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Studies on drought tolerance in maize inbred lines using morphological and molecular approaches

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Abstract

A set of hundred homozygous maize inbred lines were analyzed for drought toleranceby studying twenty-four traits related to maturity, morphological, physiological, yield, quality and few 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). These lines were subjected to different stress regimes over years leading to identification of fifteen elite lines which performed well under droughtstress showing inbuilt drought tolerance. A set of 32 SSR markers, having genome-wide coverage, were chosen for genotyping the 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. Molecular diversity however, confirmed diverse genetic nature of six lines (KDM-372, KDM-343A, KDM-331, KDM-961, KDM-1051 and KDM-1156) showing drought tolerance. Exploitation of identified elite lines in a crossing program involving all possible combinations would help to develop hybrids with inbuilt mechanism to drought tolerance. Markers viz., umc -1766, umc-1478 and phi-061 recorded PIC >8 and alleles per locus more than 9 and therefore, discriminated the set of lines more efficiently. Genotyping data complemented by morpho-physiological parameters were used to identify a number of pair-wise combinations for the development of mapping population segregating for drought tolerance and potential heterotic pairs for the development of drought tolerant hybrids.

Abbreviations

SSR = Simple Sequence Repeat or Microsatellites
PIC = Polymorphism Information Content
DARwin = Dissimilarity Analysis and Representation for Windows
CM/CML = CIMMYT Coordinated Line
HKI = Haryana karnal inbred
KDM = Karewa Dryland Maize
D2 analysis = Divergence analysis
AICRP = All India Coordinated Research Programme
SKUAST-K = Sher-e-Kashmir University of Agricultural Sciences & Technology, Kashmir
CIMMYT = International Maize and Wheat Improvement Centre
AAU = Anand Agricultural University, Anand, Gujarat
MPUAT = Maharana Pratap University of Agriculture and Technology, Udaipur
RBD = Randomized Block Design
DNA = Deoxyribo Nucleic Acid
CTAB = Cetyl- Tri Methyl Ammonium Bromide
PCR = Polymerase Chain Reaction
UPGMA = Unweighted Pair Group Method with Arithmetic mean
ANOVA = Analysis of Variance
GCV = Genotypic Coefficient of Variation
PCV = Phenotypic Coefficient of Variation
ASI = Anthesis-Silking Interval
EPP = Ears per Plant

Introduction

Drought stress is an inevitable and recurring feature of global agriculture. Drought at critical stages of crop growth and development is the major limiting factor for maize production and productivity. Denmead and Shaw (1960) recorded a reduction in grain yield of 25, 50 and

21per cent due to drought, prior to silking, at silking andafter silking, respectively, indicating that silking stage is the most critical stage for moisture stress. An estimated 80 per centof the maize crop suffers periodic yield reduction due to drought stress (Bolanos and Edmeades, 1993). Edmeades *et al*. (1995), Saindass *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. 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). Sustaining maize production and productivity with stability under drought prone environments needs both agronomic management (cultural practices and In situ water management) and genotype improvement. Development of improved maize lines capable of withstanding drought stress at critical growth stages is reliable and affordable solution for poor marginal farmers growing maize in rainfed dryland and drought prone areas. Either selection of genotypes/cultivars/lines with stability in yield over environments or selection of lines on the basis of drought stress adaptive secondary traits like ASI, EPP, root biomass, along with grain yield under managed stress can be some of the approaches for developing drought tolerant genotypes.

Material and methods

Field evaluation

A set of hundred homozygous maize inbred lines belonging to AICRP Maize Srinagar Centre along with check lines from various institutions (SKUAST-K, CIMMYT, AAU, MPUAT) were evaluated during the present study. The description of inbred lines under evaluation is given in Appendix-1. The experimental material was evaluated in factorial RBD over two years (Kharief, 2016 and 2017) with two replications. Each inbred line was planted in two row experimental plot of 1 metre length with inter and intra row spacing of 60 x 20 cm. These inbred lines were evaluated against four regimes viz;

- Well Watered (WW): water provided by flooding at 5 intervals viz; 3 weeks (21 days), 6 weeks (42 days), 9 weeks (63 days), 12 weeks (84 days) and 15 weeks (102 days) after sowing. (knee height, flowering stages and grain filling stages)
- Intermediate Stress (IS): water provided by flooding at 2 intervals viz; 3 weeks (21 days) and 6 weeks (42 days) after sowing (knee height, flowering time).
- Mild Stress (MS): water provided by flooding at 1 interval; 3 weeks (21 days) after sowing (knee height stage)
- Stress (S): No water provided at all (except at the time sowing)

Meteorological conditions for field evaluation

The meteorological data, including minimum andmaximum temperatures, relative humidity (RH) andrainfall were collected throughout the experimentalperiod for both the years (Appendix-2) assuggested by Banziger *et al*. (2000).

Pot evaluation

The same set of inbred lines were evaluated in pots for 24 days (till 4 leaf stage). The soil medium was sand clay (3:7) mixture. The experiment was laid in factorial RBD with three replications given over two years andthree moisture management regimes were followed viz.,

- Well watered: Water applied to 100% of the field capacity (Irrigated three times , after every 7 day interval till uprooting)
- Intermediate Stress: Water applied to 60% of the field capacity. (Irrigated two times , after every 7 day interval till uprooting)
- Stress: Water applied to 40% of the field capacity. (Irrigated once after 7 day interval till uprooting)

Field capacity is determined in laboratory by using a pressure plate to apply a suction of -1/3 atmosphere to a saturated soil sample. When water is no longer leaving the soil sample, the soil moisture in the sample is determined gravimetrically and equated to field capacity. In our study field capacity was determined by irrigating a test plot until the soil profile was saturated to a depth of about one metre. Then the plot was covered to prevent evaporation. The soil moisture was measured each 24 hours until the changes were very small, as the soil moisture content is the estimate of field capacity.

Agronomical and morphological traits

In field evaluation observations were recorded from each replication of the line in each treatment on traits viz., ASI, days to maturity, plant height (cm), leaf relative water content (%), canopy temperature (°C), chlorophyll content, ears plant^{-1}, kernels row^{-1}, 100 grain weight (g), grain yield plot^{-1} (g), protein content (%). In pot evaluation observations were recorded on root traits viz., seedling germination (%), number of seminal roots, number of crown roots, primary root length (cm), fresh root weight (g) and dry root weight (g). Observational data collected from both field as well as pot evaluation was used for statistical analysis to evaluate the type and

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

Source of Variation	d.f	ASI	Days to Maturity	Plant Height (cm)	LRWC (%)	Canopy temperature (°C)	Chlorophyll content (SPAD units)	Ears plant^{-1}	Kernels row^{-1}	100 grain weight (g)	Grain yield plot^{-1}	Protein content (%)
Replication	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**
Irrigation	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 × Irrigation	297	0.51**	7.03**	19.09**	118.78	1.22**	0.35	1.01*	0.11 **	2.05**	512.01**	0.07**
Irrigation × year	3	0.18*	83.96**	21026.50**	10542.30**	92.01**	218.10**	0.37**	1584.58**	595.90**	732946.10**	3.94**
Irrigationwithin Replication	7	0.04	0.80	59.35**	42.13	0.01	0.09	0.01	0.69	0.10	0.68	0.01
Irrigation within years within Replication	15	26.08**	278.96**	214103.26**	166265.59**	841.61**	1016.72**	1.82**	3035.85**	717.18**	1330511.65**	14.54**
Line × Irrigation × 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 (heritability)		0.66	0.84	0.91	0.78	0.82	0.94	0.77	0.63	0.85	0.95	0.99
Genetic Gain		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% level, respectively. σ^2g= Genotypic variance, σ^2p= Phenotypic variance, GCV= Genotypic coefficient of variation, PCV= Phenotypic coefficient of variation
d.f.= degrees of freedom, ASI= Anthesis-silking interval; LRWC = Leaf relative water content (%).

magnitude of variability and to identifydrought tolerant inbred lines.

Statistical analysis

The morphological data recorded during present investigation was subjected to following statistical and biometrical analyses:

ANOVA for all the traits in individual years (Year 1 and Year 2) and for data pooled over the years was carried out for testing variation among the inbred lines as per Verma *et al*. (1987). Genotypic and phenotypic variance was calculated using the method suggested by Johnson *et al*. (1955) for the single year and Al-Jibouri *et al*. (1958) for the data pooled over the years. The magnitude of PCV and GCV existing in a trait was worked out as per Burton (1952) Heritability (broad sense) was estimated for both single and pooled over environments as per the procedure presented by Burton and Dewane (1953), Johnson *et al*. (1955) and Hanson *et al*. (1956). Genetic advance at 5 per cent, selection intensity and expected genetic gain was worked out as per Lush (1949) and Johnson *et al*. (1955).The data was analyzed in factorial RBD with inbred lines, years and irrigation as factors in INDOSTAT software version 9.2. Further, multivariate analysis wasperformed using Mahalanobis' D^2 statistics (Mahalanobis, 1936). Treating D^2 as a generalized statistical distance, the criteria used by Toucher (Rao, 1952) was applied for determining the group constellation and clustering. The character-wise rank totals were used to calculate the per cent contribution of each character to the total divergence. Average inter- and intra- cluster distances were estimated as per the method given by Singh and Chaudhary (1985).

Molecular and genetic analysis

Following morphological and pot analysis of the hundred lines, fifteen inbred lines were identified as drought promising inbred lines. Subsequently they were subjected to SSR data analysis and cluster analysis. Extraction of plant DNA was carried out by CTAB method 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.

Phylogenetic analyses

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 was based on the neighbor joining method which was conducted using computer software program DARwin 5.0 (Perrier *et al*. 2003).

Results and discussion

Genotypic differential trait response to drought condition

As reported in Table 1, 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). Three way interactions (lines x irrigation x 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 x year, line x irrigation and irrigation x year and three way interactions *i.e.*, line x irrigation x 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 x treatment and environment x 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 under study in pooled analysis, thereby revealing the importance of environmental variance in the trait expression (Table1, 2). Kumar *et al*. (2014) observed similar results. GCV was high (> 20) for ears plant^{-1}, number of seminal roots, 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. GCV is high when values are greater than 20 (>20),

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

Sources of Variation	d.f	Germination %	Primary root length (cm)	Number of seminal roots	Number of crown roots	Fresh root weight (g)	Dry root weight (g)
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	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	1089	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(heritability)		0.84	0.84	0.77	0.95	0.95	0.95
Genetic Gain		24.71	47.39	50.77	73.69	80.7	79.7

d.f.= degrees of freedom, σ^2g= genetic variance, σ^2p= phenotypic variance, GCV (%)= genotypic coefficient of variation, PCV (%)= phenotypic coefficient of variation. *,** Significant at 5 and 1% level, respectively

medium when values are between 10 to 20 and is low when values are less than 10 (< 10). 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). 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 like 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 coupled with phenotypic selection and response of lines to water over the years confirmed identification of 15 elite lines (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). Phenotypic selection confirmed that the identified lines were superior due to their inbuilt mechanism against moisture stress with excellent parameters and positive traits. Highest desirable *per se* performance under stress conditions revealed that variability among the lines was genetic in nature and application of water had little or no effect on improving the traits under study.

Genetic diversity by molecular analysis

These identified fifteen elite drought 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. SSR primers have displayed deviation in few studies from the unexpected pattern where inbred lines are assumed to be highly homozygous and are expected to reveal only a single amplification product (allele) per locus, at least for a large majority of the loci analyzed for the locus umc-2372. 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' (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 between studies 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

Table 3 - 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	

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). Allele richness (also referred to as allelic diversity) is calculated as the average number of alleles per locus. It is a measure of genetic diversity indicative of a population's long-term potential for adaptability and persistence. A decrease in the allelic richness could lead to a reduction in the population's potential to adapt to future environmental changes, since this diversity is the raw material for evolution by natural selection (Greenbaum *et al.* 2014). Here in this study the average number of alleles per loci (11.00 to 4.00) was higher. 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 PIC value ranged from 0.886 (umc-1766) to 0.608 (Phi-051) with an average of 0.782 respectively. A PIC value of greater than. 0.7 is considered to be highly informative, whereas a value of 0.44 is considered to. be moderately informative. Clearly markers with greater numbers of alleles tend to have higher PIC values and thus are more informative (Hildebrand *et al.*1992). The PIC values provide an estimate of the discriminating power of a marker by considering not only the alleles at a locus but also relative frequencies of those alleles in the lines. The PIC value demonstrates the informativeness of the SSR loci and their potential to detect differences among the inbred lines based on their genetic relationships (Sserumaga *et al.* 2014). 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. The results were comparable with the findings of Shukla *et al.* (2014), Dubey *et al.* 2009, Nepolean *et al.* 2012, Sserumaga *et al.* 2014

Cluster analysis

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 matrix based on thirtytwo SSR markers ranged from 0.215 to 0.148 (fig-1). 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). Objective is to identify elite drought tolerant line for hybrid development but one line cannot be selected because drought is a complex QTL and one line cannot have all the QTLs . These 15 lines complement each other as we search for drought related traits as they were selected based on their genetic distance and

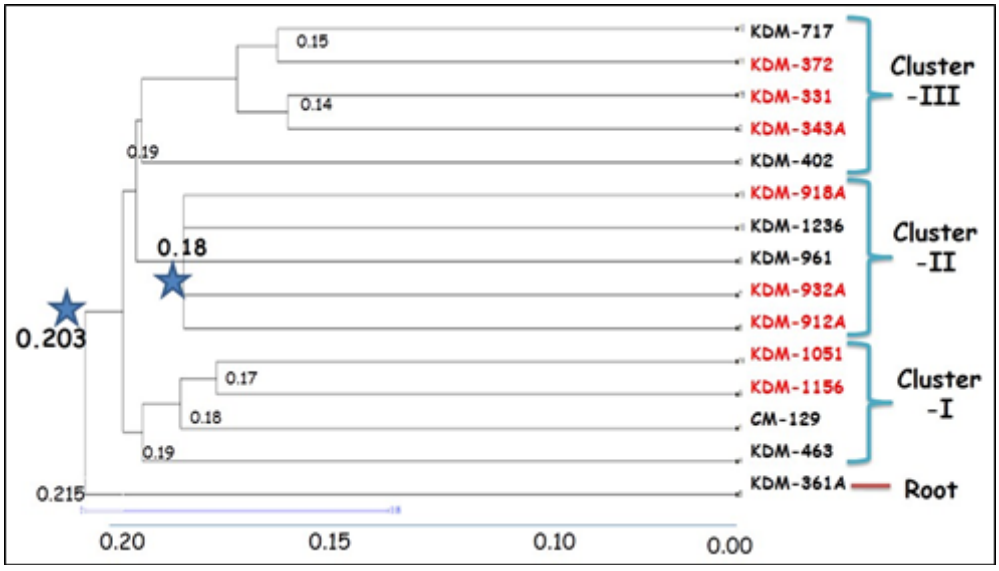

Fig. 1 - DARwin analysis of 15 elite lines for drought tolerance based on 32 SSR marker

Table 4 - 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 (Root)
			V	1	KDM-463			
			VI	1	CM-129			

Table 5 - Average inter-cluster (above diagonal) and intra-cluster (diagonal) distances among elite maize inbred lines for drought related & root traits (pooled over years

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 6 - Cluster means for morphological, maturity, physiological, yield, quality and root traits of elite maize inbred lines

	ASI	DM	PH	LRWC	CTF	CTM	EPP	KPR	100 GW	GYP	PC		G%	NSR	NCR	PRL	FRW	DRW
I	3	136.35	237.09	161.87	25.54	57.47	1.79	38.77	29.7	753.56	9.79	I	85.95	4.74	3.85	14.93	11.06	4.48
II	3	137.5	191.34	131.59	28.15	53.83	1.66	28.96	24.32	535.43	9.18	II	85.95	4.05	3.4	15.86	10.59	4.27
III	3	138.35	129.59	62.39	31.22	49.85	1.63	26.38	23.49	457.01	8.92	III	85.95	3.23	2.95	17.29	8.31	3.4
												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	3.9

ASI=anthesis silking interval, DM= days to maturity, PH=plant height (cm), LRWC= leaf relative water content(%), CTF= canopy temperature at flowering (0C), CTM= canopy temperature at maturity (0C), EPP= ears per plant, KPR=Kernels per row, 100GW= 100 grain weight (g), GYP= grain yield per plot (g), PC=protein content (%), G(%)= Germination(%),NSR= number of seminal roots, NCR= number of crown roots, PRL= primary root length, FRW= fresh root weight (g), DRW= dry root weight (g).

maturity, morphological, physiological, yield, quality, 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 cluster I and cluster III followed by a distance of 6.07 between cluster-I and cluster-II (Table-5). Maximum cluster means were observed in cluster-I (Table-6). 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 (Table-6). 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-4) 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.

Conclusions

Phenotypic selection confirmed that the identified lines exhibited stable yields under stress conditions due to their inbuilt stress adaptive secondary traits. Highest desirable *per se* performance under stress conditions revealed that variability among the lines was genetic in nature and application of water had little or no effect on improving the traits under study.The selected primers were highly polymorphic and the degree of diversity among the lines was high and PIC values were sufficient to group the population into different clusters.Cluster analysis of morphological and molecular markers distances did not show the same grouping. Morphological markers (viz., traits) were among the earliest markers used in evaluation of maize landraces. But morphological variability is often restricted, and not obvious at all plant developmental stages, strongly influenced by environment and show low polymorphism. Molecular markers are less affected by environment, estimate genetic diversity at the DNA level and high polymorphism nature of SSRs helps in DNA fingerprinting with accurate results. The higher diversity among the lines is due to long adaptation to local environmental conditions. Effectiveness of SSRs in determining genetic structure of lines should be combined with morphological traits to obtain comprehensive results in selection of drought tolerant traits and lines. Some lines were group 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. Additionally, from the genetic diversity analysis results, maize inbred lines lacking their pedigree data could be identified based on their genetic distance to make hybridization between them resulting 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 improving stress adaptive secondary traits viz., anthesis-silking interval, plant height, physiological traits,root traits along with stability in grain yield. These identified inbred lines would serve the base material for development of single cross hybrids having potential drought tolerance. In subsequent ongoing researches these inbred lines viz; KDM-372, KDM-343A, KDM-331 and KDM-1051 and KDM-1156 have the potential to be used innational multilocation trial kits and participatory plant breeding programmes with main focus on distribution to marginal farmers growing maize in dryland drought prone areas which is the main priority of drought tolerance maize.

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Appendix-1: Description of maize inbred lines under evaluation

Description	Institution	No. of Inbred lines	Colour
KDM-S9 Series	SKUAST-Kashmir	1	Yellow
KDM-S8 Series	SKUAST-Kashmir	33	Yellow
KDM-S6 Series	SKUAST-Kashmir	51	Yellow
KDM-S6 Series	SKUAST-Kashmir	3	White
KDM-S6 Series	SKUAST-Kashmir	1	Purple
CM/CML Series	IIMR, New Delhi	9	Yellow
HKI Series	MPUAT,Udaipur	2	Yellow

Appendix-2: The meteorological data, for the experimental years

month	Max temp	Min.Temp	Rainfall	RH1	RH2
Jan-15	7.19	-2.75	89.4	92.4	70.7
Feb-15	9.64	0.08	137	87.3	63.1
Mar-15	17.11	3.76	51.6	77.9	45.8
Apr-15	19.03	6.58	135.6	80.1	59.3
May-15	24.04	9.22	59	79.7	49.2
Jun-15	29.43	14.91	107.2	79.4	50.1
Jul-15	31.89	20.79	73.6	76.6	48.1
Aug-15	30.2	21.7	55.2	74.3	45.9
Sep-15	29.2	17.9	72.9	88.2	52.4
Oct-15	22.7	9.3	49.3	84.4	59.1
Nov-15	14.9	7.8	73.6	81.5	57.5
Dec-15	8.7	1.9	62.9	84.9	69
Jan-16	4.3	-3.2	67.5	94.2	79.5
Feb-16	8.6	0.07	78.9	91.1	66.4
Mar-16	15.6	3.51	38.6	78.9	44.3
Apr-16	19.3	7.01	130.5	83	53.8
May-16	22.9	8.7	43.8	81.4	56.6
Jun-16	30.4	22.4	15.2	70.1	49
Jul-16	31.3	23.4	30.1	80.3	48.1
Aug-16	31.4	20.9	27.6	74.4	42.8
Sep-16	30.1	16.2	96	88.5	64.8
Oct-16	24.3	7.8	8.9	87.3	53.2
Nov-16	16.8	6.2	12.8	82.8	53.7
Dec-16	8.1	-0.6	44.6	88.8	73