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Assessment of Variation in Agro-morphological Traits in M_3 and M_4 Maize Lines

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Abstract

A total of thirty nine M_3 and thirty seven M_4 maize lines derived from mutation breeding were evaluated for variation in agro-morphological traits at Jomo Kenyatta University of Agriculture and Technology, Farm, Juja from October 2014 to September 2015 to identify desirable mutants for use in plant breeding programmes. The experimental design was randomized complete block design in triplicates. During growth data on days to anthesis and silking, flag leaf width and length, number of leaves above upper ear, harvestable ears plant^{-1}, diameter of the ear, days to maturity and grain yield plant^{-1} were recorded. Data were analyzed using Genstat 14th edition and the means separated using LSD at 5% level of significance. Results showed significant variations for grain yield plant^{-1}, herbicide tolerance days, days to anthesis and silking, pollen shedding and flag leaf width among the assessed maize lines. The recorded variations among maize lines suggested that there existed appreciable variation that could be utilized in breeding. Grain yield plant^{-1} was significant and negatively correlated with tolerance days. Grain yield plant^{-1} and herbicide tolerance cannot be improved simultaneously and the breeder should decide which trait to improve. Overall, the results indicated that induced mutation could serve as a source of variations for use in the improvement of maize.

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Introduction

Maize (*Zea mays* L.) is the third most important cereal crop in the world, after wheat and rice. It is cultivated worldwide in an estimated area of 159 million hectares with a production of 796.46 million metric tons (USDA, 2010). In Kenya, maize is the most important cereal crop where its grains serve as a staple food and livestock feed (Vanlauwe *et al*. 2008; Nyikal *et al* 2010). It is also used as an industrial raw material to manufacture different products such as corn oil, corn flakes, corn starch, tanning material for leather industry, custard, glucose, etc. (Umar *et al*. 2015).

The average maize yield in Kenya stands at 1.5 ton/ha far below its potential of 4.9 tons ha^{1}-under research conditions (Gianessi, 2014). The low productivity could be attributed to numerous constraints, among which include high population pressure, repeated land sub-division, lack of high yield varieties, poor seed quality, high cost of certified seeds and fertilizer, abiotic (such as drought, heat, low soil fertility *etc.*) and biotic (such as weeds, bacteria, viruses, nematodes, fungi and insect pests) stresses (Macharia *et al*. (2010); Friesen and Palmer (2002); Nyoro *et al*. (2007), Macauley (2015) and Makone *et al*. (2014).

Infestation of maize fields by weeds reduces crop yields due to competition with crop for moisture, sunlight, water, space and nutrients. Heavy infestation has been reported to reduce maize yields by up to 86% (Bijanzadeh and Ghadiri, 2006).In addition, weeds infestation reduces grain quality through the presence of weed seeds, harbor insects such as aphids which are common pests in maize and diseases such as grey leaf spot, maize streak and maize chlorotic mottle virus. Other weeds such as witch weed (*Striga* spp) produce chemicals which are harmful to crop plants (Allelopathy) (Loux *et al.* 2015). Overall, weeds infestation results in yield losses of up to 34% of all agricultural production (Oerke, 2006) amounting to food consumption for a billion inhabitants (Berca, 2004)

Mutation breeding is a non- transgenic approach for the generation of elite lines in plant breeding results in increased maize productivity and herbicide tolerance through genetic improvement (Rizwan *et al.* 2015).

Crop improvement by mutation of one or few genes possessing target traits results in phenotypic changes such as grain yield, tolerance to drought, herbicides, salinity, and maturity. EMS is an important tool enhancing unique agro-morphological traits in crop plants and increasing genetic variability for qualitative and quantitative traits for yield and agronomical characters (Ndou *et al*. 2015).

EMS is an important mutation breeding tool enhancing genetic variability in crop plants such as increased grain yield $plant^{-1}$, herbicide tolerance and early maturity with desired one or several identifiable quantitative and qualitative traits for yield and other agronomical traits genotypes (Grzesiak, 2001) is crucial for maize improvement.

Development of high yielding and stable varieties is the key to cereal breeding programs including maize crop. However, Paudel (2009) reported non-significant differences of yield and yield components among maize lines. However, observed variations in maize lines results from genetic and environmental causes with the former being only the heritable cause (Oladosu *et al.*, 2014). The initial step of classifying crop germplasm is through Morphological characterization by use of quantitative traits giving an estimate in genetic diversity providing basis for further molecular characterization as reported by Smith and Smith (1989)and Bayahi and Rezgui (2015).

An understanding of the relationship between yield and other agronomic characters is important for future maize improvement programmes. Earlier studies indicated that yield plant $^{-1}$ displayed positive and highly significant correlation with vital traits such as plant height, ear height, ear diameter, number of kernels row $^{-1}$, 100 seed weight and number of kernel rows ear^{-1} (Rafique *et al.*, 2004). The authors further suggested that the aforementioned traits are the most key yield determinative characters. This implies selection for any one of such characters might result in an improvement in grain yield $plant^{-1}$. Other studies have demonstrated that a positive and significant correlation of yield plant^{-1} with number of kernels row^{-1} (Prakash *et al*. 2006); yield plant^{-1} with cob length (Sreckov *et al*. 2010); with cob girth (Manivannan 1998) and (Chinnadurai and Nagarajan 2001) and yield plant^{-1} with 100-seed weight (Satya-narayana *et al*. 1990; Chinnadurai and Nagarajan 2001). Yet in other studies, yield plant^{-1} has been reported to be negatively and significantly correlated with days to 50% anthesis and days to 50% silking (Kumar *et al*. 2011).

The aforementioned reports indicate that different studies differ with respect to variability and correlations among various traits, which could be attributed to the use of different genotypes. Thus there is a need to determine the relationship between yield and other agronomical traits in the populations being studied.

The objectives of the current study were therefore to determine variation in agro-morphological traits and analyze the correlation between yield plant^{-1}andother agronomic traits in herbicide tolerant maize lines.

Materials and methods

Experimental Siteand Soil Characteristics

The study was conducted in Juja, Jomo Kenyatta University of Agriculture and Technology Farm, from March 2015 to January 2016 under rain-fed conditions and supplemental irrigation when necessary. Juja is located 36 km North-East of Nairobi along the Thika-Nairobi highway.

It lies between latitudes 3°35"S and Longitudes of 36º35"E (GoK 1997). Juja is located in the Upper Midland Zone Four 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 (Muchena *et al*. 1978; Wanjogu and Kamoni 1986). The area receives an annual rainfall of 856mm with a bimodal distribution (Kaluli *et al*, 2011). The area has three types of soils namely, shallow clay over trachytic tuff, very shallow sandy clay over murram and deep clay (Vertisols) soils (Batjes, 2006).

Plant materials

Thirty nine (39) M_3 and 37 M_4 maize lines and a check were used in this study and were developed as follows: In August 2013, two maize hybrids namely H513 and H520, were bought from the Kenya Seed Company. About 5,000 maize kernels of each of the two maize varieties were mutagen zed using the procedures reported for wheat by Newhouse *et al*., 1992 and for sorghum by Ndung'u (2009) with minor modifications. Maize kernels were soaked in water for 12 hours at room temperature, dried in tissue paper and then placed for 6 hours in jars containing 250 ml of 0.1% EMS solution which completely immersed the seeds. The mutagen zed seeds were washed under running tap water for 10 minutes to eliminate the mutagen and dried on paper towels.

The mutagen zed seeds (M_0) were then planted in the field in JKUAT. The M_1 plants arising from these seeds were self- pollinated at anthesis and M_2 seeds bulk harvested from them at maturity. The grains were threshed and about 1.8 million M_2 seedlings drilled in furrows. Two weeks after emergence, the seedlings were sprayed with 1% glyphosate 480 SL using a knapsack sprayer. All susceptible plants died within two weeks after spraying. The surviving plants were allowed to continue growth, self- pollinated at anthesis, and each plant harvested singly at maturity to give M_3 seed. Only plants producing fifteen (15) or more seeds per cob were considered for further evaluation. The M_3 seed from each plant was divided into two portions: one portion to screen for herbicide tolerance and the other portion for agro-morphological characterization and advancement to the next generation. During characterization of M_3 lines, agronomic ally desirable plants were selected for evaluation in the M_4 generation.

The M_4 seed from each selected plant was divided into two portions: one portion to screen for herbicide tolerance and the other portion for agro-morphological characterization.

Treatments and experimental design

Screening for herbicide tolerance

Thirty nine (39) M_3 and 37 M_4 lines were used in this experiment. The M_3 lines were evaluated during the period March to August, 2015 in a randomized complete block design (RCBD) with three replications and a check variety (H520) while the M_4 lines were evaluated in September to October 2015 using randomized complete block design with two replications and a standard check variety. A random sample of 5-50 seeds from each line was drilled in single rows in furrowsof1m long. Ten days after emergence, the seedlings were sprayed with x1 glypho sate with scoring starting from 4^{th} day after spraying, the seedlings were scored for tolerance to the herbicide. The visual symptoms on susceptible plants commenced with yellowing, followed by browning, wilting and eventually death. Data on the number of days taken from spraying to eventual death of all plants in a row was recorded and used as a measure of tolerance.

Morphological characterization

Thirty nine (39) M_3 and 37 M_4 lines were used in this experiment. The M_3 lines were evaluated during the period March to August 2015 while the M_4 lines were evaluated in September 2015 to January 2016 using a randomized complete block design with three replications and a standard check variety. Each line was sown in a single row, 5 m long at inter- and intra-row spacing of 75 by 30 cm, respectively. Standard agronomic practices such as fertilization, weeding and pesticide applications were followed as per recommendations for the site. Supplemental irrigation was applied when necessary. During growth, five plants were randomly sampled from each row and tagged on which various agronomical data were recorded.

Data Collection and Analysis

Data were recorded from each experimental plot. Five plants were randomly selected from the middle of the row and pre-tagged. Data on various agro morphological characters were recorded on the pretagged plants at various stages of growth as follows:

Number of Days to Anthesis (DA):

The number of days from emergence to 50% of the plants in a line shed pollen.

Number of days to Silking (DTS):

Number of days from emergence to when 50% of plants have silked.

Days to pollen shedding (DPS):

Number of days from emergence to 50% pollen shedding was recorded when pollen shedding starts after dehiscence on central branch.

Flag leaf width (FLW):

Measured in cm using a caliper ruler at the middle of the leaf

Flag leaf length (FLL):

Measured in cm using a caliper ruler from the start of the sheath to leaf apex

Leaf above upper ear (LUE):

Number of leaves above the upper ear.

Diameter of the Ear (DOE):

Measured in cm using a caliper ruler at the middle of the cob.

Harvestable Ears Plant $^{-1}$ (HEP):

Number of ears with kernels.

Number of Days to maturity (DM):

Number of days from emergence to date when the husks changed from green to tan colour

Grain yield plant $^{-1}$ (g) (GY):

Measured in gram using electronic balance.

Tolerance days:

It was counted from the fourth day after spraying to eventual death or constant number of plant(s) survival in a line.

The mean of various agro-morphological characters over three replications was computed for each maize line and analyzed statistically. Data analysis was carried out using Genstat 14^{th} edition (Genstat Release 14.1) and the mean separated using LSD at 5% level of significance. Correlation analyses among the mean values of agro-morphological traits were conducted using the formula illustrated by Ahmad *et al.* (2010).

Results

Variation of Traits

Analysis of variance showed that all traits in the M_3 (Table 1) and M_4 (Table 3) generations were significantly different except number of leaves above the upper ear and number of harvestable ears plant^{-1}. Performance of M_4 lines Relative to their M_3 Parents.

Grain Yield and Yield related Characters

Number of Days to Anthesis (DA)

The mean values among the characterized M_3maize lines ranged from 63.0days to 97.0days with a grand mean value of 90.58days. The earliest days to anthesis was recorded in maize lines 520-56 (85.33days), 520-43 (86.0days) and 520-25 (87.5days) while 520-69 (96.33days was the latest. All the test lines had higher values than the check variety (63.0 days) (Table 2).

In evaluated M4 lines, the number of days to anthesis ranged from 85.7 days to 100.6 days with a grand mean value of 94.44days.

The earliest anthesis was recorded in line 520-51_5 (85.7 days) followed by 520-58_2 (87.4 days) and 520-51_3 (88.9 days) while the latest 50% anthesis was recorded in line 520-69_1 (100.6 days). (Table 4). All the test lines reached anthesis later than the check variety (88.5 days).

Number of Days to Pollen Shedding

The mean number of days to pollen shedding among the M_3 lines ranged from 60.33 days to 95.33 days with a grand mean value of 87.67 days. The earliest number of days to pollen shedding occurred in the check variety (60.33 days) followed by lines 520-56 (81.0 days), 520-67 (82.0 days), 520-43 (83.0 days) and 520-61 (83.0 days).

Maize line 520-31 (95.33 days) was the latest in 50% pollen shedding (Table 2). The latest number of days to pollen shedding were recorded in maize lines 520-34 (97.00 days) and 520-69 (96.33 days) (Table 2).

The mean values of days to pollen shedding for the 37 M_4 lines varied from 73 days to 92.33 days with a grand mean value of 79.7 days. Maize line 520-83_1 took the shortest time (73.00 days) to pollen shedding followed by lines 520-4_1 (73.5 days), 520-41_1 (73.5 days) and 520-51_4 (75.5 days) compared to the check variety (74.3 days). Maize line 520-61_4 (92.33 days) and 520-61_3 (88.7) attained the latest days to pollen shedding (Table 4).

Number of Days to Silking (DTS)

The mean number of days to silking among the evaluated M3 lines ranged from 72.20 days to 92.47 days with a grand mean value of 96.44 days. The earliest silking days were recorded in maize lines 520-61 (91.98 days), 520-25 (92.35 days), 520-56 (92.47 days) and 520-43 (93.47 days) compared to the check variety (76.13 days). The latest days to silking were recorded in maize lines 520-31 (102.75 days) and 520-71 (102.33 days) (Table 2).

The mean value of days to silking for 37 M4 lines 80.2 days to 103.5 days with a grand mean of 94.6 days. The earliest days to silking were recorded in maize lines 520-51_3 (85.7 days), 520-23_3 (87.4 days), 520-83_5 (88.4 days) and 520-38_5 (89.7 days) compared to the check variety (81.6 days). The latest days to silking were recorded in maize lines 520-38_3 (100.6 days) and 520-24_1 (81.6 days) (Table 4)

Flag Leaf Width (FLW)

The trait flag leaf width among the M_3maize lines ranged from 2.5cm to 11.34cm with a grand mean of 6.17cm. The highest flag leaf width was recorded in lines 520-35 (7.50cm), 520-56 (7.40cm) and 520-43 (7.27cm) and 520-34 lower than the check (10.74cm). However, the lowest flag leaf width was recorded in maize lines 520-37 (4.43cm) followed by 520-61 (4.58 cm) and 520-23 (5.18cm) (Table 2).

In the M_4lines, the mean values of flag leaf width ranged from 3.0cm to 8.4cm with a grand mean of 5.7cm. The highest flag leaf width was recorded in the lines 520-51_5 (7.3cm), 520-23_3 (6.55cm) and 520-4_5 (6.53cm) which were lower than for the check variety (9.8cm). The lowest flag leaf width was recorded in maize lines 520-83_1 (4cm). (Table 4).

Flag Leaf Length (FLL)

The flag leaf length mean values among the M_3 maize lines ranged from 17.5cm to 49.7cm with a grand mean value of 35.70cm. The longest flag leaf length was recorded in maize lines 520-71(46.52cm), 520-56 (45.63cm), and 520-83 (42.65cm) while the check variety had 46.53cm. The lowest flag leaf length was recorded in maize line 520-78 (26.53cm) (Table 2).

The flag leaf length mean values among the M_4 maize lines ranged from 17.33cm to 47.5cm with a grand mean value of 34.24cm. The longest flag leaf length was recorded in lines 520-78_3 (45.45cm), 520-4_2 (43.37cm), and 520-38_5 (40.75cm) while the check variety had 42.37cm. The lowest flag leaf length was recorded in maize line 520-38_3 (22.97cm) (Table 4).

Number of Leaves above upper Ear (LUE)

In the M_3 lines there were non-significant variations among the maize lines for number of leaves above the upper ear. The mean values ranged from 6.77 to 9.0 with a grand mean of 6.47. The highest number of leaves above the upper ear were recorded in maize lines 520-29 (7.67), 520-69 (7.6), 520-23 (7.53) and 520-62 (7.53) respectively compared with check variety (4.50). The lowest number of leaves above upper ear were recorded in maize lines 513-12 (5.67) and 520-22 (5.93). Similarly, in the M_4 maize lines, there were non-significant differences among the maize lines. The mean values ranged from 5.3 to 8.2 with a grand mean of 6.5. The highest number of leaves above upper ear were recorded in maize lines 520-24_1 (7.3), 520-81_5 (7.3), 520-61_1 (6.9) and 520-56_3 (6.9) respectively compared with check variety (8.2) (Table 4). The lowest number of leaves above upper ear were recorded in maize lines 520-83_5 (5.3) and 520-65_4 (5.7) respectively. (Table 4)

Harvestable ear Plant $^{-1}$ (HEP)

The number of harvestable ear plant^{-1}in M_3 maize lines ranged from 1.06 to 1.73 with a grand mean value of 1.42. The highest mean values were recorded in maize lines 520-4 (1.73), 520-43 (1.73), 520-51 (1.73) while the check variety had 1.47.

The lowest number of harvestable ear plant^{-1} was recorded in line 520-25 (1.06) (Table 2). Likewise, among the 37 M_4 maize lines, the number of harvestable ear plant^{-1} ranged from 1.0 to 1.7 with a grand mean value of 1.23. The maize lines with the highest values were 520-67_3 (1.70), followed by 520-4_3 (1.60), and 520-41_1 (1.50) and 520-4_1 (1.5) while the check variety had 1.3.The lowest number of harvestable ear plant^{-1} was recorded in lines 520-63_1, 520-28_2, 520-58_2 and 520-61_1 with mean values of 1.0 (Table 4).

Days to Maturity (DM)

The mean values of number of days to maturity among the evaluated M_3 maize lines ranged from 122 days to 165 days with a grand mean of 147.6 days. The earliest maturity time was recorded in lines 520-67 (133.0 days), 520-28 (139.3 days), and 520-25 (140.7 days) while the check variety recorded 122 days. The latest maturing maize line was 520-24 (165.0 days) (Table 2). In the M_4 lines, days to maturity ranged from 121.50 days to 163 days with a grand mean value of 148.5 days. The earliest maturing maize lines were 520-81_5 (131.5 days), 520-41_1 (133.03 days) and 520-81_1 (134.5 days) values that were later than for the check variety (121.5days). The latest maturity was recorded in line 520-28_1 (163.0days) (Table 4).

Diameter of the Ear (DOE)

The diameter of the ear among the M_3 maize lines ranged from 2.99cm to 4.26cm with a grand mean value of 3.94cm. The widest diameter of the ear was recorded in maize lines 520-58 (4.26cm), 520-51 (4.21cm) and 520-63 (4.18cm) values which were lower than for the check variety (4.44cm). The narrowest diameter of the ear was recorded in maize line 513-12 (2.99cm) (Table 2). In M_4 lines, the diameter of the ear varied from 3.53cm to 5.95cm with a grand mean value of 4.13cm. The widest diameter of the ear was recorded in maize lines 520-51_4 (5.95cm), 520-28_2 (4.45cm) and 520-51_5 (4.37cm) while the check variety recorded 6.95cm. The narrowest diameter of the ear was recorded in maize line 520-4_3 (3.53cm) (Table 4).

Grain Yield Plant-1 (GY)

Grain yield plant^{-1}among the evaluated M_3 maize lines ranged from 34.6g to 116.2g plant^{-1} with a grand mean value of 82.1g. The highest grain yield plant $^{-1}$ was recorded in maize lines 520-58 (116.2g), 520-61 (115.6g) and 520-28 (109.8g) which was lower than for the check variety (165.3g). The lowest grain yield plant^{-1} was recorded in lines 513-12 (34.6g) (Table 2). For M_4 lines, grain yield plant^{-1} ranged from 61.20g to 188.7g plant^{-1} with a grand mean of 94.42g. The check variety recorded the highest grain yield plant^{-1} (188.7g) while the highest test lines were lines 520-38_3 (151.5g), 520-51_5 (146.9g) and 520-78_3 (137.1g). The lowest grain yield plant^{-1} was recorded in line 520-56_3 (61.2g) (Table 4).

Herbicide Tolerance Days

Herbicide tolerance days among the M_3 maize lines ranged from 11 days to 23.67 days with a grand mean value of 17.18 days. The highest tolerance was recorded in maize lines 520-42 (23.67 days) followed by 520-25 (22.67 days) and 520-63 (22.67 days) while the check variety recorded 6.00 days. The lowest herbicide tolerance days was recorded in line 520-81 (11 days) (Table 2). For the M_4 maize lines, herbicide tolerance days ranged from 10 to 28.5 days with a grand mean of 26.5 days. The highest tolerance lines were recorded in 520-38_3 (28.5 days), 520-38_5 (27.31 days) and 520-28_4 (26 days) with check variety recording 7.00 days. The lowest tolerance was recorded in maize lines 520-83_5 (13.5 days) and 520-23_3 (14.31 days) (Table 4).

Correlation of Traits

The findings on correlation analysis are presented in tables 5 and 6 for M_3 and M_4 lines, respectively. For brevity, only correlations involving grain yield and herbicide tolerance days are reported in the text.

In the M_3 lines, grain yield plant^{-1} showed positive and highly significant ($p < 0.01$) correlation with diameter of the ear ($r = 0.68^{**}$) and days to anthesis ($r=0.24^{**}$) and significant and positive correlation with flag leaf width ($r = 0.36^{*}$) and harvestable ear plant^{-1} ($r = 0.35^{*}$). Grain yield plant^{-1} was negatively and significantly correlated with herbicide tolerance days ($r = - 0.38^{*}$), days to silking ($r = - 0.76^{**}$), days to pollen shedding ($r = -0.67^{**}$) and days to maturity ($r = - 0.47^{**}$), while non-significantly correlated with the other characters (Table 5). Likewise in the M_4 lines, grain yield plant^{-1} displayed a positive and highly significant ($p < 0.01$) correlation with number of leaves above upper ear ($r = 0.42^{**}$), significant and positively correlated with harvestable ears plant^{-1} ($r=0.28^*$), negatively and significantly correlated with tolerance days ($r= -0.27^{**}$) but was non-significantly correlated with other traits (Table 6). In the M_3 lines, there was a positive and highly significant ($p < 0.01$) correlation between herbicide tolerance days with the days to anthesis ($r = 0.24^{**}$), days to pollen shedding ($r = 0.50^{**}$) and days to silking ($r=0.52^{**}$). Results further showed grain yield plant^{-1} was highly significant and negatively correlated with ear diameter. Tolerance days revealed negative correlation with grain yield plant^{-1} ($r= - 0.27^{*}$), ear diameter ($r= -0.40^{**}$), flag leaf length ($r= -0.26^{*}$) and days to anthesis ($r= - 0.27^{*}$). Other traits in this population showed non-significant correlations (Table 5). In the M_4 lines, herbicide tolerance days showed negative correlation with grain yield plant^{-1} ($r= -0.27^{*}$) and days to anthesis ($r= -0.27^{*}$) with non-significant correlation with other traits studied (Table 6).

Table 1. Analysis of variance of yield and yield component of 39 evaluated M3 maize lines.

Characters	DF	Sum Square (SS)	Mean Square (MS)	P. Value	CV%
Tolerance days	38	1617.99	41.49	<0.001	11.1
Days to 50% silking	38	2286.05	58.62	<0.001	5.0
Days to Anthesis (50%)	38	3354.66	86.02	<.0.001	6.2
Days to Pollen Shedding (50%)	38	3735.50	95.78	< 0.001	6.6
Flag Leaf Width (cm)	38	127.716	3.275	0.004	20.7
Flag Leaf Length (cm)	38	3149.65	80.76	0.02	19.1
Leaf Number above Upper Ear	38	27.9060	0.7155	0.289	11.6
Harvestable Ear Plant $^{-1}$	38	4.0735	0.1044	0.584	23.6
Days to Maturity (80%)	38	7076.2	181.4	0.05	7.3
Diameter of the Ear (cm)	38	8.2148	0.2106	0.019	8.8
Grain Yield (g plant $^{-1}$)	38	54267.2	1391.5	0.006	32.5

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

Table 2. Mean values of yield and yield related agronomic traits of 39 M_3 evaluated maize lines.

M_3 Line	DTS	DA	DPS	FLW	FLL	LUE	HEP	DM	DOE	GY	TLD
520-4	93.93	94.00	89.67	5.67	31.6	7.40	1.73	158.67	3.95	79.30	16.00
520-6	97.28	91.67	88.00	6.57	40.47	6.50	1.13	144.00	3.82	74.60	20.33
513-12	100.67	95.00	94.67	6.50	37.28	5.67	1.22	146.00	2.99	34.60	22.00
520-14	99.52	91.00	87.67	6.67	40.38	6.27	1.48	144.33	3.65	69.00	19.67
513-16	101.20	89.50	85.50	6.49	37.35	6.70	1.30	148.50	3.84	73.30	18.33
520-22	96.60	93.67	92.33	6.23	40.27	5.93	1.07	148.33	3.67	64.80	20.33
520-23	98.13	93.67	90.00	5.10	29.47	7.53	1.67	153.00	4.15	79.90	18.33
520-24	101.35	94.33	91.00	5.54	33.57	6.35	1.22	165.00	3.86	69.70	16.67
520-25	92.35	87.50	83.83	5.18	34.84	7.02	1.06	140.67	3.83	91.50	22.67
520-27	101.87	94.00	94.00	5.20	33.96	6.80	1.20	141.33	3.22	57.40	20.33
520-28	94.87	87.67	86.67	6.83	38.13	6.47	1.47	139.33	4.01	109.80	15.00
520-29	94.97	89.33	88.33	5.25	29.63	7.67	1.50	145.00	4.09	82.90	15.00
520-31	102.75	96.00	95.33	5.70	37.82	6.87	1.32	144.00	3.94	64.60	16.67
520-32	97.82	91.67	88.67	7.00	40.11	6.74	1.27	153.00	3.76	77.90	20.33
520-34	101.40	97.00	93.33	7.03	39.83	6.77	1.67	156.67	4.09	66.80	20.33
520-35	94.93	88.33	84.00	7.50	41.3	6.73	1.47	144.00	3.93	84.10	17.33
520-36	96.78	91.33	88.67	6.30	30.62	6.04	1.40	153.33	3.81	64.10	15.67
520-37	101.47	89.50	86.00	4.43	32.00	6.37	1.08	159.17	3.61	70.60	19.67
520-38	95.80	93.33	91.33	5.37	32.00	7.47	1.60	152.00	4.26	83.50	13.00
520-41	95.07	91.33	89.33	6.33	34.13	6.93	1.33	143.67	4.01	71.00	20.67
520-42	99.00	95.67	93.67	6.40	37.23	6.80	1.47	143.67	4.04	93.50	23.67
520-43	93.27	86.00	83.00	7.27	39.37	6.98	1.73	145.00	4.11	89.90	20.00
520-47	93.73	92.67	88.33	5.53	28.37	6.80	1.47	144.33	4.15	76.10	14.67
520-50	98.10	91.33	89.33	5.97	33.38	7.03	1.23	158.67	3.88	73.10	16.00
520-51	97.40	90.67	88.33	6.17	37.13	6.67	1.73	142.00	4.21	109.10	21.33
520-56	92.47	85.33	81.00	7.40	45.53	6.13	1.40	149.33	3.95	71.20	15.67
520-58	94.87	90.67	88.00	6.03	34.13	6.33	1.40	152.00	4.13	116.20	18.00
520-61	91,98	88.00	83.00	4.58	27.21	6.69	1.39	151.33	4.07	115.60	16.00
520-62	95.83	92.00	92.33	5.37	35.23	7.53	1.53	144.67	3.88	101.20	14.67
520-63	96.60	89.33	88.67	5.60	32.17	7.13	1.48	145.33	4.18	88.50	22.67
520-65	95.93	91.00	88.33	5.80	30.33	7.40	1.27	142.67	3.92	69.10	15.00
520-67	93.53	87.67	82.00	6.45	38.18	7.05	1.70	133.00	4.14	91.70	19.33
520-69	96.33	96.33	89.33	6.03	33.13	7.60	1.53	154.33	3.87	67.50	18.67
520-70	94.93	91.33	88.33	5.80	29.27	6.33	1.53	157.67	4.08	99.00	13.33
520-71	102.33	90.00	86.00	6.97	46.52	6.13	1.20	155.00	3.73	58.40	12.33
520-72	96.07	89.67	86.67	6.07	39.33	6.83	1.47	149.67	4.16	67.70	14.00
520-78	94.07	88.00	85.00	5.50	26.53	6.60	1.47	142.00	3.91	83.00	11.33
520-81	94.87	88.67	85.00	5.53	31.13	7.40	1.53	145.33	4.13	92.80	11.00
520-83	98.67	96.00	91.67	6.76	42.65	6.82	1.40	145.67	4.13	87.00	15.00
Mean	96.44	63.00	60.33	10.74	46.53	6.47	1.47	122.00	4.44	165.3	17.18
Maximum	92.47	104.00	103.00	11.34	49.70	9.00	2.20	173.00	3.94	82.1	23.67
Minimum	72.20	90.58	87.67	6.17	35.70	6.77	1.42	147.56	4.90	6.30	11.00
Check(H520)	76.13	60.00	58.00	2.50	17.50	4.50	0.50	117.00	2.50	193.9	6.00

Note: DTS= days to 50% silking, DA= days to 50% Anthesis, DPS= days to 50% pollen shedding, FLW=flag leaf width, FLL=flag leaf length, LUE= number of leaves above upper ear, HEP=harvestable ears plant $^{-1}$, DM= days to 80% Maturity, DOE=diameter of the ear, GY=grain yield plant $^{-1}$ (g), TLD= tolerance days.

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

Characters	DF	Sum Square (SS)	Mean Square (MS)	P. Value	CV%
Days to 50% silking	36	953.61	26.48	0.05	4.1
Days to Anthesis (50%)	36	953.61	26.48	0.050	4.1
Days to Pollen Shedding	36	553.514	15.375	0.042	3.7
Flag Leaf Width (cm)	36	1738.74	48.3	0.015	14.1
Flag Leaf Length (cm)	36	39.3816	1.0939	0.027	13.8
Leaf Number above Upper Ear	36	12.1812	0.3384	0.483	9.0
Harvestable Ear Plant $^{-1}$	36	2.12312	0.05898	0.777	22.3
Days to Maturity (80%)	36	5368.62	149.13	0.05	6.2
Diameter of the Ear (cm)	36	11.74	0.3261	0.018	9.9
Grain Yield plant $^{-1}$(g)	36	37790.9	1049.70	0.040	25.6

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

Table 4. Mean values of yield and yield related agronomic traits in evaluated 37 M4 maize lines.

M4line	DTS	DA	DPS	FLW	FLL	LUE	HEP	DM	DOE	GY	TLD
520-51_4	98.50	98.5	75.5	4.20	29.40	6.7	1.2	149.0	5.95	116.6	23.00
520-83_5	98.40	98.4	76.0	5.23	39.15	5.3	1.2	151.5	4.07	104.5	13.50
520-67_5	97.55	97.6	78.5	5.25	35.90	6.5	1.1	138.0	3.93	89.1	19.00
520-4_1	91.40	91.4	73.5	5.05	36.53	6.0	1.5	161.5	3.81	98.4	22.00
520-51_3	88.90	88.9	83.5	5.50	35.31	6.7	1.4	158.0	3.587	63.1	21.00
520-65_4	97.30	97.3	76.0	6.35	40.50	5.7	1.1	159.5	4.34	96.2	21.50
520-83_1	93.00	93.0	73.0	4.00	32.05	6.0	1.2	134.5	4.23	66.2	17.50
520-23_3	96.00	96.0	78.5	6.55	39.19	6.5	1.1	151.5	3.91	108.8	14.31
520-58_1	97.00	97.0	83.0	5.60	35.15	6.8	1.1	159.0	3.90	92.0	21.50
520-51_5	94.55	94.6	78.0	5.70	34.20	6.2	1.2	145.5	4.04	90.2	22.50
520-32_5	91.90	91.9	76.0	4.20	33.05	6.5	1.1	142.5	4.32	70.9	22.00
520-41_1	99.20	99.2	73.5	5.70	33.80	6.2	1.5	133.0	4.00	74.3	17.69
520-28_2	96.30	96.3	80.5	5.70	38.66	6.5	1.3	159.5	4.45	98.4	22.23
520-67_3	90.50	90.5	81.5	5.60	34.65	5.9	1.7	150.5	4.23	98.1	23.50
520-38_5	95.10	95.1	79.5	5.15	40.75	6.5	1.3	155.5	3.58	76.3	27.31
520-28_4	98.50	98.5	84.5	6.25	31.20	6.7	1.1	154.0	4.14	79.6	26.00
5209-63_-1	96.40	96.4	82.0	6.35	34.15	6.2	1.0	147.0	4.34	65.3	24.50
520-28_2	94.70	94.7	81.0	4.35	39.27	6.3	1.0	152.5	3.97	88.7	23.50
520-28_1	92.20	92.2	79.0	5.15	26.21	6.8	1.2	163.0	4.10	99.0	16.50
520-4_5	89.70	89.7	77.5	6.53	35.33	6.5	1.3	145.5	4.12	116.4	25.50
520-58_2	87.40	87.4	78.5	5.15	32.57	6.4	1.0	153.5	4.32	77.4	21.50
520-4_3	97.50	97.5	80.0	5.76	25.15	6.0	1.6	143.5	3.53	113.5	25.00
520-56_3	89.70	89.7	79.5	5.10	33.15	6.9	1.1	155.5	3.54	61.2	21.50
520-56_1	93.80	93.8	79.5	4.59	31.40	6.3	1.2	152.0	3.86	127.3	20.00
520-38_3	95.40	95.4	82.0	5.20	22.97	6.1	1.2	152.0	3.93	151.5	28.50
520-38_2	91.10	91.1	79.5	5.60	33.40	6.2	1.3	141.5	3.77	107.2	24.50
520-58_3	99.15	99.2	80.5	4.85	25.85	6.6	1.4	151.5	4.07	107.8	24.50
520-78_3	93.10	93.1	82.0	5.90	45.45	6.0	1.4	144.0	3.85	137.1	21.00
520-61_4	95.50	95.5	92.3	6.25	33.30	6.6	1.3	137.5	3.84	84.9	21.50
520-61_3	99.30	99.3	88.7	4.60	30.65	6.3	1.1	151.0	3.92	68.5	19.50
520-4_2	92.80	92.8	88.3	5.75	43.37	6.6	1.4	140.5	3.66	87.3	22.50
520-81_5	96.00	96.0	82.0	5.28	28.18	7.3	1.3	131.5	4.20	69.1	17.50
520-51_3	93.10	93.1	77.5	5.66	35.90	6.5	1.2	160.5	4.01	85.8	24.00
520-61_4	98.50	98.5	77.0	5.10	32.80	6.9	1.0	153.0	4.15	83.9	17.00
520-51_5	85.70	85.7	78.0	7.30	37.10	6.1	1.4	140.0	4.37	146.9	24.50
520-24_1	94.50	94.5	79.5	4.90	33.27	7.3	1.1	162.5	3.76	69.9	23.50
520-69_1	100.60	100.6	79.0	6.38	37.87	6.7	1.1	141.5	4.00	103.7	19.50
Check(H520)	94.60	88.5	74.3	9.80	42.34	8.2	1.3	121.5	6.97	188.7	26.50
Mean	103.50	94.5	79.7	5.6	34.45	6.5	1.24	148.5	4.13	96.41	32.00
Maximum	80.20	100.6	92.3	9.8	45.45	8.2	1.70	163.0	6.97	188.7	10.00
Minimum	79.54	85.7	73.0	4.00	22.97	5.3	1.00	121.5	3.53	61.2	7.00

Note: DTS=days to 50% silking, DA= days to 50% Anthesis, DPS= days to 50% pollen shedding, FLW=flag leaf width, FLL=flag leaf length, LUE= number of leaves above upper ear, HEP=harvestable ears plant $^{-1}$ (HEP), DM= days to 80% Maturity, DOE=diameter of the ear, GY=grain yield plant $^{-1}$ (g), TLD= tolerance days

Note: *, **, ^{ns}: significant at $p < 0.05$, $p < 0.01$ and non-significant respectively

Table 5. Correlation coefficients of grain yield plant^{-1} with yield components among the assessed 39 maize lines.

S. No	Characters	1	2	3	4	5	6	7	8	9	10	11
1	Tolerance days	1										
2	Days to 50% silking											
3	Day to anthesis (50%)	0.51^{**}	0.24^{*}	1								
4	Days to pollen shedding (50%)	0.50^{**}	0.22^{*}	0.96^{**}	1							
5	Flag leaf width (cm)	-0.48^{**}	-0.27^{ns}	-0.62^{**}	-0.62^{**}	1						
6	Flag leaf length (cm)	0.36^{*}	0.08^{ns}	-0.29^{*}	-0.29^{*}	0.76^{**}	1					
7	Leaf number above upper ear	0.06^{ns}	0.04^{ns}	0.12^{ns}	0.09^{ns}	-0.33^{*}	-0.33^{**}	1				
8	Harvestable ear plant $^{-1}$	-0.05^{ns}	-0.17^{ns}	-0.06^{ns}	-0.10^{ns}	0.12^{ns}	0.12^{ns}	0.45^{**}	1			
9	Days to maturity (80%)	0.35^{*}	0.08^{ns}	0.59^{**}	0.51^{**}	-0.47^{**}	-0.47^{**}	-0.05ns	0.11^{ns}	1		
10	Diameter of the Ear (cm)	-0.22^{ns}	-0.20^{ns}	-0.36^{*}	-0.42^{**}	0.20^{ns}	-0.05^{ns}	0.43^{*}	0.60^{**}	-0.19^{ns}	1	
11	Grain yield plant^{1-} (g)	-0.36^{*}	-0.22^{ns}	-0.69^{**}	-0.67^{**}	0.36^{*}	-0.04^{ns}	0.15^{ns}	0.35^{*}	-0.47^{**}	0.68^{**}	1

Table 6. Correlation coefficients of grain yield plant $^{-1}$ with yield components among the assessed 37 M4 maize lines.

S. No	Character	1	2	3	4	5	6	7	8	9	10
1	Tolerance days										
2	Days to 50% anthesis	-0.17^{ns}	1								
3	Days to 50% pollen shedding	0.16^{ns}	-0.27^{ns}	1							
4	Flag leaf width (cm)	-0.31*	0.04^{ns}	0.26^{ns}	1						
5	Flag leaf length (cm)	-0.13^{ns}	-0.02^{ns}	-0.07^{ns}	0.33*	1					
6	Leaf number above upper ear	-0.10^{ns}	-0.23^{ns}	-0.11^{ns}	-0.08^{ns}	-0.06^{ns}	1				
7	Harvestable ears plant^{1-}	-0.39*	-0.11^{ns}	-0.09^{ns}	0.17^{ns}	-0.03^{ns}	0.06^{ns}	1			
8	Days to maturity (80%)	0.27^{ns}	-0.16^{ns}	0.30*	-0.12^{ns}	-0.03^{ns}	-0.23^{ns}	-0.23^{ns}	1		
9	Diameter of the ear (cm)	0.20^{ns}	-0.14^{ns}	-0.23^{NS}	-0.15^{ns}	-0.15^{ns}	0.30*	-0.17^{ns}	-0.06^{ns}	1	
10	Grain yield plant^{1} (g)	-0.27^{ns}	-0.12^{ns}	-0.27^{ns}	-0.02^{ns}	0.29^{ns}	0.42**	0.28^{ns}	-0.03^{ns}	0.16^{ns}	1

Note: *, **, ^{ns}: significant at $p < 0.05$, $p < 0.01$ and non-significant respectively

Discussion

Mutation breeding is an important process of creating genetic variation in existing varieties or creating new genotypes altogether (Kazi, 2015). Induced mutation can serve as an important source of variations for herbicide tolerance.

The characterized 39 M_3 and 37 M_4maize lines varied for number of days to anthesis pollen shedding and maturity (Table 2 and 4). 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 days to pollen shedding stages. Similar results were reported by Ghimire and Timsina (2015); Baqa *et al.* (2014) and Kinfe and Tsehaye (2015). However, Mourice *et al.* (2014) demonstrated non-significant variation for days to physiological maturity among evaluated maize lines. However, Ali *et al.* (2015) demonstrated non-significant differences among maize hybrid lines for days to pollen shedding. Earliness trait is very crucial in maize production particularly in arid and semi-arid regions, because earliness enables the maize lines to escape from late occurring drought, extremes of temperatures and late infestation of weeds, diseases and insect pests. Overall, the maize lines in this study could be exploited for future breeding programmes.

The assessed 39 M_3 and 37 M_4maize lines showed significant differences for flag leaf width and flag leaf length (Table 2 and 4). The flag leaf is usually the main source of photosynthates that are utilized in reproduction and grain filling resulting in grain yield plant^{-1} in maize.

The identified maize lines with wide and/or long flag leaves probably could have higher photosynthetic rates due to the possession of larger leaf area and greater light interception compared to the lines with smaller leaves. This probably results in the development of larger ears and consequently higher yield. Similar findings were reported by Bezaweletaw *et al.* (2006) in finger millet; Dere and Yildirim (2006); Oladosu *et al.* (2014) in rice and Ndou *et al.* (2015) in wheat.

The current study results of the assessed 39 M3 and 37 M4 maize lines exhibited significant differences with respect to days to silking. Earliness in silking characterized by appearance of earliest silks (stigmas) that are receptive to pollen allowing for faster accumulation of ear biomass hence grain yield. Significance of silking varied between.

The M3 and M4 generations most probably due to variations in genetic make-up and environmental conditions especially rainfall which could have altered growth during silking. Similar findings were reported by Borras *et al.* (2007); Baqa *et al.*, (2014) and (Ghimire and Timsina, 2015b) indicating that the trait was highly heritable.

The assessed 39 M3 and 37 M4 maize lines varied with respect to the diameter of the ear (Table 2 and 4). In this perspective, the wider the diameter of the ear, the higher the number of rows ear $^{-1}$ and larger seed size. Similarly, Rahman *et al.* (2015) reported significant phenotypic diversity for the diameter of the ear among maize genotypes.

The present results further showed highly significant differences among characterized 39 M3 and 37 M4 maize lines for grain yield plant^{-1} (Table 2 and 4). The recorded variations could be contributed by larger seed size, heavier 100 seed weight and larger leaf area. Similarly, (Mubeen, Rafique, Munis, and Chaudhary, 2015) reported significant variations for grain yield but non-significant differences in number of harvestable ears plant^{-1} among the evaluated maize genotypes. Many studies further illustrated substantial differences for grain yield among inbred lines of maize Charles *et al.* (2013); (Adebayo and Menkir, 2015). However, in contrast, Singh reported non-significant differences for grain yield among maize lines. Current results indicated the presence of sufficient phenotypic variation among maize lines. Maize lines with substantial grain yield plant $^{-1}$ and medium anthesis and maturity time while having considerable yield related traits could be exploited in future maize breeding program to generate high yielding maize varieties and utilization of the breeding materials.

There were highly significant differences among the assessed 39 M3 maize lines for herbicide tolerance days (Table 2). The lines varied in tolerance of herbicides due to the treatment of the lines with mutagens. Similarly, Forlani and Racchi (1995) reported significant differences among the maize lines to different concentrations of glyphosate.

The results further indicated that there was positive and highly significant ($p < 0.05$) correlation of grain yield plant $^{-1}$with diameter of the ear, number of harvestable ears plant^{-1} and flag leaf width. The positive correlation of grain yield plant^{-1} with yield related components is valuable for indirect selection of the ultimate grain yield plant^{-1}. Likewise, many studies reported positive and significant correlation of grain yield with diameter of the ear and number of kernel rows ear $^{-1}$Bashir *et al.* (2003), positive and significant association of grain yield with number of harvestable ears plant^{-1} (Adebayo and Menkir, 2015), grain yield with number of kernel row $^{-1}$ and ear length (Akeel *et al.* 2010), grain yield plant^{-1} with diameter of the ear (El-Badawy and Mehasen, 2011). Present results implied that increased grain yield plant^{-1} was not due to early anthesis and maturity time rather attained because of larger ear size, more number of harvestable ears plant^{-1} and broader flag leaf width.

Grain yield plant^{-1} further showed negative and significant correlation with days to anthesis, pollen shedding as well as days to maturity (Table 3). These findings were in agreement with those of Anjorin and Ogunniyan (2014) and Ghimire and Timsina (2015) who reported negative and significant association of grain yield with days to anthesis and days to maturity in maize respectively. The results contradict with those of Ghimire and Timsina (2015) who reported negative and non-significant correlation of grain yield $^{-1}$ with days to maturity among maize genotypes. The negative correlation of grain yield with days to anthesis and pollen shedding as well as days to maturity could be due to early anthesis and maturing maize lines utilize only a scanty photosynthesis produces and leads to low final grain yield production. Likewise, Anjorin and Ogunniyan (2014) reported a negative association of days to anthesis with grain yield plant^{-1}. The negative correlation among agronomic traits indicated that breeders could make priority in decision during selection of characters for future improvement of varieties because negatively associated characters cannot be improved simultaneously.

The current results confirmed there was a positive and significant ($p < 0.05$) correlation of herbicides tolerance days with the number of days to anthesis ($r = 0.51^{**}$), number of day to pollen shedding ($r = 0.50^{**}$), flag leaf length ($r = 0.36^{*}$) and days to maturity ($r = 0.35^{**}$). Tolerance days also showed negative and significant correlation with grain yield plant $^{-1}$ ($r= - 0.36^{*}$) and flag leaf width ($r= - 0.48^{**}$) (Table 5). The negative correlation indicated that there was a yield penalty for being herbicide tolerance. Stefanovic *et al.* (2010) illustrated that application of herbicide can slowdown the growth and development affecting the plant height; also affect the stages of the tassel and ear development, resulting in reduced grain yield of the tested inbred lines.

Furthermore, the number of days to maturity correlated positively and highly significantly with days to anthesis and pollen shedding. In line with this result, Zarei *et al.* (2012) showed positive and significant correlation of days to maturity with days to anthesis. Findings from this study indicated that positively and significantly correlated characters could be selected and improved concurrently. Earliness in days to anthesis and pollen shedding is direct proportional to earliness in maturity time. Thus it is possible to select and improve such important traits simultaneously.

Conclusion

Results from this study indicated that there was significant variations among the characterized 39 M_3 and 37 M_4maize lines for all the traits studied namely, days to days to anthesis and days to silking, flag leaf width and length, days to maturity, diameter of the ear, grain yield plant^{-1} and herbicide tolerance days. Grain yield plant^{-1} showed positive and highly significant correlation with diameter of the ear, harvestable ears plant^{-1} and flag leaf width. Further, the results indicated a positive and significant correlation between herbicides tolerance days with the number of days to anthesis, number of day to silking, flag leaf length and days to 80% maturity but negative and significant correlation with grain yield plant^{-1} and flag leaf width. In addition, days to maturity correlated positively and highly significantly with days to anthesis and days to pollen shedding.

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