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Analysis of drought tolerant and susceptible maize genotypes using SSR markers tagging candidate genes and consensus QTLs for drought tolerance

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Abstract

Drought stress, particularly at flowering stage, has been identified as the most important factor limiting maize production and productivity in India. In this study, a set of 24 tropical maize lines with differential responses to drought stress, including 16 lines from CIMMYT (Mexico) and eight lines from India, were characterized using 37 polymorphic microsatellite/SSR markers, including 29 SSRs tagging specific candidate genes involved in drought stress tolerance in maize. These genes, distributed on nine of the ten maize chromosomes, also colocalized with 17 'consensus QTLs' for various morpho-physiological traits associated with drought tolerance at flowering stage. The analysis using these 37 candidate gene-specific and drought 'anchor' markers tagging consensus QTLs led to unambiguous differentiation of the genotypes as well as assessment of genetic diversity in these important genetic resources. A total of 119 SSR alleles with a mean of 3.22 alleles per locus were identified. Polymorphism Information Content (PIC) of the 37 SSR loci ranged from 0.09 (*umc1627*) to 0.78 (*umc1056* and *bnlg1866*), with a mean PIC of 0.56. The study resulted in identification of eleven highly informative markers with PIC values ≥0.65, as well as five unique SSR alleles in DTPW-C9-F55-2-3, DTPW-C9-F115-1-4, DTPY-C9-F142-1-2, K64R and CML537. Pair-wise genetic similarity (GS) values, estimated using Jaccard's coefficient, ranged between 0.14 (HKI1025-K64R; HKI1025-CML247) and 0.74 (HKI-335-HKI-209), with a mean GS of 0.31, indicating high level of genetic divergence among the genotypes selected for the study. Cluster analysis revealed clear genetic differentiation of the DTP (drought tolerant population) lines developed at CIMMYT (Mexico) from those developed and identified in India (e.g. CM140). Principal Component Analysis (PCA) aided in further elucidation of the genetic relationships as well as differentiation of genotypes largely based on their phenotypic responses to drought stress. The analysis also led to identification of specific, highly informative SSR markers, namely *dupssr12* (bin 1.08), *umc1042* (bin 2.07), *bnlg1866* (bin 1.03), *umc1056* (bin 5.03), *dup13* (bin 7.04), *umc1069* (bin 8.08), *umc1962* (bin 10.03), *bnlg1028* (bin 10.06) and *umc1344* (bin 10.07), which significantly contributed to the differentiation of the drought tolerant and susceptible genotypes analysed in the study. These SSR markers could be further validated and potentially deployed in molecular marker-assisted breeding for drought tolerance in maize.

Key words: Maize, drought, SSRs, candidate genes, consensus QTL, anchor markers

Introduction

Drought and low soil fertility are among the major abiotic stresses affecting the production and productivity of maize in several countries worldwide, including India [1]. It is important to note that ~80 per cent of the total area under maize in India (amounting to ~5.3 million hectares of ~7 million hectares) is under rainfed conditions and is severely vulnerable to drought stress. The destructive impact of drought may grow as the specter of climate change becomes a reality. Climate change may increase flooding in some regions while further intensifying the frequency and magnitude of drought in others. Therefore, genetic enhancement of drought stress tolerance of maize, particularly at the flowering stage, was identified as the topmost priority of the National Agricultural Research System (NARS)[1].

The complex expression of drought tolerance makes it difficult to analyze using conventional genetic methods. Although major progress to date has been achieved through breeding [2-4], this approach not only remains slow and time-consuming. Also, breeding of varieties that are adapted to arid and semi-arid areas is not straightforward because of contradicted demands between biomass accumulated required for growing maize and stress avoidance via a reduction of transpiration under water scarcity [5]. Compounding

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these difficulties is the fact that drought occurrence is highly unpredictable over time and space [6], and diverse strategies are adopted by the genotypes to combat the stress depending on the timing, severity and stage of crop growth.

Integrating molecular approaches in breeding for drought tolerance could increase significantly the potential for genetic gain under water-limited conditions [4, 7]. The microsatellite or SSR markers are PCR-based, genetically codominant, robust, reproducible, hypervariable, informative and reasonably easy-to-use [8]. Along with the Single Nucleotide Polymorphisms (SNPs), the SSR markers, which are abundant in maize (http://www.maizegdb.org) offer significant advantages in DNA fingerprinting, genetic diversity analysis, gene/QTL mapping, and molecular marker-assisted breeding in crops like maize.

The responses of the genotypes to drought stress are governed by the activity of several genes involved in diverse pathways, including 'constitutive' QTL and 'adaptive' QTL [5]. An array of studies in the last 10-15 years led to the detection of several such QTLs influencing important component traits related to drought stress tolerance in maize [9-14]. Further, meta-analysis of such QTL data led to the identification of specific 'consensus QTL' indicating those QTL identified in several studies worldwide using different mapping populations for specific traits [5]. For example, QTL analysis of component traits, such as anthesis-silking interval (ASI) and ear number per plant, influencing drought tolerance of maize led to identification of some consensus QTLs, including one on chr.2 (bin 2.08) and another on chr.10 (bin 10.03) [14]. DNA-based markers located in such genomic (bin) locations could potentially serve as informative 'anchor' markers for molecular marker-assisted selection as well as functional genomics. In addition to the consensus QTLs, analysis of individual genes, transcriptome profiling as well as *in silico* mapping led to identification of specific candidate genes with significant influence over drought stress tolerance in maize, many of which colocalise with the consensus QTLs for drought tolerance [5, 11].

Keeping this information in view, the present study was undertaken to analyze the genetic relationships among a set of 24 inbreds with differential responses to drought stress in India, particularly at the flowering stage, using candidate gene-specific SSR markers and drought anchor SSR markers tagging consensus QTLs, for identifying potentially informative markers.

Materials and methods

A set of 24 inbred lines, including 16 DTP (Drought Tolerant Population) lines developed at CIMMYT, Mexico, and eight Indian inbreds (HKI and CM lines), were employed in this study (Table 1). These lines were selected based on their responses to drought stress at different locations in India (Delhi, Hyderabad and Pusa, Bihar) during 2006-2008. A summary of these responses are provided in Table 1.

The DTP lines were constituted at CIMMYT, Mexico, during the mid-1980s using 25 putative drought-tolerant sources, including Tuxpeno Sequia C_8, Latente, Michoacan 21, Suwan 1, crosses of CIMMYT populations 22, 32, 62, 64 and 66, landraces, Corn Belt hybrids, and germplasm from Thailand, Brazil and South Africa. Details of the selection and improvement procedure were given by Edmeades and Deutsch [15]. The DTP lines were categorised on the basis of kernel colour as DTPY (yellow) and DTPW (white). CM139 (drought susceptible) and CM140 (drought tolerant) were included in the present study as 'reference' genotypes, as these lines have been extensively characterized by the Maize Genetics Unit, IARI, at the phenotypic level, including responses to various biotic and abiotic stresses, and at the molecular level using SSR markers.

Thirty-seven SSR markers covering different bin locations of the maize genome (Fig. 1) were employed for molecular profiling of the selected genotypes, including selected set of SSR markers tagging specific candidate genes associated with drought stress tolerance in maize (Table 2), many of which also colocalise with consensus QTL identified in various studies worldwide, including India [5, 12-14]. The primer information for the selected SSR markers is available in public domain (http://www.maizegdb.org).

Genomic DNA from the selected genotypes was isolated from the leaves of three-week old seedlings using modified CTAB procedure [16]. The DNA was dissolved in Tris-EDTA buffer (1M Tris: 0.5M EDTA) and quantified using a Spectrophotometer (Bio-Tek Instruments, USA). Absorbance readings were recorded at 260nm and 280nm.The DNA quality was checked using 0.8% agarose gel electrophoresis and then diluted by Tris-EDTA buffer to the concentration 10ng/ul.

The PCR amplifications were performed in a Peltier Thermal cycler-100 or Dyad (MJ Research, USA) with a final volume of 12ul having 15-20 ng of genomic DNA. PCR mixture of 12ul contained 20ng of genomic DNA template, 10x PCR buffer, 25mM $Mgcl_2$, 10mM

Table 1. Pedigree and drought stress responses of inbred lines analysed in this study.

S.No.	Inbreds	Pedigree/source population	Source*	Response to drought stress[#]
1	DTPW-C9-F55-2-3	Derived from a pool comprising	CIMMYT	Tolerant
2	DTPW-C9-F115-1-4	25 putative drought-tolerant sources,		Tolerant
3	DTPY-C9-F46-3-1	including germplasm from Mexico,		Moderately tolerant
4	DTPY-C9-F103-5-4	USA, Thailand, Brazil and		Tolerant
5	DTPY-C9-F142-1-2	South Africa		Moderately tolerant
6	DTPY-C9-F102-4-5			Susceptible
7	DTPY-C9-F148-2-2			Susceptible
8	DTPY-C9-F46-1-2			Tolerant
9	K64R	Pride of Saline (South Africa)	INRA	Moderately tolerant
10	SCMALAWI	-NA-	INRA	Susceptible
11	CML537	MAS[206/312]-23-2-1-1-B*5	CIMMYT	Susceptible
12	CMLP1	Ac7643	CIMMYT	Moderately tolerant
13	CMLP2	Ac7729/TZSRW	CIMMYT	Susceptible
14	CML91	Pop.42 Northern Temp./German Mix	CIMMYT	Susceptible
15	CML247	Pool 24 (Tuxpeño)	CIMMYT	Susceptible
16	CML360	SA4C2F(21/26)	CIMMYT	Moderately tolerant
17	HKI 209	Pool 10	DMR	Moderately tolerant
18	HKI 335	Pool 10	DMR	Moderately tolerant
19	HKI 586	CH3	DMR	Susceptible
20	HKI 1011	BC175	DMR	Susceptible
21	HKI 1025	BC175	DMR	Susceptible
22	CM138	IPA21-10 (AD 6091)	IARI	Moderately tolerant
23	CM139	(Tarun x Makki Safed1)-Y63	PAU	Susceptible
24	CM140	J617-61	PAU	Tolerant

*CIMMYT: International Maize and Wheat Improvement Center, Mexico; INRA: Institut National de la Recherche Agronomique, Montpellier, France; DMR: Directorate of Maize Research, New Delhi; IARI: Indian Agricultural Research Institute, New Delhi; PAU: Punjab Agricultural University, Ludhiana

[#]Drought responses based on evaluation under field and rain-out shelter conditions during *Kharif*-2007 & 2009 at IARI, New Delhi, and at Maize Winter Nursery, Hyderabad, during *Rabi* 2006-07. Tolerant: short ASI (<5 days), low (<25%) leaf senescence at flowering, and low grain yield penalty (<25%) compared to well-watered conditions; Moderately tolerant: intermediate ASI (6-8 days), moderate (<50%) leaf senescence, and moderate (>25-50%) grain yield penalty; Susceptible: long ASI (>8 days or barren plants), high (>50-100%) leaf senescence, and high (>50-100%) grain yield penalty.

dNTPs, 10mM Primer and 3U/ul Taq DNA Polymerse. PCR conditions were as follows: Initial denaturing step at 94^{o}C for 2 minutes followed by 34 cycles of 94^{o}C for 1 minute, 58-62^{o}C (depending on the respective primer annealing temperature) for 2 minutes and 72^{o}C for 2 minutes. In the last cycle, primer extension at 72^{o}C for 7 minutes was given. The PCR amplified products were resolved on a 3.5% super-fine resolution agarose (Amresco, USA) gel, using a horizontal submarine gel electrophoresis system (Biorad Submarine Gel Electrophoresis Unit, USA) following the procedure described by Bantte and Prasanna [17]. A 50bp ladder (Bangalore Genei, India) was used as a molecular size standard. The gel images were captured using a Gel Documentation System (Alpha Imager, USA).

The SSR data was subjected to statistical analysis as follows. For each polymorphic SSR marker, 'Polymorphism Information Content' (PIC) was determined as described by Bantte and Prasanna [17]. PIC is a measure of allele diversity at a locus and is equal to $1\text{-}\Sigma f_i^2$, where f is the frequency of i^{th} allele. Genetic similarity (GS) based on SSR data was calculated for all possible pairs of genotypes using Jaccard's coefficient (J), based on the formula, $GS_J = N_{11}/(N_{11} + N_{10} + N_{01})$, where N_{11} is the number of alleles present in both individuals, N_{10} is the number of alleles present only in one of the pair (individual *i*), and N_{01} is the number of alleles present only in the individual *j*. The similarity matrix was further analyzed by employing UPGMA (Unweighted Paired Group Method using Arithmetic averages) clustering algorithm. Principal

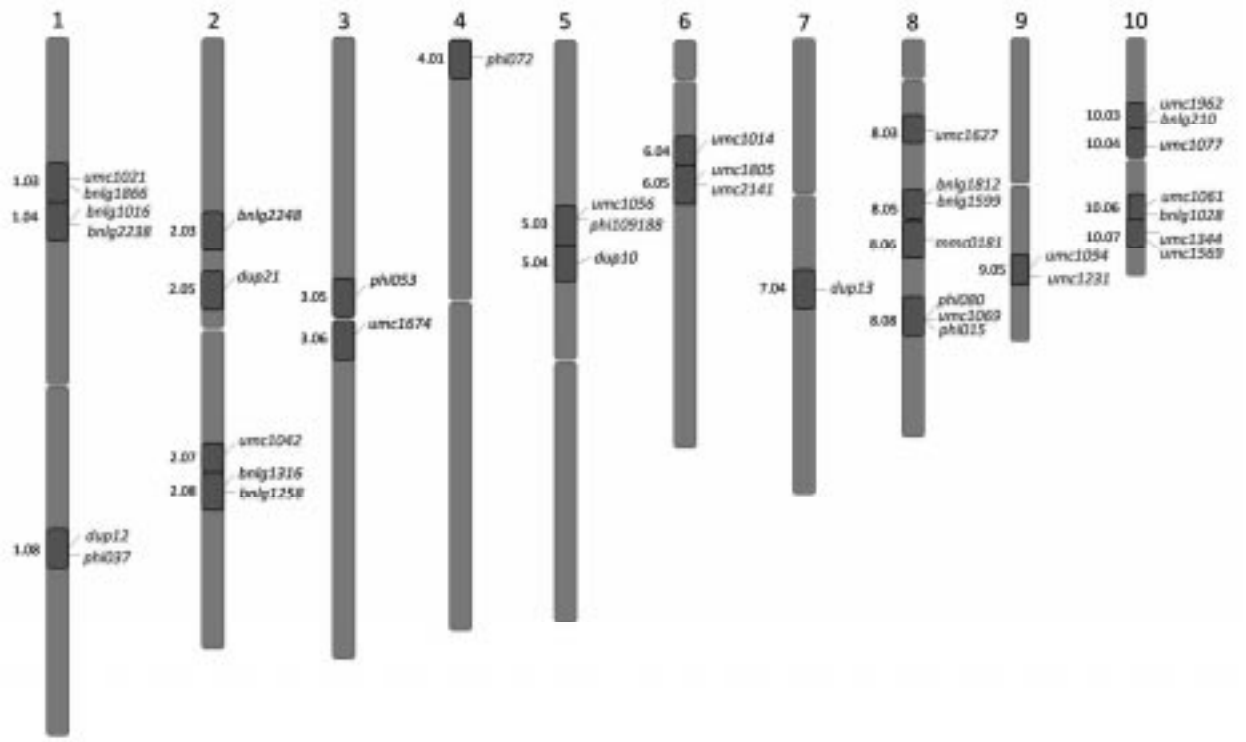

Fig. 1. Illustration of the chromosomal locations of candidate gene-specific SSRs (in red letters) colocalising with consensus QTLs, and SSR markers tagging only consensus QTLs (in blue letters) employed in the study. The bin locations of the markers are indicated on the left side of each chromosome

Component Analysis (PCA) was also undertaken to summarize the variation of the original SSR dataset, and to make a graphical presentation of the genetic relationships among the selected inbred lines. Both cluster analysis and PCA were implemented using DARwin5 software [18].

Results and discussion

Analysis of molecular polymorphisms in the 37 SSR loci led to detection of 119 alleles (Table 3; Fig. 2), with a mean of 3.22 alleles per locus. In the present study, 21 SSR loci with di-repeat motif were analysed, while the SSRs with higher repeat motifs were as follows: tri-repeat (two loci), tetra-repeat (seven loci), penta-repeat (two loci) and complex repeats (five loci). Although the tri-nucleotide or higher repeat SSR loci were reported to be less polymorphic than those with di-repeats [19], these loci were found to be significantly associated with coding regions of the genome, unlike the di-repeat SSRs.

The PIC (Polymorphism Information Content) value of a microsatellite locus is influenced by the number of alleles (allele richness) as well as distribution of these alleles across the genotypes. The PIC values of the SSR loci ranged from 0.09 (*umc1627*) to 0.78 *(umc1056* and *bnlg1866),* while the mean PIC was 0.56 (Table 3). Eleven SSR loci recorded PIC values ≥0.65 (*bnlg1028, dup12, bnlg2248, umc1042, umc1056, umc1014, dup13, bnlg1866, umc1069, umc1962* and *umc1344*); such loci could be highly useful in genotype differentiation and genetic diversity analysis. PIC is usually directly correlated to the repeat-type of the SSR locus [20]. The 21 SSR loci with di-repeat motifs recorded a mean PIC value of 0.58, while the 16 SSR loci with other repeat motifs recorded a slightly lower mean PIC value of 0.53. Significantly, four of the 11 SSR loci, namely *umc1056*, *umc1069*, *umc1962* and *umc1344*, with high PIC values (>0.65) represent those with tr- or higher repeat motifs. It is also worth mentioning that all the 11 highly informative SSR markers, except for *bnlg2248* and *umc1014*, identified in this study were candidate gene-specific, tagging important genes coding for superoxide dismutases (*SOD4, SOD9*), glutathione-S-tranferase1 (*gst1*), SNF1-related protein kinase (*SRK2E*), sucrose phosphatase (*spp1*), NACL stress protein1 (*nac1*) and cytokinin response regulator2 (*crr2*).

The level of polymorphism displayed by the 37 SSR markers in the present study was again comparable to the mean PIC value reported in some

Table 2. List of selected candidate genes for drought tolerance and associated SSR markers colocalising with consensus QTLs at specific genomic (bin) locations in maize.

Bin location	Candidate gene name*	Product	SSR markers
1.03	*hsp26* *fad8*	Heat shock protein26 Fatty acid desaturase8	*umc1021; bnlg1866*
1.04	*SOD4* *SOD9*	Superoxide dismutase4 Superoxide dismutase9	*bnlg1016* *bnlg2238*
1.08	*cpm2* *vp14(NCED)*	Chaperonin2 Vivaparous14 (9-cis-epoxy carotenoid desaturase)	*dup12; phi037*
2.07	*CBF3*	CBF3-like transcription factor	*umc1042*
4.01	*mtl1*	metallothionein1	*phi072*
5.03	*bip1*	Binding protein homolog1	*phi109188; umc1056*
5.04	*mip1* *MYB3* *bt1*	Major intrinsic membrane protein1 Drought responsive cis-acting element ADP-glucose pyrophosphorylase small subunit	*dup10*
6.05	*dhn1 (rab17)* *SOD3* *esp1*	Dehydrrin1 (responsive to abscisic acid1) Superoxide dismutase3 Embryo specific protein1	*umc1805; umc2141*
7.04	*SRK2E*	SNF1-related protein kinase	*dup13*
8.03	*SOD3c* *p5cs* *oec23*	Superoxide dismutase3c 1-Pyrroline-5-carboxylate synthetase Oxygen evolving complex 23	*umc1627*
8.05	*spp1* *vgt1*	Sucrose phosphatase Vegetative to germinal transition1	*bnlg1812; bnlg1599*
8.06-8.07	*sps1*	Sucrose phosphate synthase1	*mmc0181*
8.08	*gst1*	Glutathione-S-transferase1	*phi015; phi080; umc1069*
9.05	*sod9*	Superoxide dismutase 9	*umc1094; umc1231*
10.03	*abp4* *ppo1* *nac1*	Auxin binding protein homolog4 Polyphenol oxidase1 NaCl stress protein1	*umc1962; bnlg210*
10.06	*por2*	Porin2	*umc1061; bnlg1028*
10.07	*crr2*	Cytokinin response regulator 2	*umc1344; umc1569*

*The candidate genes associated with drought tolerance were identified based on published information and MaizeGDB (http://www.maizegdb.org)

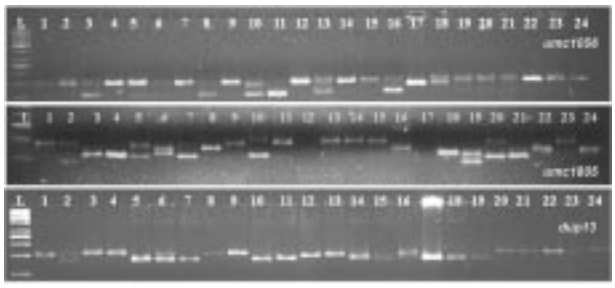

Fig. 2. SSR polymorphisms in the selected maize inbred lines illustrated for some of the loci (*umc1056*, *umc1085* and *dup13*). 'L' indicates the 50bp molecular size standard. The lane order (1 to 24) is same as the list of genotypes presented in Table 1.

earlier studies [17, 21-23]. In a study of 102 inbreds, including genotypes from the Asian countries, Mexico, USA and Germany, George et al. [24] recorded a mean PIC of 0.59 across 76 SSR loci, with a range of 0.14 to 0.83. The variation in the PIC values reported in different studies could be attributed to several reasons, including the germplasm analyzed, the nature and types of repeats in SSR loci, as well as methodology employed for allele detection.

In the present study, the number of 'rare' SSR alleles (those alleles with individual frequencies <0.10) was found to be eleven. The number of 'unique' alleles (each such allele limited to only one among the 24 selected lines) was five, which were identified in

Table 3. Details of polymorphisms in the 37 SSR loci analysed in the study

S.No.	SSR locus	Bin	Repeat motif	No. of alleles	PIC
1	*umc1021*	1.03	GT	3	0.56
2	***bnlg1866***	1.03	AG	4	0.78
3	*bnlg2238*	1.04	AG	2	0.51
4	*bnlg1016*	1.04	AG	2	0.58
5	***dup12***	1.08	AC	3	0.69
6	*phi037*	1.08	AG	3	0.40
7	***bnlg2248***	2.03	AG	4	0.65
8	*dup21*	2.05	AG	3	0.41
9	***umc1042***	2.07	GA	5	0.73
10	*bnlg1316*	2.08	AG	4	0.51
11	*bnlg1258*	2.08	AG	4	0.61
12	*phi053*	3.05	ATAC	3	0.47
13	*umc1674*	3.06	AC	2	0.49
14	*phi072*	4.01	AAAC	3	0.48
15	*phi109188*	5.03	AAAG	2	0.49
16	***umc1056***	5.03	AGCA	5	0.78
17	*dup10*	5.04	AC	5	0.64
18	***umc1014***	6.04	GA	3	0.71
19	*umc1805*	6.05	CT	5	0.33
20	*umc2141*	6.05	CT	3	0.51
21	***dup13***	7.04	CA	3	0.69
22	*umc1627*	8.03	GTAC	2	0.09
23	*bnlg1599*	8.05	AG	3	0.52
24	*bnlg1812*	8.05	AG	5	0.59
25	*mmc0181*	8.06-8.07	CA-GA	4	0.60
26	*phi015*	8.08	AAAC	3	0.54
27	*phi080*	8.08	AGGAG	2	0.47
28	***umc1069***	8.08	GGAGA	2	0.69
29	*umc1094*	9.05	Complex	2	0.48
30	*umc1231*	9.05	GA	4	0.63
31	*bnlg210*	10.03	Complex	2	0.47
32	***umc1962***	10.03	Complex	4	0.69
33	*umc1077*	10.04	Complex	3	0.34
34	***bnlg1028***	10.06	AG	3	0.70
35	*umc1061*	10.06	TCG	3	0.57
36	***umc1344***	10.07	GTTC	2	0.68
37	*umc1569*	10.07	GCA	4	0.57

*Highlighted SSR loci recorded PIC values ≥0.65.

bnlg1812, *mmc0181*, *umc1962*, *bnlg1258* and *phi037*. These unique alleles were detected in DTPW-C9-F55-2-3, DTPW-C9-F115-1-4, DTPY-C9-F142-1-2, K64R and CML537. Interestingly, four of the five unique alleles were found in the maize lines developed at CIMMYT, Mexico. The presence of unique alleles may be an indication of relatively high rates of mutation in such SSR loci [25] or introgression of alleles from some exotic germplasm [21]. Although the reasons for such high occurrence of unique alleles could not be ascertained in the present study, these alleles could be of potential value in effectively differentiating specific genotypes.

The SSR data was utilized to compute pair-wise genetic similarity (GS) values between the 24 inbreds using Jaccard's coefficient. The GS values ranged between 0.14 (HKI1025 and K64R; HKI1025 and CML247) and 0.74 (HKI-335 and HKI-209), with a mean GS of 0.31, indicating high genetic divergence among the selected genotypes. Such high genetic diversity in the tropical maize genotypes was also reported by George et al. [24]. Cluster analysis of the GS matrix using UPGMA algorithm revealed four major clusters (Fig. 3a). Cluster I comprised almost all the DTPY and DTPW lines, except for two, while Cluster II included two DTPY lines (DTPY-C9-F148-2-2 and DTPY-C9-142-1-2) along with K64R. Cluster III comprised mostly CIMMYT lines (CML91, CML537, CML360, CMLP1 and CMLP2), besides one African genotype, SCMALAWI. Almost all the genotypes, except CML247, were included in Cluster IV. As expected, high genetic divergence was noticed between CM139 and CM140, the two reference genotypes included in the study, as they were placed in two different sub-clusters in Cluster IV.

The patterns of genetic relationships displayed by cluster analysis were further elucidated and reconfirmed through Principal Component Analysis (Fig. 3b). The upper left quadrangle separated the DTPY and DTPW lines from the rest of the genotypes. It could also be observed that PCA could differentiate the drought tolerant DTPY and DTPW lines from the susceptible lines. The drought susceptible genotypes (SCMALAWI, CML247, CML91, CML537 and CMLP2) were at the bottom of the lower right and left quadrangles. The moderately tolerant HKI209, HKI335 and CML360 are in the upper portion of the bottom left quadrangle, while the drought susceptible Indian genotypes CM139, HKI1011 and HKI586 were placed together near the intersection of the upper left quadrangle. CM140 (drought-tolerant) was towards the intersection of the upper left and right quadrangles, while the susceptible genotype HKI1025 was at the extreme of the upper left quadrangle, indicating its genetic distinctness among the rest of the genotypes. Thus, the cluster and PCA patterns were largely congruent with the pedigree as well as the responses of the genotypes to drought stress, indicating the utility of the SSR markers analysed in this investigation.

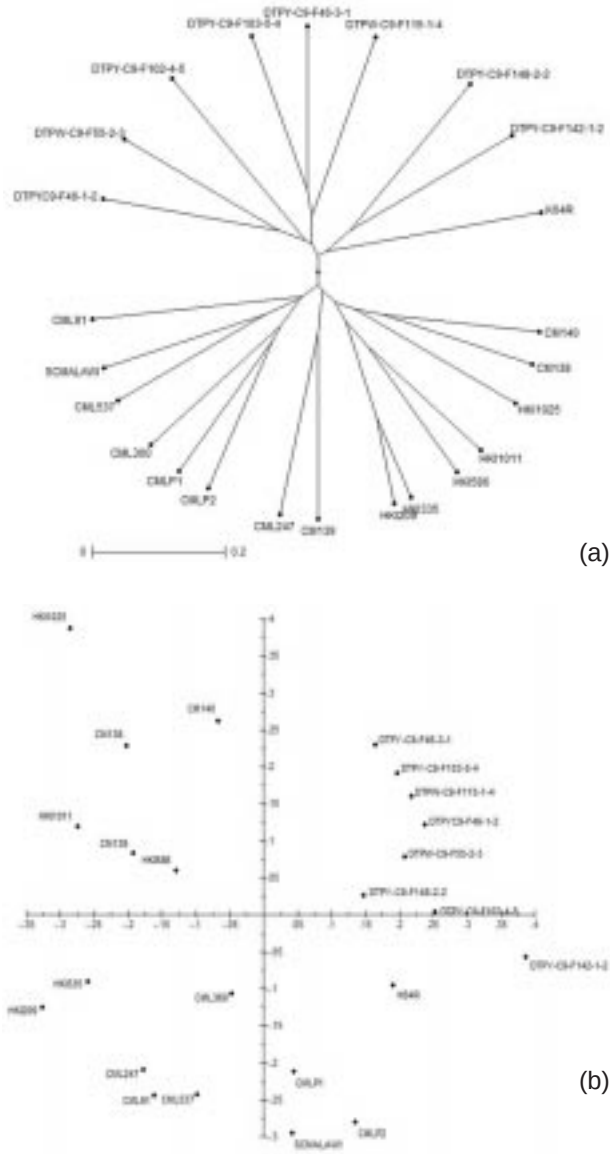

Fig. 3. Depiction of genetic relationships among the selected maize inbred lines based on (A) cluster analysis of SSR data using Jaccard's similarity coefficient and UPGMA clustering algorithm; and (B) factorial analysis of SSR data.

The highly informative SSR markers identified in this study tag some important genes involved in drought stress tolerance in maize. For example, *dup12* tags NCED (9-cis-epoxycarotenoid dioxygenase) is a gene related to ABA biosynthesis, which falls in a consensus QTL identified at bin location 1.08, through meta-analysis of various QTL mapping experiments [5]. This also colocalises with some important transcriptional factors like *CBF1/DRE1D*, involved in responses to drought and salinity. Khavkin and Coe [26] hypothesized that many significant QTLs of major effect in maize are in fact clusters of genes (e.g., genes enclosing transcription factors) regulating development, and that many of the responses of the plant to abiotic or biotic stresses rely on such clusters. Other highly informative SSR markers identified in this present investigation (such as *bnlg1028*, *dup13*, *bnlg1866*, *umc1069*, *umc1962* and *umc1344*) tag some important candidate genes involved in osmolyte biosynthesis or production of detoxification enzymes which contribute to abiotic stress tolerance at various stages and ultimately determine the phenotypic responses in terms of anthesis-silking interval (ASI), root characteristics, stay green and grain yield.

Considering the complexity of drought stress tolerance and limited success achieved so far through MAS using conventional QTL data [4,27], it is important to exploit the information related to markers tagging candidate genes and consensus QTLs, identified through meta-analysis, in the MAS strategy. The present study is a step in this direction, and had led to (i) identification of highly informative SSR markers tagging specific candidate genes and consensus QTLs associated with drought stress tolerance in maize; and (ii) understanding of genetic relationships among the selected drought tolerant and susceptible maize inbred lines differing in their responses to drought stress at flowering stage, especially in the Indian context, based on SSR data. These results could be potentially useful in planned utilization of promising drought tolerant genotypes as well as in molecular marker-assisted breeding for drought tolerance in maize.

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