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ASSESSMENT OF MOLECULAR DIVERSITY IN WHEAT (*TRITICUM AESTIVUM* L. AND *TRITICUM DURUM* L.) GENOTYPES CULTIVATED IN SEMI-ARID REGION OF GUJARAT

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ABSTRACT
Molecular diversity was assessed among wheat genotypes cultivated in semi-arid region of Gujarat employing molecular markers. In total, 18 RAPD and eight SSR markers amplified 5554 fragments with 66.83 % polymorphism and 343 fragments with 90.32% polymorphism respectively. Unweighted pair group method with arithmetic mean (UPGMA) analysis generated by RAPD, SSR and combined RAPD and SSR based on genetic distance estimates displayed two major clusters consisting of irrigated aestivum (I), irrigated and rainfed durum (II) wheat genotypes. A Jaccard's similarity coefficient of all genotypes derived from RAPD data ranged from 0.65 to 0.90 and that of SSR ranged from 0.45 to 0.94. Marker data subjected to analysis of molecular variances revealed more diversity within population. The PIC value for RAPD and SSR markers ranged from 0.91 to 0.96 and 0.57 to 0.76 respectively, with marker indexes of 11.35 and 5.35, demonstrating its utility in genetic diversity analysis. The results of PCoA analysis were comparable to dendrograms. Analysis of molecular variance (AMOVA) for combined RAPD and SSR displayed more variance within and among population than RAPD and SSR. These molecular markers can be put to use for characterization and selection of wheat genotypes suitable for cultivation in irrigated and rainfed conditions.

INTRODUCTION

Wheat (*Triticum* spp.), a self-pollinating annual plant, belonging to the family Poaceae (grasses), is globally one of the three most important crops for human as well as livestock feed (Shewry, 2009). It ranked third in the world with 86.9 million metric tons which is an increase over the past years (FAOSTAT, 2011). At least 60 million ha of wheat is grown in marginal rainfed environments in developing countries (Monneveux *et al.,* 2012). Nevertheless, the crop productivity is under the constraints of drought stress which contributes nearly 17% of yield reductions (Boyer, 1982; Ashraf and Harris, 2005). In India, hexaploid (*Triticum aestivum*) and tetraploid (*Triticum durum* and *Triticum dicoccum*) wheat spp. are cultivated (Sramkova *et al.,* 2009) and widely grown as rain-fed crop in semi-arid areas, where large fluctuations occur in the amount and frequency of rainfall events. Large part of the north-west India falls under arid and semi-region including the state of Gujarat (CFDA, 2007). Major efforts are underway worldwide to increase wheat production by extending genetic diversity and analysing key traits (Brenchley *et al.,* 2012). Understanding the genotyping characteristics and relationships of the germplasm is limited, mainly due to the polyploid nature of wheat (Haudry *et al.,* 2007). Moreover, development of high-yielding wheat cultivars under drought conditions in arid and semiarid regions has always been an important objective of breeding programs (Khamssi *et al.,* 2012). Molecular marker technology offers such approaches for improvement with respect to selection of desirable alleles. It is pre-requisite for undertaking molecular breeding activities particularly identifying and localizing important genes controlling qualitatively and quantitatively inherited traits (Varshney *et al.*, 2006). They are equally capable of tracking the introduced genomic regions in large numbers of lines for pre breeding (Allen *et al.,* 2011) and assessing the genetic diversity in the germplasm (Bahurupe *et al.,* 2013). Various molecular markers have been useful in breeding programs for assessment of genetic variability between genotypes such as restriction fragment length polymorphisms (RFLPs), random amplification of polymorphic DNAs (RAPD), amplified fragment length polymorphisms (AFLPs), inter simple sequence repeats (ISSRs), sequence characterized amplified region (SCAR), microsatellites or simple sequence repeats (SSR), single nucleotide polymorphisms (SNPs) etc. (Mallikarjuna *et al.,* 2012). Among these markers, RAPD was the first polymerase chain reaction (PCR) based marker characterized by dominant nature and requiring no sequence information (Asif *et al.*, 2005). There is a plethora of reports on genetic diversity studies employing RAPD markers in wheat (Rahman et al., 2004; Asif et al., 2005; Iqbal et al., 2007; Rashed *et al.,* 2008; Abd-El-Haleem *et al.,* 2009; Patil

et al., 2011; ElSayed and Rafudeen, 2012). Another widely utilized molecular marker is microsatellite or simple sequence repeat marker (SSR). Microsatellite or SSRs are stretches of DNA containing tandem repeating di-, tri-, or tetra- nucleotide units distributed throughout the eukaryotic genomes (Pearson and Sinden, 1998). These markers have gained considerable importance over other markers due to many desirable attributes like hyper variability, multiallelic nature, co-dominant inheritance, reproducibility, relative abundance, extensive genome coverage, chromosome specific location, amenability to automation, high throughput genotyping and their ability to associate with many phenotypes (Parida *et al.,* 2009). SSRs have been widely applied to characterize the genetic diversity in wheat and still continue to be the choice for genetic diversity analysis of trait of interest (Devos *et al.*, 1995; Prasad *et al.,* 2000; Huang *et al.,* 2001; Quarrie *et al.,* 2003; Somers *et al.,* 2004; El-Maghraby *et al.,* 2005; Eivazi *et al.,* 2007; Prasad *et al.,* 2009; Bibi *et al.,* 2010; Dodig *et al.,* 2010; Achtar *et al.,* 2010; Hao *et al.,* 2011; Kalia *et al.,* 2011; El Siddig *et al.,* 2013). The molecular markers characterize genotypes according to a particular trait and thus molecular data is a must for executing improvement programmes in crops. Selection of drought tolerant genotypes adapted to the conditions of Gujarat would preliminary require information on molecular data. Thus, molecular characterization of the local genotypes which are released varieties or widely cultivated genotypes of Gujarat would be useful for selection for molecular breeding according to the drought tolerance characteristics. In the present study, an attempt was made to characterize the irrigated and rainfed wheat genotypes of Gujarat employing RAPD and SSR markers.

MATERIALS AND METHODS

Plant material

Total 22 irrigated and rainfed wheat genotypes (Table 1), employed in the present study were procured from the Regional Research Station, Arnej, Anand Agricultural University and Main Wheat Research Station, Vijapur, Sardarkrishinagar Dantiwada Agricultural University.

DNA isolation

Total DNA was extracted from the ten days old wheat seedling of all 22 genotypes by cetyl trimethyl ammonium bromide (CTAB) method as described by Zidani *et al.,* 2005. The isolated DNA was checked spectrophotometrically at 260/280 and 260/230 ratio for quantity and quality (in terms of protein and RNA contamination) using software N.D. (V.3.3.0). To check the form of DNA (linear or sheared) and RNA contamination of isolated genomic DNA, electrophoresis was done using 0.8% agarose gel and quality was judged by viewing the image of single compact DNA band. The DNA was diluted to a working concentration of 20 ng/μL and subsequently used for PCR amplification reactions through RAPD and SSR markers.

RAPD and SSR analysis

Random primers were selected based on the previous studies of Pakniyat and Tavakol, 2007 and Gorji et al., 2010 (Table 2). Amplification reaction was performed with 25 μL volume of 2.5 PCR buffer (10 X) with 15 mM $MgCl_2$, primer (10 pmoles/μL), 0.5 dNTPs mix (10 mM each), 0.3 Taq DNA polymerase (5 U/μL), 1.5 Template DNA (20 ng/μL) (Fermentas, India). Amplification was carried in thermal Cycler (Whatman Biometra T-Gradient, Germany) with the conditions as follows; initial denaturation at 94 °C for four mins., 39 cycles of denaturation at 94 °C for 30 sec., annealing at 38 °C for 30 sec., extension at 72 °C for one min. and final extension at 72 °C for seven mins. The amplified products of RAPD were analysed on 1.8% agarose gel prepared in 1X TBE along with 1 kb standard DNA ladder (Fermentas, India). The electrophoresis was conducted at 60 V current (constant) to separate the amplified bands. The separated bands were visualized under UV transilluminator and photographed using BIORAD Gel Documentation system (BIORAD, USA).

SSR markers specific for drought stress were selected from grain genes database based on their chromosomal position (www.graingenes.org) and previous studies of Ciuca *et al.* (2009) (Table 3). Cocktail for PCR reaction was prepared by adding Taq Buffer A (10X Tris with 15 mM $MgCl_2$) followed by forward and reverse primers, dNTPs, Taq DNA polymerase (Fermentas, India) and template DNA. Cyclic amplification were carried out in the Thermal Cycler (Whatman Biometra T-Gradient, Germany) by using following thermal amplification conditions; initial denaturation at 94ºC for four mins., 39 cycles of denaturation at 94ºC for 30 sec., annealing temperature ranged between 48-58ºC (5ºC less than the temperature melting of primers) for 45 sec., extension at 72ºC for one min. and final extension at 72ºC for seven mins. The amplified products of SSR were analyzed using 2.5% agarose gel prepared in 1X TBE buffer (2.5 g agarose in 100mL 1X TBE and 3 μL ethidium bromide 10 mg/mL). The PCR amplified products (7 μL and 2 μL loading dye) were loaded into the wells along with 100 bp standard DNA ladder (marker). The electrophoresis was conducted at 100 V current (constant) to separate the amplified bands. The separated bands were visualized under UV transilluminator and photographed using BIORAD Gel Documentation system.

Coefficients of similarity were calculated by using Jaccard's similarity coefficient by SIMQUAL function and cluster analysis was performed by agglomerative technique using the UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) method by SAHN clustering function of NTSYS-pc (Rohlf, 1994). Relationships between the wheat cultivars were graphically represented in the form of dendrograms. The polymorphism rates of RAPD and SSR markers were determined using polymorphism information content (PIC) values, calculated according to the formula: PIC = 1 " $\sum P^2i$, where Pi is the frequency of the i^{th} allele (Anderson *et al.,* 1993). The resolving power (Rp) of a primer was calculated according to Prevost and Wilkinson (1999) as Rp = $\sum$ IB where IB (band informativeness) takes the value of: 1–[2 × (0.5–P)], P being the proportion of the 22 genotypes containing the band. Nei's genetic diversity (H) among wheat genotypes was calculated by using POPGENE software (Nei, 1973). Data was obtained for observed number of alleles (Na), effective number of alleles (Ne), Shannon's information index (I), expected heterozygosity (He), unbiased expected heterozygosity (uHe), number of polymorphic loci (NPL), number of band unique to a single population (NUP), percentage polymorphic loci (PPL) across

Table 1: List of wheat genotypes

Triticum aestivum Sr. No.	Irrigated	*Triticum durum* Sr. No.	Rainfed	Sr. No.	Irrigated
1	LOK-1	8	A-206	17	GW-1139
2	GW-173	9	A-9-30-1	18	GW-1245
3	GW-273	10	AR-06-1	19	GW-1255
4	GW-322	11	AR-07-7	20	GW-1260
5	GW-366	12	AR-07-30	21	GW-1265
6	GW-496	13	AR-07-33	22	HI-8498
7	HW-2004	14	DR-08-6	-	-
-	-	15	DR-06-7	-	-
-	-	16	GW-1	-	-

Table 2: List of primers used for RAPD amplification, GC content, total number of loci, the level of polymorphism, PIC value and resolving power

Primer	Primer sequence	GC(%)	Range of band size	Total no of loci	Number of polymorphic loci	Percentage of polymorphic loci	Total number of fragment amplify	PIC[a] value	RP[b]
P-1	ACACAGAGGG	60	366-6114	31	20	64.52	503	0.96	10.82
P-2	CCTCTCGACA	60	397-6985	19	17	89.47	254	0.93	8.00
P-3	TCTCAGCTGG	60	258-3412	17	11	64.70	262	0.93	5.64
P-5	CCACGGGAAG	70	396-6407	23	12	52.17	388	0.95	6.36
P-7	CTGTATCGTG	50	501-6010	22	20	90.90	322	0.94	8.91
P-11	CCATTCCCCA	60	251-4448	22	15	69.18	345	0.95	9.73
P-12	GGTGAACGCT	60	218-4080	26	20	76.92	325	0.95	9.73
P-14	TTCCGGGTGA	60	315-4737	15	12	80.00	241	0.92	3.36
P-16	CCTGGGCTTC	70	305-4762	21	16	76.20	361	0.93	7.36
P-17	CCTGGGCTTG	70	235-3476	16	10	62.50	236	0.92	5.27
P-18	CCTGGGCCTA	70	406-4390	13	07	53.85	220	0.91	3.27
P-20	TGCCCCGAGC	80	326-4508	27	15	55.55	461	0.96	9.36
P-21	TTCCCCGACC	70	314-4256	16	11	68.75	256	0.93	8.00
P-24	GAGGTCCAGA	60	277-4501	18	10	55.55	323	0.94	3.18
P-25	GAGGTCCAGA	60	236-2046	20	15	75.00	294	0.94	6.91
P-26	ATCGGGTCCG	70	152-3137	15	08	53.33	248	0.92	4.00
P-27	CCGTGCAGTA	60	267-3216	18	16	88.88	266	0.93	6.73
P-30	TACGTGCCCG	70	226-2856	14	08	57.14	249	0.92	4.09
Total		-	-	353	243	66.83	5554	0.93	-

[a]Polymorphic information content, [b]RP: Resolving power

22 wheat genotypes using GenAlEx 6.5. The RAPD and SSR data were subjected to a hierarchical analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992), using two hierarchical levels; individual and population. Principal coordinates analysis (PCoA) that plots the relationship between distance matrix elements based on their first two principal coordinates (Peakall and Smouse, 2012) was calculated. Diversity index (DI), effective multiplex ratio (EMR) and marker index (MI) were calculated according to Powell *et al.* (1996) to determine the utility of each of the marker systems. MI is defined as the product of the average diversity index for polymorphic bands in any assay and the EMR for that assay, MI = DIavp × EMR. DI for genetic markers was calculated from the sum of the squares of allele frequencies: DIn = 1-$\sum$ pi^2 (where 'pi' is the allele frequency of the ith allele). The arithmetic mean heterozygosity, Diav, was calculated for each marker class: Diav = $\sum$ Din/n, (where 'n' is the number of markers (loci) analyzed). The DI for polymorphic markers is: (Diav)p = $\sum$ Din/np (where 'np' is the number of polymorphic loci and n is the total number of loci). EMR (E) is the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay. EMR (E) = np (np/n).

RESULTS

RAPD polymorphism

In total, 5554 fragments were amplified using 18 RAPD primers with 353 loci, out of which 243 loci were polymorphic with an average of 14 polymorphic loci per primer. The highest (90.90%) polymorphism was exhibited by primer P-7, while the lowest polymorphism (52.17%) was exhibited by primer P-5 and an average 68.83% polymorphism. The minimum (152 bp) sized fragment was amplified by primer P-26, whereas maximum (6985 bp) sized fragment was amplified by primer P-2. The PIC value ranged from 0.91 (P-18) to 0.96 (P-1, P-20) (Table 2) with an average of 0.93. The maximum number of amplified fragments (503) were generated by primer P-1 with resolving power of 10.82 and the minimum number of amplified fragments (220) were generated by primer P-18 with a resolving power of 3.27. The respective values of Na, Ne, I, He, uHe, NPL, NUP, PPL and MI across all the 22 genotypes are presented in Table 4. Molecular variance within population (64%) was more than among the population (36%).

Clustering pattern of dendrogram generated by RAPD data showed two major clusters I and II having similarity coefficient

Table 3: List of primers used for SSR amplification, sequence, GC content, total number of loci, the level of polymorphism, size range of fragments and resolving power

Primer	Chromosomal position	Primer sequence	GC%	Tm(p C)	Total no of loci	Number of poly-morphic loci	Percent of poly-morphic loci	Total number of fragment amplify	PIC[a] value	RP[b]
Xbarc121	7A	F-CTGATCAGCAATGTCAACTGAA	40.9	54	5	5	100%	41	0.76	3.18
		R-CCGGTGTCTTTCCTAACGCTATG	52.2							
Xwmc603	7A	F-ACAAACGGTGACAATGCAAGGA	45.5	58	4	3	75%	45	0.58	0.64
		R- CGCCTCTCTCGTAAGCCTCAAC	59.1							
Xgwm332	7A	F- AGCCAGCAAGTCACCAAAAC	50	56	4	4	100%	43	0.70	2.09
		R- AGTGCTGGAAAGAGTAGTGAAGC	47.8							
Xwmc233	5D	F- GACGTCAAGAATCTTCGTCGGA	50	57	2	2	100%	28	0.57	0.72
		R- ATCTGCTGAGCAGATCGTGGTT	50							
Xgwm260	7A	F- GCCCCCTTGCACAAATC	58.8	54	5	4	80%	70	0.69	1.09
		R- CGCAGCTACAGGAGGCC	70.6							
Xgwm276	7A	F- ATTTGCCTGAAGAAAATATT	25	46	4	3	75%	43	0.63	1.72
		R- AATTTCACTGCATACACAAG	35							
Xwmc9	4A, 7A	F- AACTAGTCAAATAGTCGTGTCCG	43.5	55	4	4	100%	40	0.65	2.00
		R- GTCAAGTCATCTGACTTAACCCG	47.8							
Xwmc695	3A, 3B, 4B, 7A	F- GAGGGCACCTCGTAAGTTGG	60	59	3	3	100%	33	0.61	1.72
		R- GGCAGGAGCCCCTACAAGAT	60							
Total	-	-	-	-	31	28	90.32%	343	0.63	-

[a]Polymorphic information content, [b]RP: Resolving power

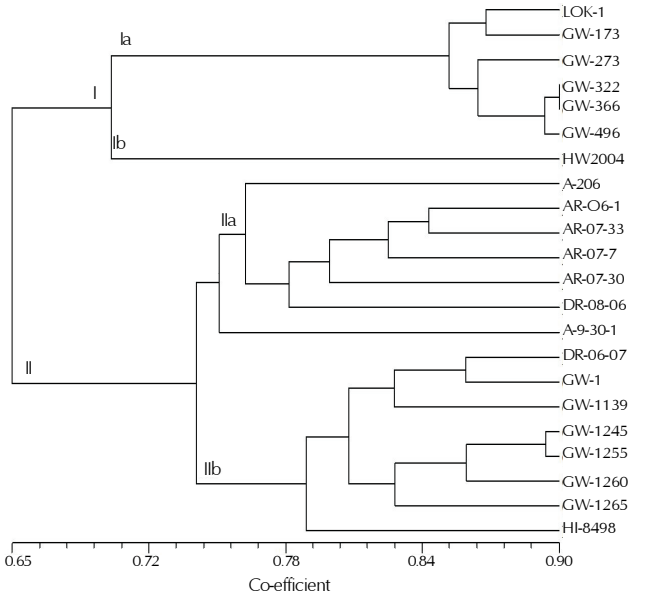

Figure 1: Dendrogram showing clustering of 22 wheat genotypes constructed using UPGMA based on Jacquard's similarity coefficient obtained from RAPD analysis

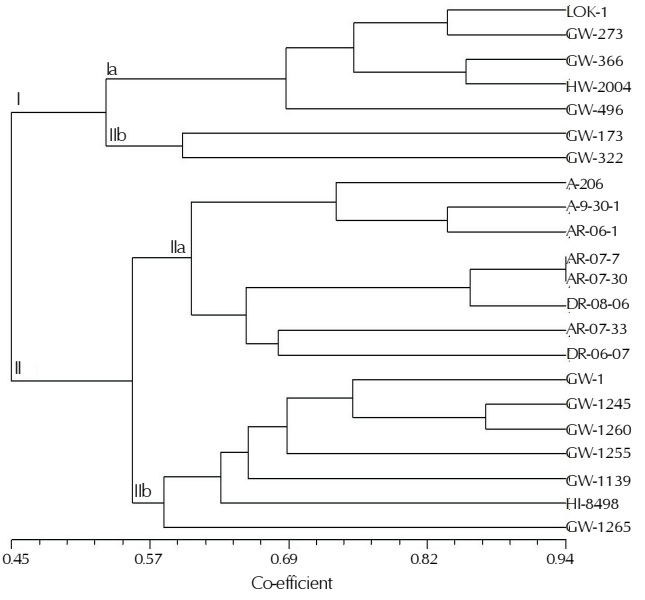

Figure 2: Dendrogram showing clustering of 22 wheat genotypes constructed using UPGMA based on Jacquard's similarity coefficient obtained from SSR analysis

of 0.65 to 0.90 (Fig. 1). Cluster I included *T. aestivum* genotypes and was further divided into two sub clusters Ia and Ib. Cluster II included *T. durum* genotypes which was further divided into two sub clusters IIa and IIb for rainfed and irrigated genotypes respectively. The results of PCoA analysis were comparable to the cluster analysis. The first and second principal coordinates explained 46.22% and 65.52% of the molecular variance, respectively.

SSR polymorphism

In total, 343 amplified fragments were generated using eight SSR primers with an average of 43 amplified fragments per primer. The average percent polymorphic loci observed were 90.32 with an average of 3.5 polymorphic loci per primer. The PIC values ranged from 0.57 (Xwmc233) to 0.76 (Xbarc121) with an average of 0.63 and the resolving power ranged from 0.72 (Xwmc233) to 3.18 (Xbarc121) (Table 3). The respective values for overall genetic variability for Na, Ne, H, I, He, uHe, NPL, NUP, PPL, MI are presented in Table 4. The first and second principal coordinates explained 38.68% and 56.79% of the molecular variance, respectively. Clustering pattern of dendrogram generated by SSR data showed two major clusters I and II having similarity coefficient of 0.45 to

Table 4: Summary of genetic variation statistics for all loci of RAPD, SSR and RAPD + SSR among the populations

Marker and Population	Sample size	Na[a]	Ne[b]	H[c]	I[d]	He[e]	uHe[f]	NPL[g]	NUP[h]	PPL[i]	MI[j]
RAPD											11.35
T. aestivum (Irrigated)	7	1.326(0.034)	1.262(0.020)	0.131(0.189)	0.222(0.015)	0.149(0.011)	0.161(0.011)	318	7	42.49%	-
T. durum (Rainfed)	9	1.346(0.034)	1.293(0.021)	0.165(0.206)	0.243(0.016)	0.165(0.011)	0.175(0.012)	321	10	43.63%	-
T. Durum (Irrigated)	6	1.394(0.033)	1.304(0.021)	0.152(0.203)	0.254(0.016)	0.172(0.011)	0.187(0.012)	328	3	46.46%	-
Mean	-	1.355	1.286	0.241	0.240	0.162	0.174	-	-	44.19%	-
SSR											5.35
T. aestivum(Irrigated)	7	1.484(0.130)	1.374(0.073)	0.212(0.210)	0.317(0.053)	0.212(0.038)	0.229(0.041)	27	6	61.29%	-
T. durum (Rainfed)	9	1.323(0.163)	1.412(0.076)	0.230(0.215)	0.338(0.054)	0.230(0.039)	0.244(0.041)	22	0	61.29%	-
T. durum (Irrigated)	6	1.161(0.154)	1.313(0.073)	0.174(0.214)	0.256(0.055)	0.175(0.039)	0.190(0.042)	22	1	45.16%	-
Mean	-	1.323	1.366	0.287	0.303	0.206	0.221	-	-	55.91%	-
RAPD + SSR											
T. aestivum (Irrigated)	7	1.318(0.032)	1.239(0.018)	0.138(0.191)	0.207(0.014)	0.138(0.010)	0.149(0.011)	348	23	41.15%	-
T. durum (rainfed)	9	1.344(0.034)	1.302(0.020)	0.170(0.208)	0.250(0.015)	0.170(0.011)	0.180(0.011)	343	10	45.05%	-
T. durum (irrigated)	6	1.281(0.035)	1.275(0.020)	0.154(0.204)	0.227(0.015)	0.154(0.010)	0.168(0.011)	335	3	40.89%	-
Mean	-	1.314	1.272	0.245	0.228	0.154	0.166	-	-	42.36%	-

[a]No. of different alleles, [b]No. of effective alleles = $1 / (p^2 + q^2)$, [c]Nei's gene diversity, [d]Shannon's information index = $-1 \times (p \times Ln(p) + q \times Ln(q))$, [e]Expected heterozygosity = $2 \times p \times q$, [f]Unbiased expected heterozygosity = $(2N/(2N-1)) \times He$, [g]Number of polymorphic loci, [h]Number of band unique to a single population, [i]Percent polymorphic loci, [j]Marker index.

0.94 (Fig. 2). Cluster I included irrigated *T. aestivum* genotypes and cluster II included *T. durum* genotypes which was further sub clustered into IIa and IIb comprising rainfed and irrigated genotypes respectively.

RAPD + SSR combined data

The average percent polymorphic for combined RAPD and SSR data was 42.36%. The respective values for overall genetic variability for Na, Ne, H, I, He, uHe, NPL, NUP, PPL, MI are presented in Table 4. Clustering pattern of dendrogram generated by combined RAPD and SSR data displayed two major clusters I and II having similarity coefficient of 0.64 to 0.90. Cluster I included irrigated *T. aestivum* genotypes and cluster II included *T. durum* genotypes which was further divided into two sub clusters IIa and IIb comprising rainfed and irrigated durum genotypes respectively (Fig. 3). AMOVA for combined RAPD and SSR displayed more variance within and among population than RAPD and SSR (Table 5). The first and second principal coordinates explained 46.09% and 65.40% of the molecular variance, respectively (Fig. 4).

DISCUSSION

Evaluation of genetic divergence and relatedness among breeding materials has significant implications for the improvement of crop plants (Chandra *et al.,* 2013). Molecular analysis by RAPD displayed specific amplified products unique to irrigated aestivum and durum genotypes. These markers can be employed to screen the aestivum and durum wheat genotypes as they follow distinct clusters. The cluster analysis distinguishes genotypes on the basis of their diversity and could be used as basis of selection of genotypes for crop improvement (Bharose *et al.,* 2014). These RAPD markers can be used for determination of association with drought tolerance which corroborates with the results reported by ElSayed and Rafudeen, 2012 in wheat genotypes. Our results are also in agreement with earlier results of Hashad *et al.* (2005); Fadly *et al.* (2007); Gorji *et al.* (2010). Pakniyat and Tavakol (2007) observed specific bands present in drought tolerant cultivars associated with drought tolerance in wheat which corroborates with the present study. The SSR markers were able to distinguish the aestivum and rainfed and irrigated durum genotypes. The polymorphism exhibited among irrigated *T. aestivum* and rainfed and irrigated *T. durum* consistent with the earlier findings by Younes (2009). SSR markers were selected based on chromosomal position associated with drought tolerance. Drought tolerance in wheat is associated with chromosome 7A (Morgan and Tan, 1996; Galiba, 2002; Cattivelli *et al.,* 2002). Membrane stability has been suggested as a useful measure of drought tolerance in wheat breeding programs. Morgan (1991) located a gene for osmoregulation ('or') on chromosome 7A. Tian-Mei *et al.* (2010) established association of SSR markers located on the 5D and 3B with drought tolerance in wheat cultivars. The markers selected from these chromosomes showed PIC value more than 0.5, pointing out its utility in distinguishing the genotypes according to their response to water deficit conditions (Sonmezoglu *et al.,* 2012). Recent study by Siddig *et al.*, 2013 showed that SSR markers were associated with drought tolerance in wheat. Dendrograms generated through RAPD, SSR and combined RAPD and SSR indicated major

Table 5: Analysis of molecular variance (AMOVA) based on RAPD and SSR individually and in combination, among the populations of wheat. Levels of significance are based on 1000 iteration steps

Among population	Within population					
d.f.	2			19		
Marker	RAPD	SSR	RAPD + SSR	RAPD	SSR	RAPD + SSR
S.S.D.	304.716	36.420	374.159	568.921	65.262	601.159
Variance component	16.938	2.044	21.507	29.943	3.435	31.640
Percentage	36%	37%	40%	64%	63%	60%
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

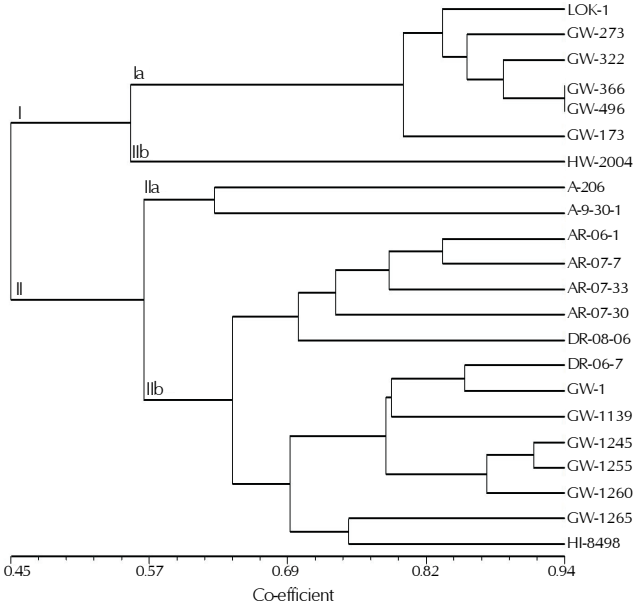

Figure 3: Dendrogram showing clustering of 22 wheat genotypes constructed using UPGMA based on Jacquard's similarity coefficient obtained from RAPD + SSR analysis

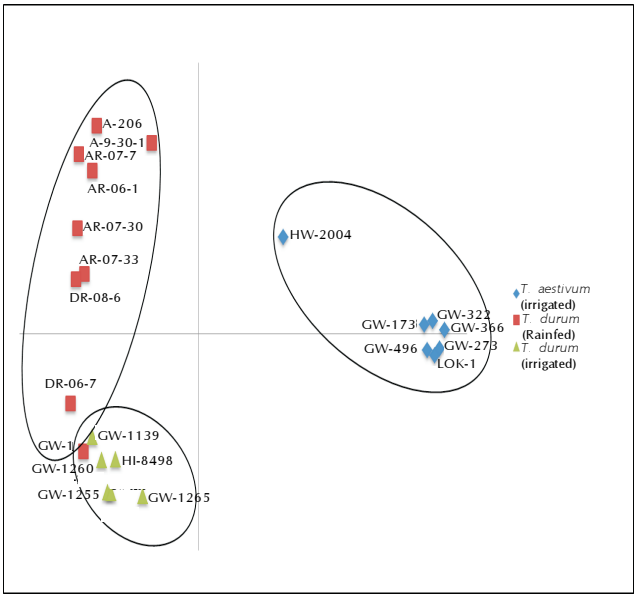

Figure 4: Two-dimensional plot of principal component analysis (PCoA) of 22 wheat genotypes using RAPD + SSR analysis

three clusters consisting irrigated aestivum, rainfed durum and irrigated durum genotypes. The rainfed durum genotypes, DR-06-7 and GW1 clustered with irrigated durum genotypes which can be ascribed to the common parentage of this genotype with the irrigated durum genotypes. The similarity coefficient for RAPD (0.65 to 0.90), SSR (0.45 to 0.94) and combined RAPD and SSR (0.64 to 0.90) indicated more diversity for SSR markers. Our results are in agreement with earlier studies of Ciuca and Petcu (2009); Dreisigacker *et al.* (2004) in assessing wheat genetic diversity. The mean values of Na, Ne, H, I, He, uHe, NPL, NUP were found to be higher for SSR than RAPD. The molecular markers employed in the present study demonstrated high levels of polymorphism among the rainfed and durum genotypes which is in agreement with the studies of Bousba *et al.*, 2012. AMOVA assisted in partitioning of the overall variations among and within population. Based on AMOVA, more variance was observed within the population which is in agreement with the results of Prasad *et al.*, 2009 in wheat cultivars. The principal co-ordinate analysis was consistent with the clustering pattern obtained by dendrograms.

CONCLUSION

In conclusion, the present study revealed distinguishing differences in the aestivum and durum genotypes grown under rainfed and irrigated conditions of Gujarat. The information provided by the molecular markers in wheat would be beneficial for breeding for drought tolerance and selection of genotypes for cultivation in the rainfed areas of Gujarat.

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