Morphological and molecular characterization of maize inbred lines showing variability for drought tolerance

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ABSTRACT. A set of hundred maize inbred lines were analyzed for resilience to moisture stress for twenty-four traits related to maturity, morphological, physiological, yield, quality and root traits. Evaluation confirmed a wide range of variability revealing significant response of main effects (lines, irrigations and years and their respective digenic and trigenic interactions). Fifteen elite identified lines performed well under moisture stress conditions showing inbuilt tolerance towards moisture stress. A set of 32 SSR markers, having genome-wide coverage, was chosen for genotyping the selected 15 inbred lines. These markers generated a total of 239 polymorphic alleles with an average of 7.47 alleles per locus. The minimum and maximum PIC value was 0.886 and 0.608 with a mean of 0.782. The coefficient of genetic dissimilarity ranged from 0.215 to 0.148. DARwin derived cluster analysis grouped 15 elite maize lines in three major clusters with five lines each in cluster-III and II and four lines in cluster-I with KDM-361A as root (at origin point. Molecular marker data however, confirmed diverse genetic nature of six lines (KDM-372, KDM-343A, KDM-331, KDM-961, KDM-1051 and KDM-1156) with favorable drought resilient traits with no yield penalty under moisture stress. Exploitation of these identified elite lines in involving all possible combinations helped to develop single cross hybrids under moisture stress conditions.

Key words: Genetic diversity; SSRs; Drought; D2 analysis.

INTRODUCTION

Maize grain yield is limited by various factors and is a problem of immediate practical implication and among various abiotic stresses; inadequate water availability at critical stages of crop growth and development is the major limiting factor for its production and productivity (Naveed *et al*., 2013; Aslam *et al*., 2014). Denmead and

Shaw (1960) recorded the reduction in grain yield by 25, 50 and 21% due to moisture stress prior to silking, at silking and after silking, respectively, indicating that silking stage is the most critical stage for moisture stress. They also reported that early stress has an indirect effect on grain yield by reducing the size of the assimilatory surface at the time of ear development, while stress imposed after the ear emergence has more direct effect through reducing the assimilation in the critical period when daily assimilation is being used in grain production. An estimated 80 per cent of the maize crop suffers periodic yield reduction due to drought stress (Bolanos and Edmeades, 1993). Edmeades *et al*. (1995), Dass *et al.* (2001) and Cakir (2004) revealed that drought may occur at any stage of maize growth, but when it coincides with the flowering and grain filling periods it causes yield losses of 40-90 per cent. The critical stages of growth which particularly affects the ability of maize to produce grain are determined at the early growing season, at flowering and during mid-to-late grain filling (Heisey and Edmeades, 1999). Bolanos and Edmeades (1993) reported that there is always a risk of drought stress at all the stages because of the unpredictable nature of mean monthly rainfall.

Understanding the genetic basis of drought tolerance in crop plants based on various morpho-physiological traits is a pre-requisite for a geneticist/breeder to evolve superior genotype through either conventional breeding methodology or biotechnology methodology (Singh, 1978). Unluckily, ecological stresses such as water scarcity and high temperature stresses are going to confine the maize production. Water deficiency occurs in most parts of the world every year having overwhelming effect on maize production. Edmeades *et al*. (1992) reported that the average loss due to drought alone is estimated to 15.0 million tonnes per year. These losses represent 17 per cent of well watered environment and it can be as high as 60 per cent in severely drought affected regions. Increased rainfall variability accentuated by climate change will have severe effects on maize production which is grown predominantly as a rainfed crop in India.

In maize crop during knee height stage the uppermost ear and tassel initiation starts and kernel row number per ear is determined, tillers (suckers) begin to emerge at this time with degeneration and loss of lower leaves and nodal root system gets established. Moisture stress at knee height stage affects plant height, photosynthetic rate, Leaf Area Index (LAI) and total biomass (Ritchie *et al*., 2005). Tassel begins when the last branch of tassel is visible, but the silks have not emerged. Tassels normally appear 2 to 3 days before silk emergence. The silking stage begins when the silk is visible outside the husk. Pollen falls onto the silks to potentially fertilize the ovules and each ovule produces an individual kernel. Moisture stress at this time can cause the desiccation of silks or pollen grains, reduction in pollen production, pollen viability and results in barren tips or loss of entire ears causing yield reduction (80-90%), which increases the frequency of kernel abortion and reduces seed set. The plant provides nutrients for reproductive growth instead of vegetative growth during the grain filling stage. Once kernels have reached the dough stage of development, further yield losses resulting from water stress will occur mainly from reductions in kernel dry weight accumulation Once grain has reached physiological maturity water stress will have no further physiological effect on final yield (Lauer, 2007).

Present study was conducted to identify lines with low yield penalty under drought-stress conditions (especially at anthesis and silking interval) as compared to well-watered conditions with stability over years. Under stress conditions, selection for grain yield has often been considered inefficient because of the increase in environmental variance relative to genetic variance, which decreases heritability in yield. Hence, selection for secondary traits, correlated to grain yield having high heritability may increase selection efficiency (Bolanos and Edmeades, 1993). Progress in grain yield under stress has been achieved through selection for reduced anthesis-silking interval (ASI), reduced barrenness and improved kernel set (Campos *et al*., 2004). Number of ears per plant, kernels per plant and anthesis-silking intervals (ASI) are considered as the most important drought adaptive traits, followed by plant height (Edmeades *et al*., 1997). Phenotyping of inbreds was done by using morphological, maturity, physiological, yield and yield attributing traits, quality along with early seedling and root traits. Genotyping data of identified elite lines was complemented by phenotyping data which was used for development of drought tolerant hybrids.

MATERIAL AND METHODS

A set of 100 maize inbred lines developed and maintained at AICRP (All India Coordinated Research Project) Maize Srinagar Centre along with the checks from CIMMYT (International Maize and Wheat Improvement Centre) Mexico, AAU, Anand and MPUAT, Udaipur were evaluated in factorial RBD with three factors viz., inbred lines, years and irrigations to evaluate the impact, interaction and influence of each factor. Indigenous inbred lines were at different stages of selfing mostly at S5. Year factor was studied for understanding the interaction and stability of the lines over the years. Two experiments were conducted over two years viz.,

(a) Field experiment: Maize lines with two replications in two row experimental plot of 1meter length (60 × 20 cm with recommended package of practices) were evaluated against four moisture management regimes viz;

- **Well Watered (WW):** Watered at all the critical growth stages (grain filling, flowering and kneeheight stage).
- **Intermediate Stress (IS):** Watered at flowering and kneeheight stage.
- **Mild Stress (MS):** Watered only once at kneeheight stage.
- **Stress (S):** No water provided after sowing (complete rainfed).

(b) Pot experiment: Maize inbred line was sown in pots with two replications in a soil mixture of clay and sand in the 3:7 ratio. These lines were evaluated at early seedling stage for three weeks and were maintained in laboratory having optimal temperatures by giving three treatments to the plants. First, well watered treatment in which plants were watered on regular weekly basis thereby maintaining 100% of the field capacity (FC) of the plants, second, intermediate stress in which plants were watered regularly for two weeks and stress was imposed after 14 days by stopping irrigation after two weeks, maintaining 60% of the field capacity. In third treatment, stress was applied to the plants by withdrawing irrigation after one week maintaining 40% FC. All the treatments were given irrigation required for germination and after twenty four days observations were recorded on various seedling and root traits in all the treatments after giving subsequent drought cycles.

The meteorological data, including min and max temperatures, relative humidity (RH) and rainfall were recorded over the years. Observations were recorded on anthesis-silking interval, days to maturity, plant height (cm), leaf relative water content (%), canopy temperature (°C), chlorophyll content (SPAD Units), ears plant^{-1}, kernels row^{-1}, 100 kernel weight (g), grain yield plot^{-1} (g), protein content (%) and root traits viz., germination (%), number of seminal roots, number of crown roots, primary root length (cm), fresh root weight (g) and dry root weight (g). Total chlorophyll content measured in SPAD units. Field Capacity is the amount of soil moisture or water content held in the soil after excess water has drained away and the rate of downward movement has decreased. This usually takes place 2–3 days after rain or irrigation in pervious soils of uniform structure and texture. Here field capacity of each treatment mixture was measured on volume basis. The RWC was calculated by using the following formula:

$$\text{Fresh weight} - \text{Dry weight}/\text{Turgid weight-Dry weight} \times 100$$

Observational data collected from both (a) and (b) experiment was subjected to analysis of variance for all the characters in the individual years and for the data pooled over the years was carried out for testing variation among the lines as per the procedure suggested by Verma *et al*. (1987) through windostat version 9.1 statistical package to generate information on components of variability, heritability, expected genetic gain under different treatments. Lines possessing genetic variability for drought related traits in the pots and having per se superiority over the population mean were identified as potential lines having inbuilt drought tolerance and hence were further selected for molecular studies

(c): Molecular studies: Observational data recorded was used to evaluate the type and magnitude of variability and to characterize identified inbred lines with inbuilt drought tolerance at molecular level. In the third year, fifteen identified drought promising inbred lines were subjected to SSR data analysis and cluster analysis. Extraction of plant DNA was carried out by CTAB (Cetyl-Tri Methyl Ammonium Bromide) method as described by Murray and Thompson (1980) from a pool sample of 15 seedlings leaves. Genetic diversity studies were carried with the help of forty micro-satellite markers (four per chromosome) retrieved from www.maizegdb.org standardized as per Warburton *et al*., (2001). PCR amplifications were performed using thermal cycler (Eppendorf, Hamburg, Germany) and resolution of amplified PCR products was done using 3.5% agarose gel. After the initial screen, eight SSR markers which did not amplify were rejected from the experiment. Based on the electrophoretic banding pattern of 32 SSR markers, pair wise genetic distance amongst genotypes were estimated and a dendrogram was generated using UPGMA clustering. Phylogenetic reconstruction based on the neighbor joining method was conducted using computer software programme Dissimilarity Analysis and Representation for Windows (DARwin) 5.0 (Perrier *et al*., 2003).

RESULTS AND DISCUSSION

ANOVA revealed highly significant mean sum of squares for the maize inbred lines under study for all the maturity, morphological, physiological, yield, quality and root traits in pooled over years analysis, thus indicating significant difference among the lines for all the traits. Mean sum of squares due to years and irrigations were also significant for all the traits indicating differential responses of maize lines for these traits

over years and different moisture management regimes (Table 1).

Table 1. Analysis of variance for drought related traits (Pooled over years).

S.V	d.f	Mean squares										
		ASI	DM	PH	LRWC	CT	CCF	EPP	KPR	100GW	GYP	PC
Rep	1	0.04	1.75	19214.12**	1774.84**	0.34	412.44**	0.81**	719.21**	103.83**	71.80	0.43**
Year	1	155.00**	564.53**	282454.50**	44426.25**	1132.10**	802.26**	5.04**	14551.78**	3034.52**	5398729.00**	57.74**
Irri	3	78.54**	1120.22**	948795.20**	805287.00**	3738.55**	4460.39**	6.81**	8502.75**	1943.63**	4120011.00**	49.40**
Lines	99	8.86**	452.36**	1132.90**	2194.18**	22.75**	596.43**	1.13**	133.28**	64.67**	70061.61**	13.72**
Lines × year	99	1.06**	5.49**	8.54 **	4.39	0.03	0.09	0.93**	1.10**	1.35*	803.26**	0.04**
Line × Irri	297	0.51**	7.03**	19.09**	118.78	1.22**	0.35	1.01*	0.11 **	2.05**	512.01**	0.07**
Irri × year	3	0.18*	83.96**	21026.50**	10542.30**	92.01**	218.10**	0.37**	1584.58**	595.90**	732946.10**	3.94**
Irri within Rep	7	0.04	0.80	59.35**	42.13	0.01	0.09	0.01	0.69	0.10	0.68	0.01
Irri within years within Rep	15	26.08**	278.96**	214103.26**	166265.59**	841.61**	1016.72**	1.82**	3035.85**	717.18**	1330511.65**	14.54**
Line × Irri × Year	297	0.26**	4.41**	0.32	1.46	0.02	0.03	0.01	0.10	0.05	239.74**	0.01
Error	1485	0.26	5.06	6.91	37.18	0.30	2.20	0.02	4.72	0.65	204.75	0.01
σ^2g		0.53	27.95	70.37	134.81	1.4	37.14	0.07	8.03	4	4366.05	0.85
σ^2p		0.83	33.02	77.29	172	1.7	39.34	0.09	12.75	4.66	4570.81	0.86
GCV (%)		16.24	3.54	5.3	12.63	3.76	14.78	23.38	11.01	9.44	16.01	12.18
PCV (%)		19.85	3.85	5.55	14.26	4.15	15.22	26.61	13.87	10.19	16.38	12.2
h^2		0.66	0.84	0.91	0.78	0.82	0.94	0.77	0.63	0.85	0.95	0.99
GG		27.37	6.71	10.41	23.03	7.02	29.59	42.33	18	18.01	32.24	25.04

*, ** Significant at 5 and 1% probability level, respectively. (Note: Rep= Replication, Irri=Irrigation, Anthesis-silking interval=ASI; Days to maturity=DM; Plant height (cm)= PH; Leaf relative water content (%)=LRWC; Canopy temperature (˚C)=CT; Chlorophyll content (SPAD units)=CC, Ears plant^{-1} =EPP; Kernels row^{-1} =KPR; 100-grain weight (g)= 100 GW; Grain yield plot^{-1} (g)= GYP; Protein content (%)=PC).

Three-way interactions (lines × irrigation × year) were observed to be significant for all the traits except for leaf relative water content, canopy temperature, chlorophyll content, ears plant^{-1}, kernels row^{-1}, 100 grain weight and protein content. Differential response of lines was observed over years as exhibited from the two way interactions i.e., line × year, line × irrigation and irrigation × year and three way interactions i.e., line × irrigation × year. Dubey *et al*. (2010) reported presence of significant genetic variation for all the traits under drought conditions revealing importance of locations/seasons, environments, location/season × treatment and environment × treatment interaction for almost all the characters. Significant differences among the inbred lines for majority of the traits over different moisture management regimes and over years indicated the presence of

wide genetic variation amenable for breeding for drought tolerance. Results were in conformity with Maiti *et al.* (1996), Chapman *et al*. (1997); Banziger *et al*. (2000); Mehdi *et al*. (2001); Zaidi *et al*. (2004), Saindass *et al*. (2001); Asghar and Khan (2005); Qayyum *et al*. (2012) and Umar *et al.* (2015).

Components of phenotypic variability were higher than the corresponding estimates of genotypic variability for all the traits in pooled analysis, thereby revealing the importance of environmental variance in the trait expression (Table 1). Kumar *et al*. (2014) observed similar results. Genotypic coefficient of variation was high (> 20) for ears plant^{-1}, number of seminal roots, and number of crown roots, fresh root weight, dry root weight and primary root length thus indicating presence of sufficient inherent genetic variance over which selection could be effective. Saleem *et al*. (2007); Qayyum *et al*. (2012) and Ali *et al.* (2013) observed similar results. However, moderate values of GCV (10-20) were recorded for ASI, leaf relative water content, chlorophyll content, kernels row^{-1}, 100 grain weight, grain yield plot^{-1} and protein content. Similar results of moderate GCV were observed by Alake *et al.* (2008); Salman *et al.* (2011) and Kumar *et al*. (2014). High to moderate GCV for these traits indicated sufficient variability and offers scope to improve these traits through phenotypic selection. Days to maturity, plant height and canopy temperature showed low GCV estimates (<10) therefore, there is a limited scope of selection (Azam *et al*., 2014; Praveenkumar and Sridevi, 2014). High estimates of heritability along with higher genetic advance are usually more useful than either of these parameters taken alone in predicting the resultant effect of selecting the best individuals (Johnson *et al*., 1955). Genetic advance being the function of heritability, selection intensity and phenotypic standard deviation indicates the magnitude of improvement in the desired direction that can be expressed in a particular character by selecting a certain proportion of population. Heritability (b.s.) was observed to be higher (> 60%) for all the traits suggesting that selection for improvement of these characters would be effective through phenotypic selection. Similar results were reported by Aminu and Izge (2012), Kumar *et al*. (2014) and Azam *et al*. (2014). High heritability estimates is indicative to preponderance of additive gene action. High values of heritability indicate character is less influenced by environmental effects. High estimates of broad-sense heritability for most of the traits revealed that variations were transmitted to the progeny and indicated potential for developing high yielding varieties through selection of desirable plants in succeeding generations (Aminu and Izge, 2012). However, the selection for improvement of such characters may not be useful because broad sense heritability is based on total genetic variance which includes additive, dominant and epistatic variances. Thus, heritability values coupled with high genetic advance would be more reliable and useful on correlating selection criteria. High heritability estimates with high genetic gain were observed in present set of lines for traits namely anthesis-silking interval, leaf relative water content, chlorophyll content, ears plant^{-1}, grain yield plot^{-1} protein content and root related traits. Similar results were reported by Ram Reddy *et al.* (2012). High heritability estimates coupled with moderate genetic gain were observed in present set of lines for traits like plant height, kernels row^{-1} and 100 grain weight. Low estimates of genetic gain were revealed for days to maturity and canopy temperature.

Components of variability, phenotypic selection and selection index (viz., Smith Hazel Index described in Gazal et al., 2017) coupled with the response of lines to water over the years interms of mean values confirmed identification of fifteen elite lines viz., KDM-463, KDM-912A, KDM-717, KDM-343A, KDM-961, KDM-932A, KDM-1051, KDM-402, KDM-918A, KDM-1156, KDM-1236, KDM-372, CM-129, KDM-331 and KDM-361A. These elite lines had superiority in drought related traits viz., narrow anthesis silking interval (ASI), early maturity, good plant height, high leaf relative water content, good amount of chlorophyll content, low canopy temperature and root traits as compared to the population lines under stress conditions (Table 2).

Table 2. Analysis of variance for root related traits over years (Pooled over years).

S.V.	d.f	G%	PRL	NSR	NCR	FRW	DRW
Replications	1	7.08	61.16**	0.04	0.11	14.61**	10.08**
Year	1	12.07*	4.16**	13.97**	12.91**	7.43**	1.36**
Irrigations	2	3818.51**	13.16**	318.20**	185.87**	169.34**	27.12**
Lines	99	927.95**	148.01**	5.73**	4.41**	65.97**	10.70**
Lines × year	99	10.48**	0.10**	0.12**	0.17**	0.18**	0.02**
Line × irrigation	198	27.50**	0.85**	0.19**	0.22**	0.46**	0.07**
Irrigations × year	2	5298.10**	3.47**	65.19**	12.02**	382.64**	61.60**
Irrigation within replication	5	9.94	0.01	0.01	0.02	0.02	0.01
Irrigation within years within replication	11	1663.83**	8.96**	70.99**	37.17**	102.38**	17.17**
Lines × irrigation × year	198	27.35**	2.11**	0.13**	0.13**	0.90**	0.13**
Error	1089	13.86	0.56	0.08	0.10	0.28	0.04
σ^2g		76.17	0.47	0.35	12.28	5.47	0.88

σ^2p	90.03	0.56	0.46	12.85	5.76	0.93
GCV (%)	13.04	25.1	27.94	36.58	40.18	39.65
PCV (%)	14.18	27.39	31.69	37.41	41.22	40.64
h^2	0.84	0.84	0.77	0.95	0.95	0.95
GG	24.71	47.39	50.77	73.69	80.7	79.7

(Note: G%= Germination, PRL= Primary root length (cm), NSR= Number of seminal roots, NCR= Number of crown roots, FRW= Fresh root weight (g), DRW= Dry root weight (g)) *, ** Significant at 5 and 1% level, respectively

On comparing the yield and yield attributing traits very low yield penalty was recorded in these elite lines. Ears per plant and kernels per cob was more with quality and heavier grains as compared to population mean. Highest desirable *per se* performance under stress conditions revealed that variability among the lines was genetic in nature. (Table 3).

Table 3. Performance of elite lines over population mean in stress moisture management regime for drought related traits (Pooled over years)

Elite lines	ASI	% decrease	PH	% increase	CC	% increase	EPP	% increase	GYP	% increase
CM-129	3.00	-42.64	124.83	14.43	15.86	53.36	1.62	62.00	483.39	59.15
KDM-331	3.00	-42.64	123.64	13.34	15.81	52.93	1.62	62.00	475.48	56.54
KDM-343A	3.00	-42.64	122.47	12.27	15.34	48.33	1.57	57.00	407.50	34.16
KDM-361A	3.00	-42.64	123.70	13.39	16.29	57.50	1.67	67.00	485.34	59.79
KDM-372	3.00	-42.64	124.74	14.35	16.05	55.22	1.67	67.00	480.65	58.25
KDM-402	3.00	-42.64	123.23	12.96	15.85	53.31	1.62	62.00	445.16	46.56
KDM-463	3.00	-42.64	123.38	13.09	15.68	51.64	1.56	56.00	436.11	43.58
KDM-717	3.00	-42.64	123.00	12.75	15.51	50.00	1.56	56.00	439.50	44.69
KDM-912A	3.00	-42.64	122.97	12.72	15.32	48.16	1.61	61.00	430.03	41.58
KDM-918A	3.00	-42.64	122.32	12.12	14.84	43.54	1.55	54.75	376.81	24.06
KDM-932A	3.00	-42.64	121.83	11.68	15.14	46.37	1.64	63.50	403.50	32.84
KDM-961	3.00	-42.64	122.14	11.96	15.04	45.41	1.64	63.50	400.48	31.85
KDM-1051	3.00	-42.64	124.06	13.72	15.68	51.60	1.59	58.50	464.04	52.77
KDM-1156	3.00	-42.64	122.33	12.13	14.43	39.56	1.54	53.50	375.24	23.54
KDM-1236	3.00	-42.64	121.57	11.44	14.39	39.19	1.54	53.50	366.61	20.70
Elite mean	3.00	-42.64	123.08	12.82	15.41	49.03	1.60	60.00	431.32	42.00
Population mean	5.23		109.09		10.34		1.00		303.74	

(Note: % decrease and increase over population mean ; ASI= anthesis-silking interval, PH=Plant height (cm), CC=chlorophyll content, EPP= Ears plant^{-1}, GYP= Grain yield plot^{-1})

These identified fifteen elite droughts promising inbred lines were studied for genetic diversity using SSR markers which suggested that the heterozygosity level in the inbred panel was low. The mean value of heterozygosity was 0.06 revealing that most of the loci attained homozygosity. However, for the loci umc-2372 the heterozygosity was 0.60. The presence of heterozygosity arises due to few causes including residual heterozygosity, pollen or seed contamination, mutation at specific SSR loci, or amplification of similar sequences in different genomic regions due to duplication (Bantte and Prasanna, 2003). In cross-pollinated crop, pollen or seed stock contamination during maintenance could be the most plausible explanation for the residual heterozygosity which is not uncommon in maize. As a result, inbred lines tend to segregate for a few loci/characters despite repeated cycles of selfing over many generations. Mutations at specific SSR loci, and amplification of similar sequences in different genomic regions due to duplications possibly explains the occurrence of 'double -bands' (Bantte and Prasanna, 2003; Semagn *et al.*, 2006) when analyzed with locus umc-2372. However, the low heterozygosity in the inbred lines revealed that they have been maintained properly and the reported heterozygosity was inherent. The 32 SSR markers produced as many as 239 alleles with an average of 7.47 alleles per locus in the 15 genotype panel (Table 3). Differences and similarities in the numbers of

alleles could be explained mainly due to the size of the samples under study, the methodologies employed for detection of polymorphic markers which influence allelic differences, expected diversity or uniformity based on pedigrees, and most importantly, use of di-tri-and tetra-repeat types of SSR used in the studies. Di-nucleotide SSR primers are known to yield a significantly higher number of alleles per marker than SSRs with longer repeat motif and also they are often not used in general because of the difficulty in accurately sizing alleles (Choukan *et al.*, 2006; Adetimirin *et al*, 2008). The average number of alleles per loci (11.00 to 4.00) obtained in the present study was higher considering the number of genotypes examined in this study. Major allele frequency ranged from 0.53 for SSR marker Phi-051 to 0.13 for SSR marker umc-1424 with a mean of 0.29. The results were in accordance with the previous report (Dubey *et al*., 2009; Nepolean *et al*., 2012; Sserumaga *et al*., 2014). The PIC value ranged from 0.886 (umc-1766) to 0.608 (Phi-051) with an average of 0.782. Apart from being a different marker system which can detect polymorphisms, the ability to resolve the alleles also plays a crucial role in detecting the number of alleles. PIC and alleles per locus indicated that selected primers were highly polymorphic and the degree of diversity among the lines was high and PIC was sufficient to group the population into different clusters. These findings were comparable with the findings of Mishra and Singh (2012) and Shukla *et al*. (2014). Representative gel picture depicting SSR profile across 15 elite maize lines is shown in Figure 1. (Table 4).

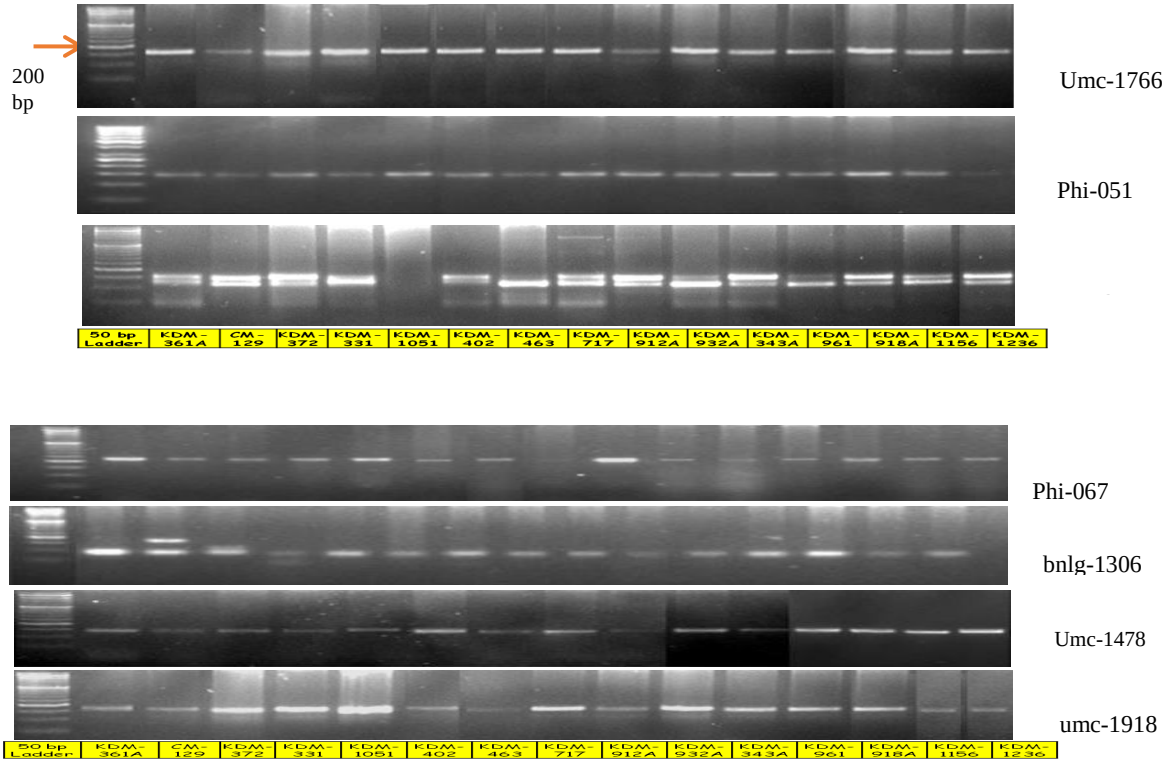

Figure 1. Representative gel pictures depicting SSR profile across 15 elite maize lines.

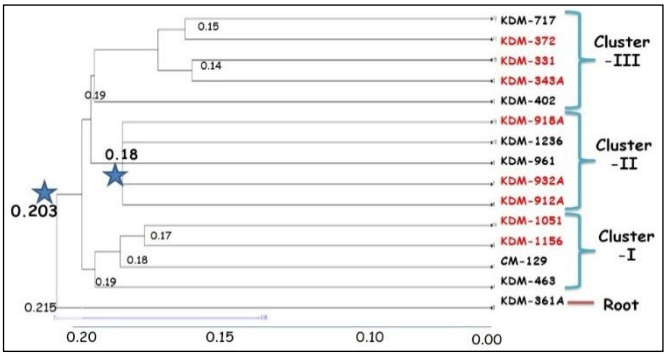

Figure 2. DARwin analysis of 15 elite lines with drought tolerance based on 32 SSR markers.

Table 4. Summary statistics of the genotyping assay for the maize inbred lines

S. No.	Marker	Bin location	Major Allele Frequency	Alleles per locus	Heterozygosity	Polymorphic Information Content (PIC)
1	umc-2383	1.02-1.03	0.27	7.00	0.00	0.815
2	umc-1664	1.06	0.14	4.00	0.00	0.698
3	umc-1147	1.07	0.27	7.00	0.00	0.772
4	umc-1823	2.03	0.30	7.00	0.00	0.775
5	umc-1026	2.04	0.33	6.00	0.00	0.762
6	umc-2372	2.06	0.37	8.00	0.60	0.719
7	umc-2144	2.08	0.33	7.00	0.06	0.750
8	umc-1594	3.09-3.1	0.27	7.00	0.07	0.796
9	bnlg-1621	4.06	0.27	6.00	0.07	0.762
10	umc-1478	5.01	0.27	10.00	0.47	0.830
11	umc-1766	5.01	0.40	11.00	0.13	0.886
12	bnlg-1306	5.07	0.27	8.00	0.13	0.878
13	umc-1918	6.03	0.33	6.00	0.13	0.751
14	umc-1762	6.06	0.33	7.00	0.07	0.711
15	umc-1063	6.07	0.30	7.00	0.07	0.803
16	umc-1018	6.01	0.17	6.00	0.00	0.805
17	phi-452693	6.04	0.33	7.00	0.00	0.775
18	umc-1424	6.06	0.13	8.00	0.00	0.857
19	phi-129	6.05	0.33	8.00	0.00	0.787
20	umc-1002	6	0.27	8.00	0.00	0.795
21	phi-051	7.05	0.29	8.00	0.00	0.608
22	umc-1036	7.02	0.27	8.00	0.00	0.805
23	umc-1708	7.04	0.27	6.00	0.00	0.781
24	bnlg-1056	8.08	0.40	7.00	0.00	0.713
25	umc-1141	8.06	0.33	6.00	0.00	0.751
26	umc-1415	8.04	0.20	7.00	0.00	0.814
27	umc-1786	8.01	0.33	9.00	0.00	0.810
28	phi-067	9.01	0.33	7.00	0.00	0.786
29	phi-061	9.03	0.53	10.00	0.00	0.804
30	umc-1077	10.04	0.20	7.00	0.00	0.850
31	mnc-0501	10.02	0.27	9.00	0.00	0.807
32	bmc-1655	10.03	0.33	7.00	0.07	0.756
Total				239		
Mean			0.29	7.47	0.02	0.782
Range			0.53-0.13	11.00-4.00	0.60-0.00	0.886-0.608

The fifteen elite maize inbred lines were analyzed for dissimilarity coefficient using DARwin 5.0 version computer software (UPGMA analysis) which is more robust and gives significance levels for tree construction. DARwin derived cluster analysis grouped 15 elite maize lines in three major clusters with five lines each in cluster-III and II and four lines in cluster-I with KDM-361A as root. The dissimilarity coefficients based on thirty-two SSR markers data ranged from 0.215 to 0.148 (Figure 2).

Of the pair wise combinations generated from fifteen elite inbred lines, KDM-361A showed highest dissimilarity index (0.215) and lines KDM-343A and KDM 331 showed lowest dissimilarity index (0.148) indicating that KDM-361A had 0.78 similarity index with other inbred lines and the lines KDM-343A and KDM 331 had 0.85 similarity index which confirms that these inbred lines were closely related. Minimum genetic distance between KDM-343A and KDM 331 was a good indication confirming the efficiency of SSR markers to distinguish closely related inbred lines (Dubreuil and Charcosset, 1999). These fifteen moisture stress tolerant elite inbred lines were selected based on their genetic distance and maturity, morphological,

physiological, yield, quality, and seedling and root traits for development of new moisture stress tolerant hybrid.

D^2 analysis classified the 15 elite lines for maturity, morphological, physiological, yield and quality traits into three clusters with four lines viz; KDM-961, KDM-1051, KDM-1156 and KDM-1236 in cluster-I, four lines viz; KDM-918A, KDM-932A, KDM-912A and KDM-717 in cluster-II and rest seven lines viz; KDM-361A, KDM-402, KDM-463, KDM-372, KDM-343A, KDM-331, CM-129 in cluster-III (Table 4). Maximum inter-cluster distance (D^2) value (17.28) was recorded between clusters I and cluster III followed by a distance of 6.07 between cluster-I and cluster-II (Tables 5 and 6).

Table 5. Comparison among 15 elite maize lines based on phenotypic (D^2 statistic) and molecular diversity (using SSR markers).

D^2 analysis among 15 elite lines for maturity, morphological, physiological, yield and quality traits			**D^2 analysis among 15 elite lines for seedling and root traits**			**DARwin analysis of 15 elite lines based on 32 SSR markers**		
Cluster No.	**Number of lines**	**Inbred line**	**Cluster No.**	**Number of lines**	**Inbred line**	**Cluster No.**	**Number of lines**	**Inbred line**
I	4	KDM-961, KDM-1051, KDM-1156, KDM-1236	I	3	KDM-1236, KDM-1051, KDM-1156	1	4	KDM-1051, KDM-1156, CM-129, KDM-463
II	4	KDM-918A, KDM-932A, KDM-912A, KDM-717.	II	5	KDM-402, KDM-717, KDM-912A, KDM-918A, KDM-932A	2	5	KDM-918A, KDM-1236, KDM-961, KDM-932A, KDM-912A
III	7	KDM-361A, KDM-402, KDM-463, KDM-372, KDM-343A, KDM-331, CM-129	III	4	KDM-361A, KDM-372, KDM-343A, KDM-331	3	5	KDM-717, KDM-372, KDM-331, KDM-343A, KDM-402
			IV	1	KDM-961	4	1	KDM-361A
			V	1	KDM-463			
			VI	1	CM-129			

Table 6. Average inter-cluster (above diagonal) and intra-cluster (diagonal) distances among elite maize inbred lines for drought

Drought related traits				**Root traits**						
Cluster	**I**	**II**	**III**	**Cluster**	**I**	**II**	**III**	**IV**	**V**	**VI**
I	**0.75**	6.07	17.28	**I**	**0.19**	1.13	8.19	0.38	1.27	8.63
II		**1.09**	6.23	**II**		**0.25**	3.98	1.53	0.47	4.30
III			**1.11**	**III**			**0.26**	9.34	4.56	0.57
				IV				**0.00**	1.05	9.10

Table 7a. Cluster means for morphological, maturity, physiological, yield and quality traits of elite maize inbred lines.

Clusters	**ASI**	**Days to Maturity**	**Plant height (cm)**	**Leaf relative water content (%)**	**Canopy temperature at flowering (˚C)**	**Chlorophyll content at flowering (SPAD Units)**	**Ears plant^{-1}**	**Kernels row^{-1}**	**100 Grain weight (g)**	**Grain yield plot^{-1}**	**Protein content (%)**
I	3.00	136.35	237.09	161.87	25.54	57.47	1.79	38.77	29.70	753.56	9.79
II	3.00	137.50	191.34	131.59	28.15	53.83	1.66	28.96	24.32	535.43	9.18
III	3.00	138.35	129.59	62.39	31.22	49.85	1.63	26.38	23.49	457.01	8.92

Table 7b. Cluster means for root traits of elite maize inbred lines

Clusters	Germination (%)	Number of seminal roots	Number of crown roots	Primary root length (cm)	Fresh root weight (g)	Dry root weight (g)
I	85.95	4.74	3.85	14.93	11.06	4.48
II	85.95	4.05	3.40	15.86	10.59	4.27
III	85.95	3.23	2.95	17.29	8.31	3.40
IV	85.95	4.92	3.58	16.72	12.32	4.97
V	85.95	4.33	3.08	17.97	11.74	4.76
VI	85.95	3.38	2.75	19.97	9.62	3.90

Maximum cluster means for all the traits were observed in cluster-I (Tables 7a and 7b). For root traits, D^2 analysis classified these 15 elite lines into six clusters with three lines viz; KDM-1236, KDM-1051 and KDM-1156 in cluster-I, five lines viz; KDM-402, KDM-717, KDM-912A, KDM-918A and KDM-932A in cluster-II, four lines viz; KDM-361A, KDM-372, KDM-343A and KDM-331 in cluster-III; and KDM-961 in cluster-IV, KDM-463 in cluster-V and CM-129 in cluster-VI (Table 4). Maximum inter-cluster distance (D^2) value (9.34) was recorded between cluster III and cluster IV followed by 9.10 between cluster-IV and VI and 8.63 between cluster-I and cluster-VI (Table 5). Maximum cluster means was observed for cluster IV and V (Tables 7a and 7b).

Comparative analysis of the genetic diversity based on phenotypic variance (D^2 statistics) and genetic distance (GD) at the molecular level using SSR markers (Table 3) revealed that the phenotypic distance and the genotypic distance did not define the same pattern of clustering. Based on the two approaches lines KDM-1051, KDM-1156 were grouped into cluster -I, lines KDM-912A, KDM-918A, KDM-932A were grouped into cluster -II and lines KDM-372, KDM-343A, KDM-331 grouped into cluster -III. But lines KDM-961, KDM-1236 KDM-717, KDM-361A, KDM-402, KDM-463 and CM-129 showed scattered distribution across clusters generated through the two approaches. Few lines which were scattered and grouped into separate clusters is possibly due to the inherent lack of correlation between the loci underlying morphological divergence and genetic divergence. As the phenotypic divergence reflects small fraction of genes and genotype × environment interactions whereas the genetic divergence (done with the help of molecular markers) provides more precise and potentially more representative portrayal of divergence for the genome as a whole therefore better reflecting genetic divergence.

CONCLUSION

Inbred lines viz., CM-129, KDM-372, KDM-331, KDM-1051, KDM-402, KDM-463, KDM-717, KDM-912A, KDM-932A, KDM-343A, KDM-961, KDM-918A, KDM-1156 and KDM-1236 were clustered together as evident from the genetic distances and also from the dendrogram generated by SSR marker as well as in the dendrogram generated by phenotypic traits. This may be due to narrow diversity among them or because they may have originated from same source or pedigree. This is in agreement with earlier findings of Kumar *et al.* (2012) and Shukla *et al.* (2014) who demonstrated the correspondence of SSR marker distance with pedigree information in maize. Also, from the genetic diversity analysis results, maize inbred lacking their pedigree data could be identified based on their genetic distance to make hybridization between them to result in the development of a good hybrid. Hence, it could be concluded that the inbred lines viz; KDM-372, KDM-343A, KDM-331 and KDM-961 could be crossed in all possible combinations for improvement of seedling and root traits and for improvement of important moisture stress related traits viz; anthesis-silking interval, plant height, physiological traits and grain yield to develop composites or synthetics or single cross hybrids.

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