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Relationship between genetic distance among drought tolerant maize inbred lines with heterosis and specific combining ability under well-watered and managed drought conditions

Maize (*Zea mays* L.) is the third most important cereal grain, after rice and wheat, and is the world's largest grown cereal (Edmeades, 2013). Maize production in tropical regions is under threat from various stresses including drought, high temperatures and diseases. Drought is the major abiotic stress affecting maize production world-wide (Edmeades, 2013). Climatic changes may increase the incidence of droughts in some regions worsening the plight of maize farmers, especially those practising rain-fed crop agriculture. Improving drought tolerance in maize by breeding is one of the approaches to enhance sustainability of maize crop production under water limiting environments (Carena, 2011). In view of the need for high yielding drought tolerant maize hybrids, and the need to reduce costs in hybrid development, and to expedite drought tolerant hybrid deployment, use of aiding technologies such as molecular markers might be the solution. The aim of this study was to estimate molecular genetic distance among 12 drought tolerant tropical maize inbred lines and analyse its association with heterosis and specific combining ability in respect of grain yield and kernels per ear under well-watered and drought conditions.

All fieldworks were done at Agricultural Research Station, Arabhavi in Karnataka, India. Twelve drought tolerant tropical maize inbred lines (Table. 1) were hybridized following Griffing Method II of diallel mating in the 2012 *kharif* season. Sixty-six single-cross hybrids and twelve selfed progenies (parents) were generated and evaluated in the 2012-2013 *rabi* season. During evaluation of hybrids and their parents, there were two equal sections in the field of which one portion was well-watered throughout the growing period, and in the other portion there was imposition of severe drought stress by withdrawing irrigation water at 36 days after planting. In each of the environments (well-watered and drought stressed), the inbred line parents were laid out in a 4 x 3 alpha lattice design replicated twice, and the hybrids were laid out in a 11 x 6 alpha lattice design also replicated twice. Data were recorded for number of kernels per ear (KPE), and grain yield (GY) in tons per hectare (t ha^{-1}). Griffing (1956) method II model 1 was followed for combining ability analysis. The computations were done in WINDOSTAT version 8.5 and heterosis over mid-parent value, and specific combining ability of the crosses were determined.

Research Laboratory - 1, Department of Genetics and Plant Breeding, College of Agriculture, University of Agricultural Sciences, Dharwad was used for molecular marker genotyping of the twelve inbred lines that were involved in diallel mating. Twenty-two simple sequence repeat (SSR) primer pairs that were used for molecular genotyping are presented in Table 2. These primers were primarily chosen for being associated with drought tolerance traits either as anchor markers for quantitative trait loci (QTL) regions, or for being identified as drought tolerance candidate gene-specific SSRs in previous studies (Mohammadi *et al.,* 2008), and for showing high polymorphic information content. The SSR primers chosen are found on Maize GDB (http://www.maizegdb.org/ssr.php).

Deoxyribonucleic acid (DNA)was extracted from twelve drought tolerant inbred lines following a method that employs a detergent called cetyltrimethyl-ammonium bromide (CTAB) with some modifications. The quality of DNA was assessed visually after gel electrophoresis using 0.8% agarose gel with 1X TAE buffer. The gel was stained with ethidium bromide solution (10µg/ml) and the gel images were visualised using an Alpha Imager Gel Documentation System (Alpha InfoTech, USA). Most of the DNA samples were contaminated with ribonucleic acid (RNA), and in order to get rid of RNA, the DNA samples were treated with 2 µl of the enzyme RNAse at a concentration of 10 mg/ml. The samples were incubated at 37 °C for 30 minutes, and thereafter, 65 °C for 15 minutes.For amplification of primers by polymerase chain reaction (PCR), the SSR reaction mixture in each PCR tube consisted of 1 µl of template DNA (20 ng) and 19 µl of PCR master mix. The PCR master mix consisted of the following components: 2 µl of Taq buffer, 2 µl of dNTP mix, 1 µl of $MgCL_2$ at 2.5 mM, 0.5 µl each of forward and reverse primers, 0.2 µl of Taq DNA polymerase, and 12.8 µl of sterile distilled water. The total reaction mixture volume for amplification was 20 µl. Amplification of SSR primers was carried out on Mastercycler Gradient

Table 1. Drought tolerant inbred lines that were used in diallel crossing and for which genetic distances were estimated

Code	Inbred line	Pedigree	Origin
G1	DMR-M-81	CI – 4	International Centre for Maize and Wheat Improvement (CIMMYT)
G2	M4	KDMI – 16	All India Coordinated Maize Improvement Project (AICMIP), ARS Arabhavi
G3	FA6	ARYP – 73	AICMIP, ARS Arabhavi
G4	DMR-M-83	CI – 5	CIMMYT
G5	GPM36	ARYP – 36	Indian Institute of Maize Research, ICAR, Winter nursery, Hyderabad (IIMR, Hyderabad)
G6	DMR-M-88	CM – 501	ICAR – Indian Institute of Maize Research (IIMR), New Delhi
G7	DMR-M-84	KDMI – 10	AICMIP, ARS Arabhavi
G8	M39	ARYP – 39	AICMIP, ARS Arabhavi
G9	M53	ARYP – 53	Zonal Agricultural Research Station, Mandya, University of Agricultural Sciences, Bengaluru
G10	FA3	ARYP – 70	AICMIP, ARS Arabhavi
G11	GPM43	ARYP – 43	IIMR, Hyderabad
G12	GPM53	ARYP – 53	IIMR, Hyderabad

Table 2. Nucleotide sequences of 22 simple sequence repeat primer pairs and the annealing temperatures used during amplification by polymerase chain reaction

Sl. No.	PRIMER	SEQUENCE (52 – 32)	Annealing Temperature (°C)
1	umc1917F†	ACTTCCACTTCACCAGCCTTTTC	61.55
	umc1917R	GGAAAGAAGAGCCGCTTGGT	
2	umc2025F	CGCCGTAGTATTTGGTAGCAGAAG	61.65
	umc2025R	TCTACCGCTCCTTCGTCCAGTA	
3	umc1122F	CACAACTCCATCAGAGGACAGAGA	61.75
	umc1122R	CTGCTACGACATACGCAAGGC	
4	umc1862F	ATGGGCACATGAAAAAGAGACATT	61.4
	umc1862R	CCCATGAGAAGAGTGAAGACAACA	
5	umc1719F	CCTGGAAGCACCACTGATACTAGC	62.4
	umc1719R	AGCTCCAGCCTGCCTACCAG	
6	umc1447F	TAATACTCCTACTAACGGCGCTGC	61.25
	umc1447R	TCTGTCTCCCATGCCTGAAATAAT	
7	umc1432F	GGCCATGATACAGCAAGAAATGAT	61.5
	umc1432R	TACTAGATGATGACTGACCCAGCG	
8	umc2189F	CGTAAGTACAGTACACCAATGGGC	60.7
	umc2189R	ACACCGACTACAAGCCTCTCAACT	
9	umc1542F	TAAAGCTATGATGGCACTTGCAGA	61.4
	umc1542R	CATATTTGCCTTTGCCCTTTTGTA	
10	bnlg1179F	GCGATTCAGTCCGCAGTAGT	59.3
	bnlg1179R	GTACTGAACAAACCGTGGGC	
11	bnlg1014F	CACGCTGTTTCAGACAGGAA	59.25
	bnlg1014R	CGCCTGTGATTGCACTACAC	
12	bnlg2132F	GGCGAGAGAGGCAAAGTTAA	58.85
	bnlg2132R	GTCGCACAAGGGGATCAC	
13	bnlg1118F	CAGAGTTGATGAACTGAAAAAGG	57.65
	bnlg1118R	CTCTTGCTTCCCCCCTAATC	
14	bnlg2190F	TCCTCCTTCATCCCCTTCTT	58.9
	bnlg2190R	CCCAGTATCATTGCCCAATC	
15	bnlg1327F	TCTCTCTCGCGTGTGTGC	59.05
	bnlg1327R	TGGGTCTCCTTCTCCGTCTA	
16	umc1069F	AGAGAATCCCCAAGCAAACAAAC	61.8
	umc1069R	CTTCATCGGAGCCATGGTGT	
17	umc2237F	CTCAGCTACAGGAGCGAAGAGG	61.9
	umc2237R	GTCACTGCACGATCCATCACAT	
18	umc1852F	CTGTTTGCTATCTCCAAGTCTGCAT	61.75
	umc1852R	TTGCATGTAGTCACCTCGATGTTC	
19	umc1042F	AAGGCACTGCTACTCCTATGGCTA	61.2
	umc1042R	CTGACCTTTGAATTCTGTGCTCCT	
20	bnlg1866F	CCCAGCGCATGTCAACTCT	59.95
	bnlg1866R	CCCCGGTAATTCAGTGGATA	
21	dupssr12F	CAGGTACTACGTGCCGTG	55.05
	dupssr12R	CTAGAGACAAACGAGGCTAGG	
22	bnlg1035F	TGCTTGCACTGTCAGGAATC	59.05
	bnlg1035R	CAGCTCTGACACACCACACA	

† F = forward primer, R = reverse primer

Table 3. Dice (1945)'s molecular genetic dissimilarity index matrix of twelve maize inbred lines based on simple sequence repeats marker data

Genotype	DMR-M-81	M4	FA6	DMR-M-83	GPM36	DMR-M-88	DMR-M-84	M39	M53	FA3	GPM43
M4	0.490										
FA6	0.333	0.444									
DMR-M-83	0.469	0.423	0.308								
GPM36	0.333	0.259	0.407	0.346							
DMR-M-88	0.373	0.222	0.333	0.462	0.185						
DMR-M-84	0.542	0.333	0.255	0.347	0.373	0.294					
M39	0.417	0.294	0.451	0.347	0.373	0.373	0.375				
M53	0.478	0.510	0.429	0.489	0.469	0.469	0.522	0.391			
FA3	0.458	0.333	0.294	0.388	0.333	0.294	0.292	0.375	0.478		
GPM43	0.524	0.378	0.378	0.442	0.422	0.378	0.381	0.381	0.300	0.238	
GPM53	0.574	0.480	0.320	0.375	0.480	0.360	0.234	0.447	0.467	0.319	0.366

(Eppendorf) thermal cycler. The amplification conditions were as follows: (i) initialization step at 94 °C for 5 minutes, (ii) 1 minute denaturation at 94 °C, (iii) 1 minute for primer annealing (annealing temperatures are shown in Table 2) (iv)1 minute for primer extension at 72 °C. The regular PCR cycle, that is from (ii) to (iv) was repeated for 30 times before the final step, which is (v) completion of primer extension at 72 °C for 5 minutes. The PCR products were electrophoresed on a gel (2% Agarose + 2% Metaphor) stained with ethidium bromide (10µg/ml). Gel electrophoresis was done in 1X TAE buffer at 100V for about three hours and visualised using an AlphaImager Gel Documentation System (Alpha Innotech, USA). Photographs were taken from the gels using the gel documentation system and saved for later use during marker scoring. The SSR primer amplicons were scored by giving code '1' for present and '0' for absent and a binary data matrix was produced for the 22 markers and their alleles, and twelve genotypes. Dice (1945) molecular genetic dissimilarity indices (genetic distances) were determined among the 12 maize inbred lines using DARWIN version 6.0.12 software.

The genetic distance between any two maize inbred lines was determined as:

$$d_{ij} = \frac{b+c}{2a + (b+c)}$$

Where,

d_{ij} = dissimilarity between inbred line i and inbred line j

a = number of bands present in both inbred lines i and j

b = number of bands present in inbred line *i* but absent in inbred line j

c = number of bands absent in inbred line *i* but present in inbred line j.

The genetic distance matrix was used to group the inbred lines into clusters using the UPGMA (unweighted pair-group method us-ing the arithmetic averages) method.

Trait parameters *viz.,* mean per se performance, specific combining ability and mid-parent heterosis values for number of number of kernels per ear, and grain yield per plot (GY), for each pair of crosses involving the twelve inbred lines were determined as mentioned earlier. Pearson's correlation coefficients between genetic distance (GD) and the trait parameters were determined in SAS version 9.3 following the PROC CORR procedure (Anon., 2010).

Dice's molecular genetic distance between two parental inbred lines of each cross is shown in Table 3. The genetic distances ranged from 0.185 for the cross [DMR-M-88 / GPM36] to 0.574 for the cross [GPM53 / DMR-M-81], and the average distance was 0.385.Genetic distance indices were rather low, in contrast to distance indices reported in other studies. However, it is important to point out that in this investigation, all inbred lines were drought tolerant; and they were genotyped using informative SSR markers, most of which had been linked to performance in respect of various traits under drought (Table 2). Thus the drought tolerant inbred lines could be having a high level of similarity in respect of the SSR loci.

The dendrogram showing grouping of the twelve inbred lines using the UPGMA (unweighted pair-group method using the arithmetic averages) method (Fig.1). Six groups were identified at 41% distance level. The largest group consists of four inbred lines GPM53, DMR-M-84, DMR-M-83, and FA6. The second largest group consists of three members, which are DMR-M-88, GPM36 and M4. Next is a group with two members GPM43 and FA3. The remaining three groups contain single members M53, M39, and DMR-M-81.

Pearson's correlation coefficients between molecular genetic distance with grain yield and kernel set trait parameters under well-watered and under severe drought conditions are

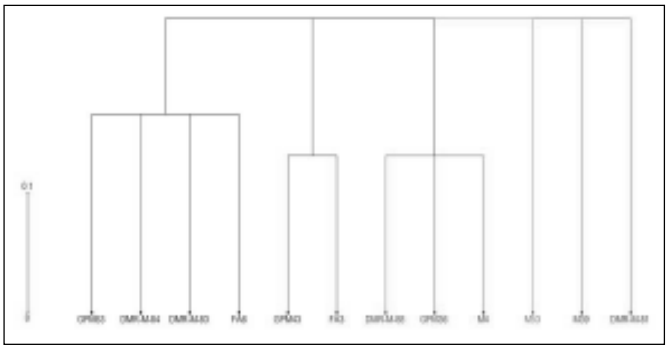

Fig. 1. A UPGMA tree showing grouping of 12 drought tolerant maize inbred lines at 41% distance level based on Dice dissimilarity indicescalculated using 22 SSR marker data. Scale bar (0 - 0.1) indicates genetic distance

Table 4. Pearson's correlation coefficients between molecular genetic distance with grain yield (t ha^{-1}) and number of kernels per ear trait parameters under well-watered conditions (above diagonal) and under severe managed drought (below diagonal)

	GD	KPE	GY	KPEsca	GYsca	KPEmph	GYmph
GD		-0.116	-0.068	0.002	-0.008	-0.025	-0.019
KPE	-0.012		0.282*	0.787**	0.168	0.554**	-0.082
GY	-0.121	0.490**		0.360**	0.832**	0.297*	0.558**
KPEsca	-0.060	0.879**	0.627**		0.376**	0.870**	0.302*
GYsca	-0.122	0.541**	0.910**	0.678**		0.367**	0.783**
KPEmph	-0.204	0.720**	0.555**	0.868**	0.595**		0.428**
GYmph	-0.246*	0.388**	0.742**	0.568**	0.789**	0.731**	

* Significant at 5 % level of probability, ** Significant at 1 % level of probability

GD – genetic distance, KPE – number of kernels per ear, GY – grain yield per plot, KPEsca – specific combining ability for KPE, GYsca – specific combining ability for GY, KPEmph – mid parent heterosis for KPE, GYmph – mid parent heterosis for GY

preserented in Table 4. In general, higher magnitude correlations were observed under severe drought conditions than under well-watered conditions. Under well-watered conditions, genetic distance was not significantly correlated to any trait parameter but the correlation coefficients were low to negative.The lower correlation coefficients between molecular genetic distance and number of kernels per ear and grain yield (t ha^{-1}) trait parameters under well-watered conditions could have been due to the fact that variation in the traits for which the SSR markers are linked to and the traits' contribution to grain yield (t ha^{-1}) is more under drought stress. However, molecular genetic distances remain constant across all environments. However, under severe drought stress there was significant and negative correlation between genetic distance and mid-parent heterosis for grain yield (GYmph) ($r = -0.246$, $p < 0.05$); other correlations between GD and trait parameters were low, negative and not significant. Fernandes *et al.* (2015) highlighted that for there to be a positive association between genetic distance and mid-parent heterosis, the parentage must be different in terms of allelic frequency controlling the plant trait under study. However, in this investigation the parents used showed a high level of similarity at the informative SSR loci associated to grain yield and related traits. The high level of similarity is reflected in the low genetic distance indices (Table 4), therefore there was lack of allelic frequency differences. The lack of allelic frequency differences among parental lines was most probably the cause of the negative association between molecular genetic distance and mid-parent heterosis (Fernandes *et al.,* 2015). This explanation should be valid as reason for the negative though non-significant correlation coefficients between genetic distance and other trait parameters including mid-parent heterosis for number of kernels per ear, and mean trait values and specific combining ability values for both grain yield (t ha^{-1}) and number of kernels per ear. A negative correlation between molecular genetic distance and mid-parent heterosis can be observed if the markers used are not associated with quantitative trait loci (QTLs) that determine the trait under study (Lanza *et al.,* 1997). To prevent that, the markers used in this study, as mentioned earlier, were selected for being linked to important traits for productivity under drought such as anthesis-silking interval, grain yield and delayed leaf senescence. The significant negative correlation between genetic distance and mid-parent heterosis for grain yield (t ha^{-1}), together with the non-significant negative correlation coefficient between GD and mean grain yields (t ha^{-1}) and specific combining ability effect, under drought, suggests that some crosses involving similar inbred lines gave higher and positive mid-parent heterosis, positive specific combining ability values, and higher *per se* performance.

Correlation coefficients among mean trait value, mid-parent heterosis and specific combining ability for the three traits were positive and in most cases significant under well-watered conditions - the only not significant correlation coefficient was between kernels per ear (KPE) and specific combining ability for grain yield (GYsca). Under severe drought, all correlation coefficients among mean trait value, mid-parent heterosis and specific combining ability for the three traits were positive and significant.The significant positive correlation coefficients between mean trait values for grain yield (t ha^{-1}) and number of kernels per ear, and between mean trait values for each of the traits and sca effect of the other trait, and between sca effects of the two traits show that the traits are strongly interrelated at phenotypic and genetic levels. This was true especially under drought. The significant positive correlation between mean trait values and specific combining ability for each trait show that non-additive gene action was important for expression of the traits.

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References

Anonymous, 2010, SAS System for Windows. Version 9.3. SAS institute Inc., Cary, NC, USA.

Carena, M. J., 2011, Germplasm enhancement for adaptation to climate changes. *Crop Breed. Appl. Biotechnol.,* 11: 56-65.

Edmeades, G. O., 2013, Progress in Achieving and Delivering Drought Tolerance in Maize - An Update by. *The International Service for the Acquisition of Agri-Biotech Applications,* 1-44.

Fernandes, E. H., Schuster, I., Scapim, C. A. and Vieira, E. S. N., 2015, Genetic diversity in elite inbred lines of maize and its association with heterosis, 14(2): 6509-6517

Griffing, B., 1956, Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.,* 9: 463-493.

Lanza, L. L. B., De Souza, C. L., Ottoboni, L. M. M., Vieira, M. L. C. and De Souza, A. P., 1997, Genetic distance of inbred lines and prediction of maize single-cross performance using RAPD markers. *Theor. Appl. Genet.,* 94 (8): 1023-1030.

Maize GDB. Maize Genetics and Genomics Database. Retrieved from http://www.maizegdb.org/ssr.php

Mohammadi, S. A., Prasanna, B. M., Sudan, C. and Singh, N. N., 2008, SSR heterogenic patterns of maize parental lines and prediction of hybrid performance. *Biotechnol. Biotechnol. Equip.,* 22 (1): 541-547.