19	-0.03	0.17	0.28*	1.00					
NGE	0.02	-0.11	0.08	-0.01	0.25	-0.03	-0.28*	-0.05	0.00	-0.30*	1.00				
DM	-0.16	0.05	0.30*	-0.08	-0.06	-0.03	0.03	-0.12	-0.03	-0.23	0.08	1.00			
PH	-0.21	-0.35*	-0.10	-0.07	0.10	0.10	-0.01	-0.27*	0.02	-0.07	-0.26	0.07	1.00		
TEP	-0.28*	-0.16	-0.06	-0.17	0.21	-0.12	0.24	0.01	0.27*	0.59**	-0.23	0.07	0.13	1.00	
TLD	-0.27*	-0.04	0.32*	-0.26	0.03	0.05	-0.10	0.07	-0.27*	0.19	0.00	0.16	0.02	0.13	1.00

Table 6. Principal component analysis of various agro-morphological traits in M_3 maize lines.

Parameters	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	8.955	2.626	1.8	1.5	1.4	1.1
Total variance %	40.70	11.94	8.19	6.62	6.31	4.93
Cumulative variance %	40.70	52.64	60.83	67.45	73.76	78.69
Factor loading by various traits						
Days to anthesis	**-0.29**	0.17	0.22	0.01	0.12	-0.15
Days to pollen shedding	**-0.27**	0.15	**0.28**	-0.03	-0.02	-0.15
Days to silking	-**0.30**	-0.04	**0.25**	0.08	0.04	-0.09
Days to tasseling	**-0.30**	0.06	0.18	0.06	-0.04	0.02
Ear diameter	0.19	**0.36**	-0.09	0.06	0.04	-0.03
Ear length	0.21	0.07	**0.47**	0.16	0.04	0.02
Flag leaf length	0.12	**-0.38**	**0.31**	-0.15	**0.33**	-0.01
Flag leaf width	**0.24**	**-0.28**	0.12	-0.13	**0.26**	-0.12
Grain yield plant^{-1}	**0.28**	0.15	-0.02	0.04	-0.21	0.20
Harvestable ears plant^{-1}	0.13	**0.43**	0.14	0.02	**0.35**	-0.05
Grains ear^{-1}	**0.25**	-0.14	**0.26**	**0.32**	-0.21	0.09
Days to maturity	-0.21	0.09	0.00	0.14	0.01	**-0.43**
Plant height	**0.26**	0.10	0.11	-0.15	0.23	-0.22
Total ears plant^{-1}	0.05	**0.29**	**0.39**	**-0.35**	-0.13	0.09
Tolerance days	-0.18	-0.07	**0.26**	-0.11	0.06	**0.31**

Principal component analysis

Results on principal component analysis in M_3 (Table 6) showed that the first six components with eigenvalues >1 accounted for 78.69%. The proportion of variance accounted by each of the first six principal components were 40.70, 11.94, 8.19, 6.62, 6.31 and 4.93% respectively. The PC1 accounted for 40.70% of the variation which was associated with plant height, days to tasseling, days to silking, flag leaf width, number of grain ear^{-1} and grain yield plant^{-1} as main positive contributors. The PC2 contributed 11.94% of the variation and was positively associated with ear diameter, harvestable ears plant^{-1} and total ears plant^{-1} and negatively associated with flag leaf length and width. The PC3 accounted for 8.19% of the variation and was positively attributed to days to silking, ear length, flag leaf length, grain ear^{-1}, total ears plant^{-1} and tolerance days.

The PC4 explained 10.02% of the variation and was mainly attributed positively to grains ear^{-1} and negatively associated with total ears plant^{-1} as the major contributors. The PC5 accounted for 6.31% of the variation which was associated with flag leaf length and width as the main positive contributors and negatively associated with number of grains row^{-1}. The PC6 explained 4.93% of variation among genotypes attributed positively to herbicide tolerance while negatively associated with ear height and days to physiological maturity contributed negatively.

M_4 generation principal component analysis results (Table 7) revealed that the first seven components with eigenvalues greater than one accounted for 71.28%. The proportion of variance accounted by each of the first seven principal components were 24.93, 10.10, 9.11, 8.74, 7.31, 5.63 and 5.46% respectively. The PC1 accounted for 24.93% of the variation which was associated with grain yield plant^{-1}, plant height and grains ear^{-1} as main positive contributors while days to tasseling, silking, anthesis and pollen shedding were the main contributors in the negative direction. The PC2 contributed 10.10% of the variation and was positively associated with ear diameter and length, total and harvestable ears plant^{-1} and negatively associated with flag leaf length and width. The PC3 accounted for 9.11% of the variation and was negatively attributed to days to silking and tasseling, ear length, flag leaf length, grain ear^{-1} and tolerance days.

The PC4 explained 8.74% of the variation and was mainly attributed positively to grains ear^{-1} and negatively associated with total ears plant^{-1} as the major contributors. The PC5 accounted for 7.31% of the variation which was associated with flag leaf length and width as well as harvestable ears plant^{-1} the main positive contributors. The PC6 explained 5.63% of variation among genotypes attributed positively to herbicide tolerance while negatively associated with days to physiological maturity. PC7 accounted for 5.46% of the total variation attributed by tolerance days as the main negative contributor.

Cluster analysis

Cluster analysis of the 39 M_3 and 37 M_4 maize lines and

Table 7. Principal Component Analysis of various agro-morphological traits in M_4 maize lines.

Parameters	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	5.485	2.221	2.005	1.923	1.608	1.238	1.200
% total variance	24.93	10.10	9.11	8.74	7.31	5.63	5.46
Cumulative variance %	24.93	35.03	44.14	52.88	60.19	65.82	71.28
Factor loading by various traits							
Days to anthesis	**-0.28**	0.17	0.22	0.01	0.12	-0.15	-0.05
Days pollen shedding	**-0.27**	0.15	**0.28**	-0.03	-0.02	-0.15	-0.05
Days to silking	**-0.30**	-0.04	**0.25**	0.08	0.04	-0.09	0.04
Days to tasseling	**-0.30**	0.06	0.18	0.06	-0.04	0.02	0.05
Ear diameter	0.19	**0.36**	-0.09	0.06	0.04	-0.03	0.16
Ear length	0.21	**0.77**	**0.47**	0.16	0.04	0.02	-0.17
Flag leaf length	0.12	**-0.38**	**0.31**	-0.15	**0.33**	-0.01	0.16
Flag leaf width	0.24	**-0.28**	0.12	-0.13	**0.26**	-0.12	0.02
Grain yield plant^{-1}	**0.28**	0.15	-0.02	0.04	-0.21	0.20	-0.03
Harvestable ears plant^{-1}	0.13	**0.43**	0.14	0.02	**0.35**	-0.05	-0.10
Grains ear^{-1}	**0.25**	-0.14	**0.26**	**0.32**	-0.21	0.09	-0.05
Days to maturity	-0.21	0.09	0.00	0.14	0.01	**-0.43**	0.08
Plant height	**0.26**	0.10	0.11	-0.15	0.23	-0.22	0.16
Total ears plant^{-1}	0.05	**0.29**	**0.39**	**-0.35**	-0.13	0.09	0.03
Tolerance days	-0.18	-0.07	**0.26**	-0.11	0.06	**0.31**	**-0.41**

the check based on the standardized values of agronomic traits was performed by Unweighted Pair Group Method with Arithmetic mean (UPGMA) method and dendrograms constructed (Figures 1 and 2). The resulting dendrogram of M_3 lines revealed three main clusters (I, II and III) at a genetic distance of 0.77. Cluster I comprised the check (H520), cluster II contained line 513-12 while cluster III had the rest of the test lines.

Similarly, cluster analysis of M_4 grouped the evaluated lines into three clusters. Cluster I contained the check (H520), cluster II had line 513-12-4 while cluster III had the rest of the test lines.

DISCUSSION

Plant genetic resources plays an important role in sustainable agriculture and food supply, especially after shortages due to the increasing population and global climate change. Utilization of plant genetic resources (PGR) is one of the important tool in sustainable methods of crop improvement. Conservation and utilization of plant genetic resources are valuable to meet future needs and rising concern of food security (Shehzad and Okuno, 2014). This is aimed at alleviating food crisis through new crop release that are tolerant to biotic and abiotic stresses that retards crop growth hence impinging on overall yields (Jaleel et al., 2009).

Mutation breeding involves genetic improvement of crops including maize for various economic traits through the use of induced mutations achieved by chemical or physical treatments followed by selection for heritable changes of particular genotypes for genetic enhancement of crop plants (Kazi, 2015) especially in traits with very low genetic traits variation level (Abtahi and Arzani, 2013). This results in improved yields and enhanced quality of the novel varieties for subsequent use in breeding, improved harvest index from heterosis in hybrid cultivars, increased response to agronomic inputs, and consumer preference (Roychowdhury and Tah, 2013).

Agronomic traits variation in M_3 and M_4 maize lines

Plant height

There were considerable differences among the M_3 and M_4 maize lines for the trait. Tallness in maize is an important factor that has direct effect on inter-nodal length, ear height, number of leaves and number of ears which directly impacts on yield (Okuyama et al., 2004). Plant height is highly controlled mostly by genetic makeup and to a larger extent by environmental factors (Anderson et al., 2019; Szoke et al., 2002; Zsubori et al.,2002). However, tall plants have been prone to lodging and low yielding as the limited assimilates are competed for somatic cells enlargement resulting in luxuriant vegetative growth and increased plant height in rice (Oladosu et al., 2014). Short plants are exposed to natural a predator which devours maize in the field and low photosynthates lowering overall yields.

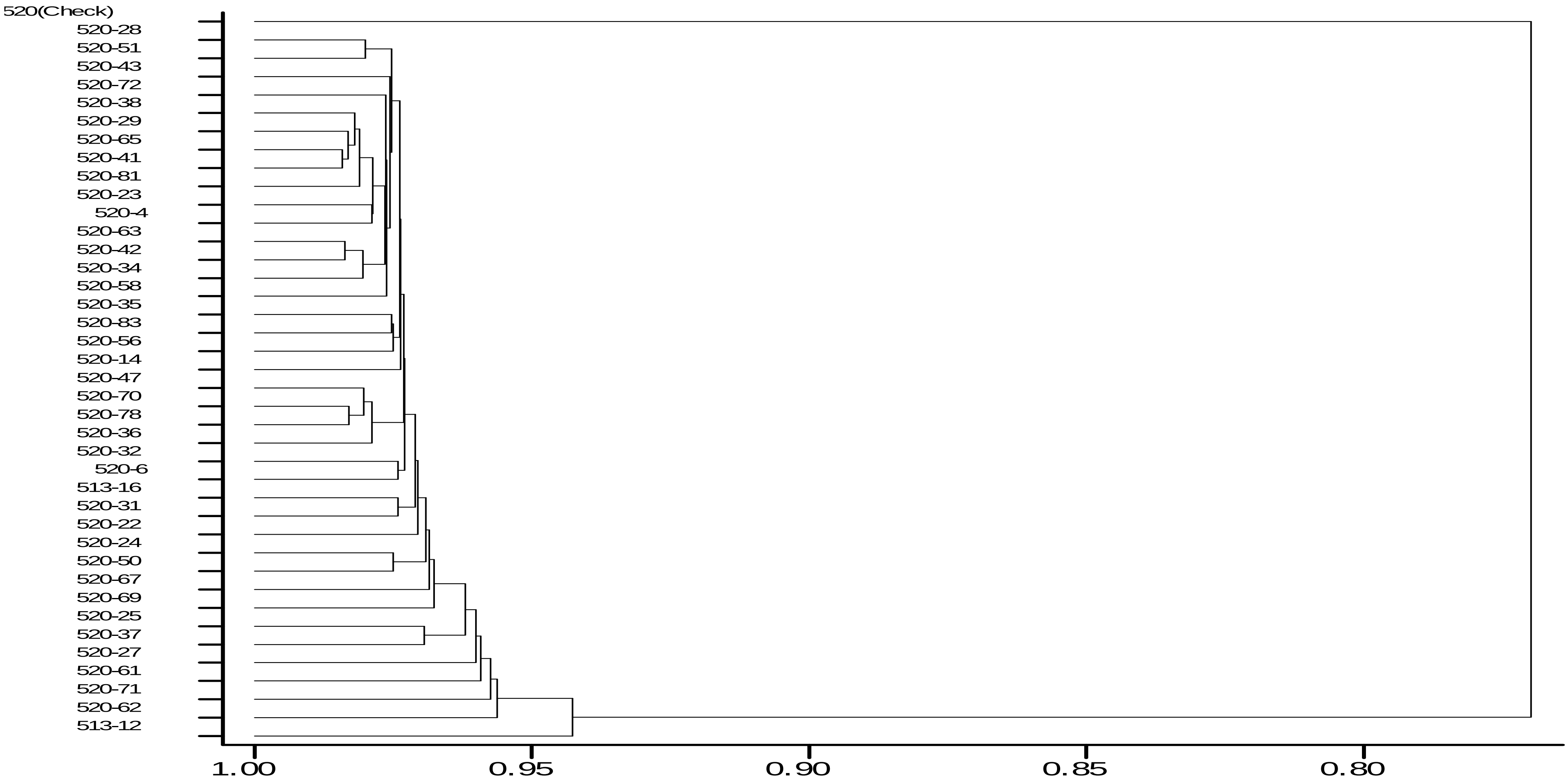

Figure 1. Phenetic dendrogram generated using morphological data of 40 maize lines depicting their relationships based on UPGMA clustering comparisons.

Therefore, the breeder should systematically and carefully select lines with intermediate plant height to minimize lodging and competition of photosynthates enhancing yields. This would help farmers to overcome challenges in bridging the structural production deficit in Kenya.

Ear diameter

The evaluated 39 M_3 and 37 M_4 lines showed variability with respect to ear diameter. Ear diameter is a crucial trait in maize since it determines the number and weight of grains that can be supported by the ear without competition for food hence an attempt should, therefore, be made for an effective selection of this trait.

Existence of variations among the lines for this trait could provide the maize breeder with opportunity to develop varieties with desired ear

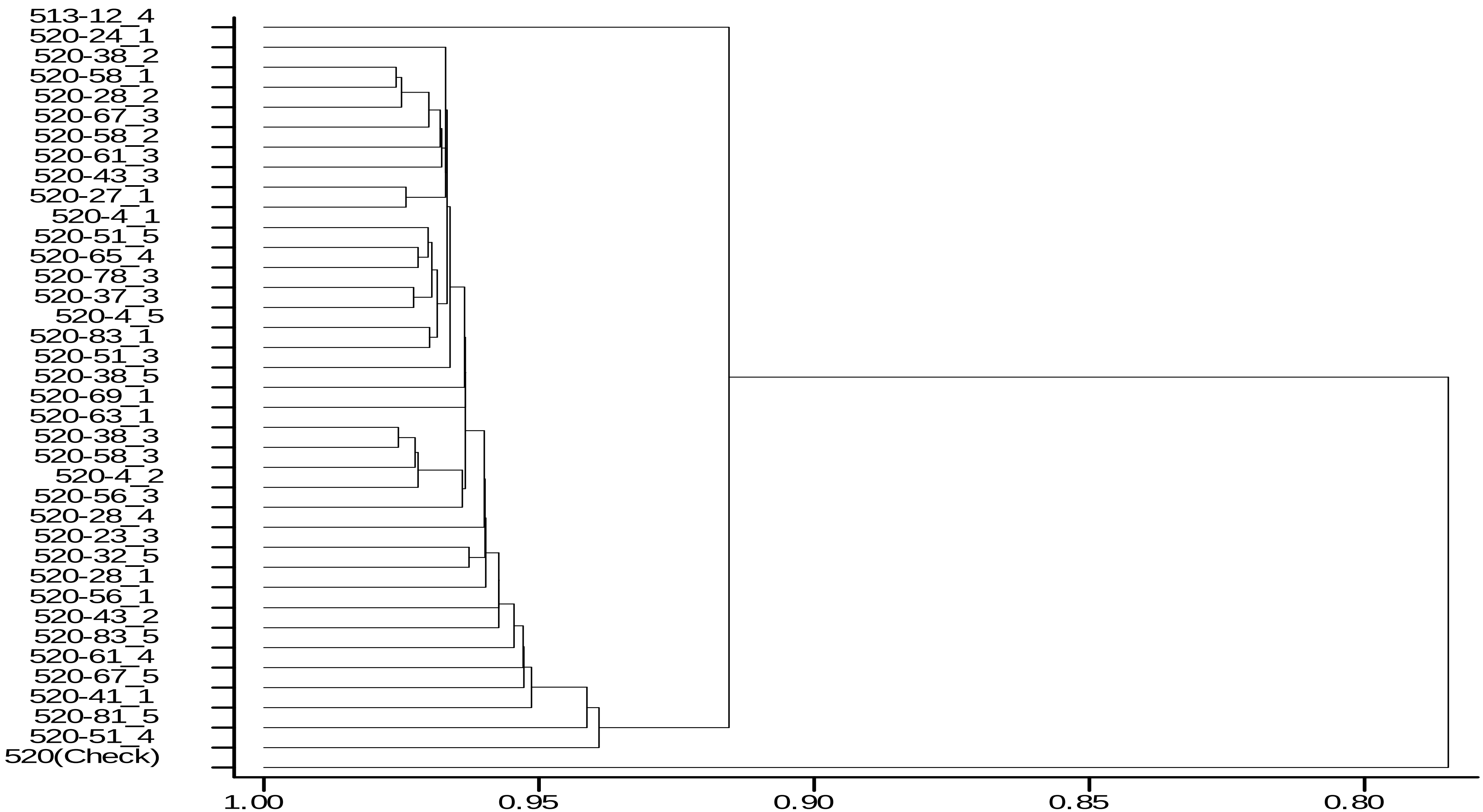

Figure 2. Phenetic dendrogram generated using morphological data of 38 M_4 maize lines depicting their relationships based on UPGMA clustering comparisons.

diameter. Similarly, Kashiani et al. (2010) and Rahman et al. (2015) reported similar findings gleaned in support of the present study results.

Days to anthesis and pollen shedding

Earliness in anthesis is very crucial in maize production particularly in arid and semi-arid regions, because earliness enables the maize lines to escape from late occurring biotic and abiotic stresses. The characterized 39 M_3 and 37 M_4 maize lines varied for number of days to anthesis and days to pollen shedding (Tables 2 and 3). In the current results, maize lines could be grouped into early, moderate and late in days to anthesis and pollen shedding. Likewise, Shrestha (2014), Malik et al. (2011), Shamim et al. (2010) and Singh and Chauhan (2010) reported significant variations among maize lines for days to anthesis and pollen shedding stages. However, Ali et al. (2015) reported non-significant differences among maize hybrid lines for days to pollen shedding. Thus, lines with moderate flowering and considerable grain yield plant^{-1} can be exploited for future breeding program along with the existing landraces having desirable other traits.

Days to silking

Days to silking along with other traits are usually utilized by plant breeders as basis of determining maturity in maize (Rahman et al., 2012; Peiris and Hallauer, 2005) whereby in semi-arid regions with short growing seasons and in high potential areas with multiple cropping seasons, the early silking varieties are the most desirable.

Previous studies by Vega et al. (2002) indicated that mutations reduce the duration of the juvenile vegetative phase of development and cause early flowering. This results in short to medium statured plants with early flowering allowing the variety to escape late drought experienced in semi-arid areas.

Days to tasseling

The mean for days to tasseling among the lines showed variability. Days to tasseling is an important agronomic trait in maize with late tasseling lines contributing to higher grain yield plant^{-1}. Similarly, many researchers (Hussain and Hassan, 2010; Shah et al., 2012; Reddy et al., 2012; Baqa et al., 2014; Shrestha, 2014; Kinfe and Tsehaye, 2015) concurred underscoring the idea that days to tasseling was important in maize breeding.

Herbicide tolerant maize experiences early but reduced pollen production due to shorter tassels and fewer tassel branching resulting in reduced kernel set. This problem worsens under drought and low N stress conditions. Thus, shorter and lighter tasseled genotypes are recommended to improve grain yield without compromising on tassel size to ensure sufficient pollen availability, especially under stress environments (Dari et al., 2017).

Flag leaf length and width

M_3 and M_4 lines showed significant differences for flag leaf width and length (Tables 2 and 3). The size of flag leaf could probably be genetic which has direct effect on yield plant^{-1} making it an important trait in selection and improvement of maize. Flag leaf usually provides the main sources of photosynthesis utilized in reproduction and grain filling period. The identified lines with broader and longer flag leaf probably could benefit in the process of photosynthesis due to larger leaf surface area and more light interception. Similar findings were reported by Dere and Yildirim (2006) and Singh (2011) who concluded that flag leaf contributes most of the assimilates stored in grains in poaceae like wheat and barley compared to other leaves.

Therefore, genetic manipulations geared towards alteration of flag leaves with optimal shape and size for efficient light trapping resulting to faster growth and improved crop performance through improved photosynthesis that would be quite amenable by improving photosynthetic efficiency (Mathan et al., 2016).

Days to physiological maturity

Earliness is one of the prime breeding aims in maize as most farmers generally seek for early maturing varieties in order to enable the crop to mature within the growing season and give optimum yield, allowing escape from abiotic and biotic stresses and multiple seasons cropping facilitation. The current study showed considerable differences among the 39 M_3 and 37 M_4 lines in maturity (Tables 2 and 3). The lines differed in maturity can be classified into early, medium and late in maturity. Days to maturity was probably attributed to genetic make-up, environmental factors and mutagenic effects. Medium maturing lines could be utilized in parentage selection for further hybridization and improvement of maize in Kenya. Usually, early maturing varieties are shorter and late maturing ones are taller (Garba and Namo, 2013), thus, the maize breeder ought to decide either to select lines for earliest maturity or high yielding lines. Thus, development of hybrids with better uniformity in maturity could allow for further incorporation with other favorable traits making the hybrid, composite or synthetics better adapted to different habitats especially length of growing season, machine harvesting, herbicide tolerance and weeding (Nzamu, 2018). Hybrids have also been developed to increase maize yield in many other areas of the world. Similar results were reported by Baqa et al. (2014), Ghimire and Timsina (2015b), and Kinfe and Tsehaye (2015). However, Mourice et al. (2014) demonstrated non-signifi-cant variation for maturity among evaluated maize lines.

Number of grains ear^{-1}

The number of grains ear^{-1} of the characterized maize lines showed significant differences among the 39 M_3 and 37 M_4 lines (Tables 2 and 4). This could possibly be as a result of varietal differences among the maize lines evaluated. The maize breeder could exploit the genetic variability to select and breed for novel varieties, synthetics or composites with higher grains ear^{-1} for higher grain yield improvement in future. Similarly, Tulu (2014) revealed that the trait plays a vital role in enhancement of grain yield and a lot of emphasis should be given to this trait during selection. Seka et al. (2019) elucidated that large number of grains ear^{-1} results from delayed leaf aging, enhanced plant growth rate especially around silking period, increased dry matter accumulation in developing grains alongside better radiation use efficiency during the grain filling period for attainment of higher grain yield plant^{-1} contributed more by the trait than 100 seed grain weight at optimum plant density. However, Singh and Chauhan (2010) reported non-significant variations among the genotypes for this trait.

Herbicide tolerance days

There were highly significant differences among the assessed 39 M_3 and 37 M_4 lines for herbicide tolerance (Tables 2 and 3) respectively. The lines varied in glyphosate tolerance due to mutagenic effects. The lines could be categorized into highly tolerant, medium and low tolerant. Maize breeders could utilize the most tolerant lines to develop herbicide tolerant hybrids, synthetics or composites leading to excellent weed control measure. This would enhance yields, reduce environmental pollution, reduce soil compaction, low cost of production and enhance soil microbial activities (Powell et al., 2009; Rizwan et al., 2015). Likewise, Forlani and Racchi (1995) reported significant differences among the maize lines to different concentrations of glyphosate.

Grain yield plant^{-1}

Grain yield plant^{-1} is a quantitative trait and the result of various physiological and biochemical processes in the plant. Grain yield plant^{-1} is one of the main criteria for identifying and selecting superior varieties for release to the farmers. The present results showed highly significant differences among characterized 39 M_3 and 37 M_4 maize lines for grain yield plant^{-1} (Tables 2 and 3). The trait could be categorized into high, medium and low yielding cultivars. Higher grain yield plant^{-1} among the progenies than the parental lines in the breeding programmes is the main aim for the development of high yielding varieties. Higher grain yield plant^{-1} among the maize lines indicates the potential of specific lines to convert the photosynthates into dry matter. Plant breeder could utilize the valid morphological differences indicating existing inheritable genetic variability among the maize lines already existing in the material to facilitate and maintain long term future breeding programmes (Ahmad et al., 2011; Sharma et al., 2014). However, Singh and Chauhan (2010) reported non-significant differences for maize lines for grain yield.

Moreover, Nadolska-Orczyk et al. (2017) revealed that mutation plays a vital role in enhancement of grain yield and a lot of emphasis should be given to this trait during selection. This implies that the lines found to have considerable level of observable variations within the genotypes provides good opportunity for improving the trait among the tested lines geared towards maize improvement.

Correlation of yield and herbicide tolerance with other agronomic traits

Grain yield improvement can be attained through direct selection for grain yield plant^{-1} or by indirect selection through yield related characters. Characters to be considered for indirect selection of yield should be positively and significantly correlated with seed yield. The study results showed positive and significant correlation of grain yield plant^{-1} with ear diameter, flag leaf length and width, grains ear^{-1}, plant height and harvestable ears plant^{-1}. This implied that increased grain yield plant^{-1} could be due to tall plant height, enlarged ear diameter, more grains ear^{-1} and total number of harvestable ears plant^{-1}. According to Kashiani et al. (2010) and Saleh et al. (2002), characters with positive and significant correlation with yield could be used for indirect selection of high yielding genotypes without evaluating for yield *per se.* The results of Sujiprihati et al. (2002) and Mallikarjuna et al. (2003) affirmed the results in this study for plant height and grains ear^{-1}, days to tasseling, silking and maturity.

The results from this study further showed negatively significant correlation of grain yield plant^{-1} with days to tasseling, silking, maturity and tolerance days. Negative correlation of grain yield plant^{-1} with days to silking and tasseling could be due to the fact that early flowering genotypes utilize only a short period for photosynthesis and this leads to low ultimate grain yield. Overall, short growth duration gives low yields compared to medium and long growth duration. These results contradicts with those of Poudel et al. (2015) whose findings illustrated positive and significant correlation with days to silking and tasseling in maize suggesting that with more days to tasseling and silking there would be more vegetative growth and less time for reproductive growth which consequently resulting in less yield. Borrás et al. (2007), Ghimire and Timsina (2015a) and Kanagarasu et al. (2012) findings concurred in totality with the current study. Sujiprihati et al. (2002) affirmed these results for days to tasseling and silking and maturity in maize.

Grain yield plant^{-1} in M_4 exhibited significant and positive correlation with grains row^{-1}, 100 seed weight and number of leaves above upper ear, total ears plant^{-1}, harvestable ears plant^{-1} and flag leaf width. According to Abdulkhaleq and Tawfiq (2014), characters with positive and significant correlation with yield could be used in indirect selection of high yielding genotypes without evaluating yield *per se*. Similar studies in maize have reported significant and positive in hundred grain weight was positively correlated with grain yield (Zarei et al., 2012), number of spikes plant in barley (Tofiq et al., 2015), total ears plant^{-1} and 100 seeds weight in maize (Aisha et al., 2015) and leaves above upper ear significant while total ears plant^{-1} non-significant correlation with grain yield plant^{-1} in maize.

Glyphosate tolerance in M_3 was positively and significantly correlated with days to pollen shedding, tasseling and silking. This indicated that genes for higher herbicide tolerance inhibits early flowering by interference of the shikimate pathway responsible for aromatic amino acids formation (Forlani and Racchi, 1995) prolonging maturity duration which would increase days to tasseling and silking. The herbicide-tolerant maize would offer farmer's opportunities to fight weeds while preserving the topsoil, provide herbicide application flexibility, reduction in herbicide cost and to use herbicides with preferred environmental characteristics.

In M_4 lines, herbicide tolerance showed negative correlation with grain yield plant^{-1} with non-significant correlation with other traits studied (Table 7). This implied that an increase in tolerance, led to a lowered grain yield plant^{-1}. Williams et al. (2014) reported similar results for herbicide tolerance with grain yield while Kariuki et al. (2016) reported significant negative correlation for grain yield plant^{-1} in maize.

Principal component analysis

Grain yield plant $^{-1}$, plant height, days to tasseling and silking, grains ear^{-1} and flag leaf width were the most important traits contributing for the overall variability recorded among the M_3 and M_4 lines. PCA grouped together the maize lines with similar morphology which displayed that the total variation was clearly distributed throughout the agronomical traits. The effect of environmental and mutagenic effects contributed to the diversity of lines. Silking, tasseling and maturity days, grain yield plant^{-1}, flag leaf width and herbicide tolerance were the major contributors to PC1. Anjorin and Ogunniyan (2014) previously reported similar results for days to tasseling, silking and maturity, grain yield plant^{-1} and plant height in M_3 and days to maturity, silking and tasseling and grain yield plant^{-1} in M_4 respectively.

In PC 2, the major agronomic traits were those related to foliar and ear aspects. In this investigation, size has the biggest bearing to the overall genetic variability. This implies that the lines had tremendous genetic differences which may be of a great potential generic source for the future maize breeding strategies (Ndou et al., 2015).

Overall, PCA analysis of M_3 and M_4 showed great variations, caused by several agronomical traits. The presence of the variability has the potential to be used for the selection and development of productive varieties.

Cluster analysis

The present findings allowed the M_3 and M_4 lines to be classified into three distinct groups each. Cluster I in comprising of only H520 (check) in both M_3 and M_4 was characterized by tall statured plants, late tasseling and silking, long and wide flag leaf. Moreover, the lines had medium total ears plant^{-1}, harvestable ears plant^{-1}, long ears, wide ears and grain yield plant^{-1}, high grains ear^{-1} and late maturing lines.

Cluster II in both M_3 and M_4 lines comprising of H513-12 and its progeny H513-12_4 respectively were characterized by short statured plants, medium tasseling and silking, medium flag leaf length and flag leaf width and medium number of grains ear^{-1}. The lines also had medium sized ear length and narrow ear diameter, low grain yield plant^{-1} and medium maturing.

Cluster III lines constituting the rest of lines in both M_3 and M_4 respectively were characterized by medium statured, flag leaf length and width, late tasseling and silking, short ears, medium and grains ear^{-1}. Additionally, the lines had narrow ear diameter, high number of ears plant^{-1} and harvestable ears plant^{-1} accompanied with medium maturing and grain yield plant^{-1}.

Cluster I comprised the highest yielding lines, late maturing, longest cob and larger leaf area. Low yielding was grouped in cluster III and associated with early maturity, shortest plants, narrow ear diameter, shortest ear length and medium leaf area. Almost similar findings were reported previously by Chanda et al. (2014) that the highest yielding lines are those associated with longer days to maturity, longest cob and large leaf area. The lowest yielding lines were those associated with cluster 4, associated with early maturity, shortest plant height, shortest cob length, smallest leaf area and lightest kernel weight. It is also evident that the little fluctuation noted between the results from previous studies may be because of the difference in the genetic make-up of experimental materials (Iqbal et al., 2014) and change in the environmental conditions.

Therefore, it is clear from the cluster analysis that the maize lines investigated has tremendous genetic diversity which may be of great potential source for the future maize breeding strategies. Different quantitative traits preferably plant height, ear length and diameter, ears plant^{-1}, days to tasseling, silking and pollen shedding as well as flag leaf size in combination of few or more can be useful for breeding programmes.

Conclusion and Recommendation

The study reveals that there are significant differences in performance of different mutated inbred lines suggesting a substantial genetic variability among the maize lines evaluated. Therefore, any improvement of these characters would result in a substantial increment on grain yield. It also indicates that the inbred lines could be promising parents for efficient exploitation in future specific breeding programmes for herbicide tolerant hybrids development.

Therefore, maize breeders interested in generation of commercial and food maize genotypes can utilize the information generated in this study. Moreover, the maize lines generated can be advanced in similar multi-locations to test their stability.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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