GENETIC DIVERSITY IN WHEAT GENOTYPES USING SIMPLE SEQUENCE REPEAT (SSR) MARKERS

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ABSTRACT

A total of 73 genotypes including 65 hexaploid (*Triticum aestivum* L.) and 8 tetraploid (*Triticum durum* L) wheat varieties were used in this study. Eleven microsatellite markers were used to test the genetic diversity of wheat genotypes, yielding a polymorphism. The total number of detected alleles was 89 with an average allele number of 8.1 per locus. The maximum number of polymorphic alleles (9 bands) was obtained by the primer SSR-9 while the minimum number (1 allele) was recorded for primer SSR-11 with an average 5 alleles per primer. Polymorphism percentage ranged from as low as 50 % (SSR-1, SSR-2 and SSR-5) to as high as 100% (SSR-10 and SSR-11) with an average of 68.24 %. Eleven alleles at molecular size [219 bp and 188 bp (SSR-1), 239 bp (SSR-3), 635 bp and 503 bp (SSR-4), 358 bp and 331 bp (SSR-8), 250 bp, 226 bp and 210 bp (SSR-9) and 532 bp (SSR-11)], were present only in bread wheat genotypes, while one allele at molecular size 312 bp generated with primer (SSR-6) was unique to durum wheat genotypes. These alleles could be used as marker to distinguish the durum wheat from the bread wheat genotypes. Polymorphism information content (PIC) value ranged from 0.06 (primer SSR-6) to 0.2 (primer SSR-11) with an average value of 0.13**.** These results reflect the wide range between the frequencies of alleles at loci of SSR studied in the tested wheat varieties. The dendrogram grouped the 73 wheat genotypes according to their ploidy levels into two main clusters. Cluster 1 included the durum wheat genotypes, while cluster 2 contained all bread wheat genotypes.

Keywords: durum wheat, bread wheat, SSR markers, wheat genotypes

INTRODUCTION

Wheat is an important cereal crop used as a major human consumable commodity in most areas of the world. The species of *Triticum* are grouped into diploids (2n=2x=14), tetraploids (2n=4x=28) and hexaploids (2n=6x=42). *Triticum aestivum*, common bread wheat, contains 3 different but genetically related genomes (A, B and D) with a total genomic size of $1.7x10^{10}$ base pairs, illustrating the complex nature of wheat genome. Durum wheat or macaroni wheat (*Triticum durum* or *Triticum turgidum* subsp. durum) is the only tetraploid species of wheat of commercial importance that is widely cultivated today. It is an allotetraploid species with 2n = 4x = 28 (AABB genome) that originated through intergeneric hybridization and polyploidization involving two diploid grass species *T. urartu* (2n = 2x = 14, AA genome) and a B-genome that is diploid related to *Aegilops speltoides* (2n = 2x = 14, BB genome) **(Kihara 1944 and McFadden and Sears 1946).**

Several molecular markers like random amplified polymorphic DNAs (RAPD), inter simple sequence repeats (ISSR) and simple sequence repeats (SSRs) are presently available to assess the variability and diversity at molecular level **(Palombi and Damiano, 2002).** Hence, keeping in view the importance of these aspects the present study has been planned. This is considered as the most effective method for qualifying the degree of genetic diversity among the genotypes included in the study.

Genetic diversity is one of the key factors for improvement many crop plants including wheat. Plant breeders rely on the availability of genetic diversity during selection in cultivar development. The efficiency of genetic gain by

selection can be improved if the patterns of genetic diversity within a population of breeding lines are

known. Genetic similarity and or distance estimates among genotypes are helpful in the selection of parents to be used in a breeding program **(Van-Becelaere *et al.,* 2005).** Genetic diversity can be assessed from pedigree analysis, morphological traits or using molecular markers **(Pejic *et al.,* 1998).** However, diversity estimates based on pedigree analysis have generally been found inflated and unrealistic **(Fufa *et al.,* 2005).** Genetic diversity estimates based on morphological traits, on the other hand, suffer from the drawback that such traits are limited in number and are influenced by the environment **(Maric *et al.,* 2004).**

Molecular markers are useful tools for estimating genetic diversity as these are not influenced by environment and do not require previous pedigree information. Among the molecular markers techniques, random amplified polymorphic DNA (RAPD) which introduced by **Williams *et al.* (1990).**

DNA markers are technology that can increase breeding progress, especially for traits that are difficult to select under field conditions and that are controlled by multiple genes. Microsatellites are repeating sequences of 2–6 base pairs of DNA (SSRs; Simple sequence repeats) and are among the most stable markers of genetic variation and divergence among wheat genotypes because they are multiallelic, chromosome-specific and evenly distributed along chromosomes. Microsatellite genotyping is used for genetic biodiversity, population genetics at the level of relatedness, genome mapping, as markers for pathogens, etc. The main objective of the present investigation was **to study the** genetic relationship among wheat genotypes based on SSR markers.

MATERIALS AND METHODS

Materials: A total of 73 genotypes including 65 hexaploid (*Triticum aestivum* L.) and 8 tetraploid (*Triticum durum* L) wheat varieties were used in this study (Table 1).

A-DNA extraction

Genomic DNA was extracted from young leaves of 2-weeks-old seedlings, bulked from 5 different plants per genotype following the CTAB procedure described by **Murray and Thompson (1980)** with some modifications. The quantity and quality of genomic DNA was tested by the spectrophotometer and agarose gel electrophoresis.

B-Microsatellite markers analysis:

Eleven wheat microsatellite markers for eleven loci representing at least one microsatellite marker from chromosomes (2A, 3A, 3B, 3D, 4A, 4B, 4D, 5D, 7A, 7B and 7D) **(Table 2)** were selected for genotyping **Röder *et al.* (1998).** The primer sequence of Xtaglgap was described by **Devos *et al.* (1995)**. All Gatersleben Wheat Microsatellites (Xgwm) used were dinucleotide repeats, whereas Taglgap has a trinucleotide motif. Microsatellite amplifications were carried out as reported by **Röder *et al.* (1998)**. Polymerase chain reaction and fragment analysis were performed according to **Devos *et al.* (1995)** and **Röder *et al.* (1998) (Table 2).** Fragment detection for SSR markers was carried out as given in **Röder *et al.* (1998).** GWM designation, chromosomal location, motif and fragment size location in 'CS' (bp) of the amplified loci were reported by **Röder *et al.* (1998).**

Each PCR was carried out in a about 25 µl reaction volume containing double distilled deionizer H_2O, 10x buffer, $MgCl_2$, dNTPs, Taq polymerase, and both primer pairs according to the primers profile. The PCR amplification of wheat genomic DNA was done by incubating the DNA samples for 5 minutes at 94°C, then 45 cycles comprising 94°C for 60 seconds, annealing of primer for 60 seconds at 58-60°C and the extension for 60 seconds at 72°C. The final extension was carried out for 10 minutes at 72°C. The PCR products were electrophorized on 2.5% of agrose gel containing 8 µl ethidium bromide, at 80 volts for 3-3.5 hours and observed under a UV transilluminator.

C. Band scoring and data analysis

Banding pattern of the SSR markers in the individuals were scored as presence (1) and absence (0) of the band. The pairwise comparisons between the tested genotypes were used to calculate the coefficient of genetic similarity matrix (Gs) according to Nei and Li (1979). The similarity matrix was subjected to cluster analysis using un weighted pair group method with arithmetic means (UPGMA) clustering procedure and a dendrogram was generated using the software package MVSP (Multi-Variate Statistical Package).

To evaluate the efficiency of selected primers for investigation of genetic diversity, the polymorphism information content (PIC) was calculated as: [PIC = 1- Σ fi^2] where, *fi* is the frequency of the i^{th} allele (Weir 1990). The Resolving power (Rp) of each primer was calculated according to Prevost and Wilkinson (1999) as: Rp = ΣIb where, Ib = band informativeness, Ib = 1- [2×(0.5-p)] and p = total number of bands present.

RESULTS AND DISCUSSION

Microsatellites combine many desirable marker properties including abundant, high levels of polymorphism and information content (PIC), high reproducibility, co-dominance, rapid and simple genotyping assays, uniform genome coverage, and specific polymerase chain reaction (PCR) based assays **(Röder *et al.,* 1998).** Furthermore, the analysis of microsatellites based on PCR is simple to perform. In wheat, simple sequence repeats (SSR) have been successfully used in a wide range of applications such as genotype identification **(Prasad *et al.,* 2000; Zeb *et al.,* 2009** and **Salem and Mattar, 2014),** diversity studies **(Bo¨rner *et al.,* 2000; Huang *et al.,* 2002; Zeb *et al.,* 2009; Salem *et al.,* 2008; Akfirat and Uncuoglu 2013; Bousba *et al.,* 2013; Amir *et al.,* 2014** and **Khavarinejad, 2014)** and quantitative trait loci (QTL) analysis **(Bo¨rner *et al.,* 2002** and **Salem *et al.,* 2007).**

Eleven microsatellite markers were used to test the genetic diversity of seventy-three of wheat genotypes (65 bread wheat and 8 durum wheat) **(Table 2 and Figs. 1, 2).** All microsatellite markers allowed identifying DNA fragments amplified polymorphic patterns from genomic extracts of wheat genotypes, yielding a polymorphism. The total number of detected alleles was 89 with an average allele number of 8.1 per locus. The number of alleles per locus ranged from one for the SSR-11 locus to 14 for the SSR-1 locus **(Table 2).** Their fragment size ranged from 1007 bp in SSR-7 to 93 bp in SSR-10. Different numbers of SSR alleles have been detected in wheat using microsatellite markers. **Prasad *et al.* (2000)** found 7.4 averages allele numbers in 55 elite of wheat genotypes. **Röder *et al.* (2002)** detected an average of 10.5 alleles per marker from 19 wheat microsatellites in 502 European wheat varieties. Whereas, **Huang *et al.* (2002)** used 26 microsatellites to investigate 998 Genbank accessions of wheat originating from different 68 countries and thereby recorded an average allele number of 18.1. **Khlestkina *et al.* (2004)** detected average allele numbers of 6.6 in 54 common spring wheat varieties. **Akfirat and Uncuoglu (2013)** found an average allele number of 3.9 in seven Turkish winter bread wheat genotypes.

The genotype (G29) displayed the highest number of alleles (77 alleles) followed by G28, G49 and G52 (76 alleles, each), while G6 (49 alleles) followed by G4 (50 alleles) revealed the least number of alleles **(Table 3).** These variations in number of bands amplified by different primers influenced by variable factors such as primer structure and number of annealing sites in the genome **(Kernodle *et al.,* 1993).**

The maximum number of polymorphic alleles (9 bands) was obtained by the primer SSR-9 while the minimum number (1 allele) was recorded for primer SSR-11 with an average 5 alleles per primer. Polymorphism percentage ranged from as low as 50 % (SSR-1, SSR-2 and SSR-5) to as high as 100% (SSR-10 and SSR-11) **(Table 65).** Average polymorphism across all the 73 wheat genotypes was found to be 68.24 %. A significant correlation (0.80, $p<0.01$) was observed between the total

Table 1: Pedigree of tetraploid and hexaploid wheat genotypes used in the study.

Code	Name	Pedigree	Code	Name	Pedigree	Code	Name	Pedigree
G1	SOHAG 1	GDOVZ469/JO//61.130/LDS	G26	Nour	selected early maturing inbred line (F14) derived from a cross between Shenap*Sakha69	G51	ICR-35	ICARDA
G2	SOHAG 3	MEXICALI/MAGHREBI 72//51792/DURUM#6	G27	1x15	Advanced breeding line derived from inter population-interenvironmental cross between early segregates selected in two contrasting enviroments	G52	ICR-39	ICARDA
G3	BANI SEWEF 1	JO"S"/AA"S"/FG"S"	G28	Line 6	Advanced long spike, short statured inbred line derived from a cross between two landraces collected from dry areas in Upper Egypt (Omara, 1994)	G53	ICR-DH18	ICARDA
G4	BANI SUEF 5	DIPPER-2/ BUCHEN-3	G29	L.S.15 (Long spike 15)	An advanced long-spike inbred line (F14) derived from a cross among landraces collected from stress areas in Upper Egypt (Omara,1994)	G54	ICR-49	ICARDA
G5	SVEVO	Cimmyt's Line / Zenit	G30	SIDS 1	HD2173/PAVON"S"//1158.57/MAYA 74 "S"	G55	HD-38	ICARDA
G6	Ciccico	APPULO/VALNOVA(F6)//VALFORTE/PA TRIZIO(F5)	G31	SIDS 4	MAYA"S"/MON"S"//CMH74A.592/3/GIZA 157*2	G56	Madden	Gamenya//Gabo*3/Khapstein
G7	WK-12	The landraces were originally collected from farmers' fields near Dandara Temple at Qena Governorate in 1993 (Omara, 1994) and were grown since then every year in order to ascertain the stability of the black glume character.	G32	SIDS 12	BUC//7C/ALD/5/MAYA74/ON//1160.147/3/B B/GLL/4/CHAT"S" /6/MAYA/VUL//CMH74A.63014*SX	G57	ALTAISKAYA 50	Unknown
G8	LOCAL SBW	unknown	G33	Lerma Rojo 64	((Yaqui 50*(Norin 10*Brevor))*Lerma 52)*(Lerma Rojo)2	G58	HONGMANGM AI	OROFEN/DIZIUZHAO
G9	GIZA 157	GIZA155//PIT62/LR64/3/TZPP/KNOTT	G34	TOBARI-66	TEZANOS-PINTOS-PRECOZ/SONORA-64-A[114][144][145][39][1765];	G59	SAHEL 1	NS732/PIMA//VEE#5
G10	GIZA 160	CHENAB70/GIZA155	G35	SALAMBO 80	PATO//CC/INIA	G60	Ejaseed-3	Unknown
G11	GIZA 163	F61.70/BON//CNO67/7C	G36	BACANORA T 88	JUP/BJY//URES	G61	CONDOR	WW15*2/3/PJ/GB56//TZPP/NAI60
G12	GIZA 164	KVZ/BUHO//KAL/BB	G37	Sonora 64	YAKTANA-54//NORIN-10/BREVOR/3/2*YAQUI-54	G62	MIRONOVCHA NKA	WEIHENSTEPHEN M./MIRONOV-YA 808
G13	GIZA 165	CIANO F 67/MARIS FUNDIN//MONCHO	G38	DEBEIRA	HD2160/5/TOB/CNO67//BB/3/NAI60*2//TT/S N64/4/HD1954	G63	NASMA	BT1149/BT2511 or BT1149//Florence/Aurore C
G14	GIZA 168	MIL/BUC//SERI	G39	EL NIELAIN	S948.A1/7*SANTA ELENA	G64	GOUMRIA-15	ICW94-0029-0L-6AP-3AP-2AP-0APS-0AP
G15	SAKHA 8	CNO67//SN64/KLRE/3/8156	G40	MEXIPAK65	PENJAMO62/GABO55	G65	Arrehane	L222 (KLDN)
G16	SAKHA 69	INIA/RL4220//7C/3/YR	G41	PAVON F 76	VCM//CNO/7C/3/KAL/BB	G66	Saada	BUTTE//BUTTE/ARTHUR71
G17	SAKHA 92	NAPO//INIA/WREN	G42	KBG-01	300-SM-501-M/HAR-1709	G67	Marchouch	KAL/CNO//2*8156/3/BT908
G18	SAKHA 93	SAKHA 92/TR 810328	G43	CHAM 4	FLK/HORK	G68	HUBARA-5	Unknown
G19	SAKHA 94	OPATA/RAYON/3/JUP/BJY//URES	G44	CHAM 6	W3918A/JUP	G69	BerKum	Unknown
G20	GEMMIZA 7	CMH74.630/5X//SERI82/3/AGENT	G45	GHAM-8	ICARDA	G70	Canada-462	Unknown
G21	GEMMIZA 9	ALD'S'/HUAC'S'//CMH74.630/5X	G46	Reyne 28	ICARDA (CIMMYT breeding line)	G71	Canada-515	Unknown
G22	Gemmieza 10	Maya 74	G47	Aguilal	SAIS*2/KS85241-14	G72	GURAB-2	ICARDA
G23	Shandaweel1	Site//Mo/4/Nac/Th.Ac./3*Pvn/3/Mirlo/Buc	G48	ATTILA	ND/VG9144//KAL/BB/3/YACO/4/VEE#5	G73	GK BENCE	ATR/SAVA//LIB
G24	Misr 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR	G49	ICR-DH	ICARDA			
G25	US3-2 (LIRA SA 92)	KVZ/TRM//PTM/ANA	G50	ICR-33	ICARDA			

Table 2. The codes and sequence of SSR primers used for amplification with the number of Total bands (TB), polymorphic bands (PB), percentage of polymorphism (PP), polymorphism information content (PIC) and resolving power (RP) for each primer.

Primer code		Primer sequence	TB	PB	PP	PIC	RP
1-Xgwm160	SSR-F	5′–GAATTTACAAACTTCCTTGAGC–3′	14	7	50	0.15	2.79
	SSR-R	5′–AAAAATATACAGCAGTAATCATCTG–3′					
2-Xtaglgap	SSR-F	5′–TTGCTTGGTTGAAGGATTACTTC–3′	8	4	50	0.07	0.63
	SSR-R	5′–CCCTCGTAGGAGACCTTCTTT–3′					
3-Xgwm577	SSR-F	5′–GGGAGGCTGAGGGAATTGTC–3′	4	3	75	0.2	1.04
	SSR-R	5′–AGTGCCGCTGAATTCAGTGAAA–3′					
4-Xgwmc695	SSR-F	5′–AAGAGGCAGAGATGGAGTTC–3′	11	6	54.5	0.10	1.29
	SSR-R	5′–TCCCTGACACAGACGAGAT–3′					
5-Xwmc596	SSR-F	5′–TGCAAAGCATCACGGAGA–3′	8	4	50	0.07	0.68
	SSR-R	5′–ATACACGGTGGAAGTTGGC–3′					
6-Xgwm497	SSR-F	5′–GTAGTGAAGACAAGGGCATT–3′	13	6	46.15	0.06	0.93
	SSR-R	5′–CCGAAAGTTGGGTGATATAC–3′					
7-Xgwm260	SSR-F	5′–CACGAAGAGATATCACCCCGAG–3′	9	7	77.78	0.16	1.97
	SSR-R	5′–GGATGTCTGCGAGCCTTTCATAT–3′					
8-Xgwm174	SSR-F	5′–TTTCTTCCGCATCAAGAGATCC–3′	7	4	57.14	0.12	1.04
	SSR-R	5′–CCTCAGGCTATGGCACAGAAT–3′					
9-Xgwm635	SSR-F	5′–TTGCTTGGTTGAAGGATTACTTC–3′	10	9	90	0.14	1.64
	SSR-R	5′–CCCTCGTAGGAGACCTTCTTT–3′					
10-Xgwm573	SSR-F	5′–GGGAGGCTGAGGGAATTGTC–3′	4	4	100	0.14	0.66
	SSR-R	5′–AGTGCCGCTGAATTCAGTGAAA–3′					
11-Xgwmc182	SSR-F	5′–AAGAGGCAGAGATGGAGTTC–3′	1	1	100	0.20	0.22
	SSR-1R	5′–TCCCTGACACAGACGAGAT–3′					
Average			8.09	5.00	68.24	0.13	1.17

Table (3): Number of amplified DNA-fragments in seventy-three wheat genotypes investigated with eleven SSR primers.

Genotypes	SSR-1	SSR-2	SSR-3	SSR-4	SSR-5	SSR-6	SSR-7	SSR-8	SSR-9	SSR-10	SSR-11	Total
G1	7	6	2	7	7	9	5	4	4	0	0	51
G2	7	6	2	7	7	9	7	4	4	0	0	53
G3	7	6	2	7	7	9	5	4	4	0	0	51
G4	7	6	2	7	7	9	4	4	4	0	0	50
G5	7	6	2	7	8	10	5	5	4	0	0	54
G6	7	6	2	7	5	9	5	4	4	0	0	49
G7	7	7	2	7	7	9	6	4	4	0	0	53
G8	7	6	2	7	8	10	6	5	4	0	0	55
G9	12	7	3	11	7	9	4	6	8	2	1	70
G10	12	7	3	11	7	8	5	6	8	2	1	70
G11	12	7	3	11	7	8	5	6	8	2	1	70
G12	12	7	3	11	7	8	5	6	8	2	1	70
G13	13	7	3	11	7	8	5	6	8	2	1	71
G14	13	7	3	11	8	9	4	7	8	2	1	73
G15	11	7	3	11	7	8	4	6	7	2	1	67
G16	11	7	3	11	7	8	4	6	7	2	1	67
G17	11	7	3	11	7	8	5	6	6	2	1	67
G18	11	7	3	11	7	8	4	6	7	2	1	67
G19	11	7	3	11	8	9	5	7	7	2	1	71
G20	9	7	3	11	7	9	5	6	8	1	1	67
G21	9	7	3	11	7	9	4	6	8	1	1	66
G22	9	5	3	11	7	9	7	6	8	1	1	67
G23	12	7	4	11	7	8	4	6	8	2	1	70
G24	12	7	4	11	7	8	4	6	8	2	1	70
G25	13	8	4	9	7	9	4	7	7	2	1	71
G26	13	8	3	9	7	9	4	7	7	2	1	70
G27	13	8	4	9	7	9	3	7	7	2	1	70
G28	13	8	3	10	8	9	5	7	9	3	1	76
G29	13	8	4	10	8	9	5	7	9	3	1	77
G30	12	7	4	11	7	8	4	6	8	2	1	70
G31	13	7	3	10	7	8	3	6	8	2	1	68
G32	12	7	3	11	7	8	3	6	8	2	1	68
G33	12	7	3	11	7	8	4	6	8	2	1	69
G34	14	7	4	11	7	8	4	6	8	2	1	72
G35	14	7	4	11	8	9	4	7	7	2	1	74
G36	14	7	4	11	7	8	3	6	7	2	1	70
G37	12	7	3	11	8	9	4	7	7	2	1	71
G38	14	7	4	11	7	8	4	6	7	2	1	71
G39	13	7	4	11	8	9	4	7	8	2	1	74
G40	13	7	3	11	7	8	5	6	8	2	1	71
G41	13	7	3	11	8	9	5	7	7	2	1	73
G42	13	7	3	11	7	8	5	6	7	2	1	70
G43	12	7	4	10	8	9	4	7	8	2	1	72
G44	12	7	4	10	8	9	4	7	8	2	1	72
G45	12	7	4	10	7	8	3	6	8	2	1	68
G46	11	7	3	11	7	8	4	6	8	2	1	68
G47	11	7	3	11	7	8	5	6	8	2	1	69
G48	12	7	3	11	7	8	6	6	8	2	1	71
G49	12	8	3	11	8	9	5	7	9	3	1	76
G50	12	7	2	11	7	8	5	6	9	2	1	70
G51	12	7	2	10	7	8	5	6	9	2	1	69
G52	12	7	4	11	8	9	6	7	9	2	1	76
G53	12	7	3	11	7	8	5	6	9	2	1	71
G54	12	8	2	11	7	8	4	6	9	2	1	70
G55	12	7	4	11	7	8	4	6	9	2	1	71
G56	12	7	4	11	7	8	4	6	8	2	1	70
G57	13	8	2	10	8	9	5	6	7	1	1	70
G58	13	7	3	11	8	9	5	7	7	2	1	73
G59	14	7	3	11	7	8	5	6	8	2	1	72
G60	12	7	4	11	7	8	5	6	8	2	1	71
G61	12	7	3	11	7	8	3	6	8	2	1	68
G62	13	7	3	10	7	8	4	5	7	1	1	66
G63	13	7	3	11	7	8	4	6	8	2	1	70
G64	14	7	3	11	7	8	5	6	8	2	1	72
G65	14	7	3	11	7	8	5	6	8	2	1	72
G66	14	7	2	11	7	8	5	6	8	2	1	71
G67	13	7	4	11	7	8	4	6	8	2	1	71
G68	12	7	3	11	7	8	4	6	8	2	1	69
G69	13	7	2	11	7	8	4	6	8	2	1	69
G70	12	7	4	11	7	8	3	6	8	2	1	69
G71	12	7	3	11	7	8	3	6	8	2	1	68
G72	12	7	3	11	7	8	2	6	7	2	1	66
G73	12	7	3	11	8	9	4	7	8	2	1	72
Total	854	510	225	756	526	616	324	440	542	128	65	4986
Average	12	7	3.1	10	7.2	8.4	4.4	6	7.4	1.8	0.9	68.31

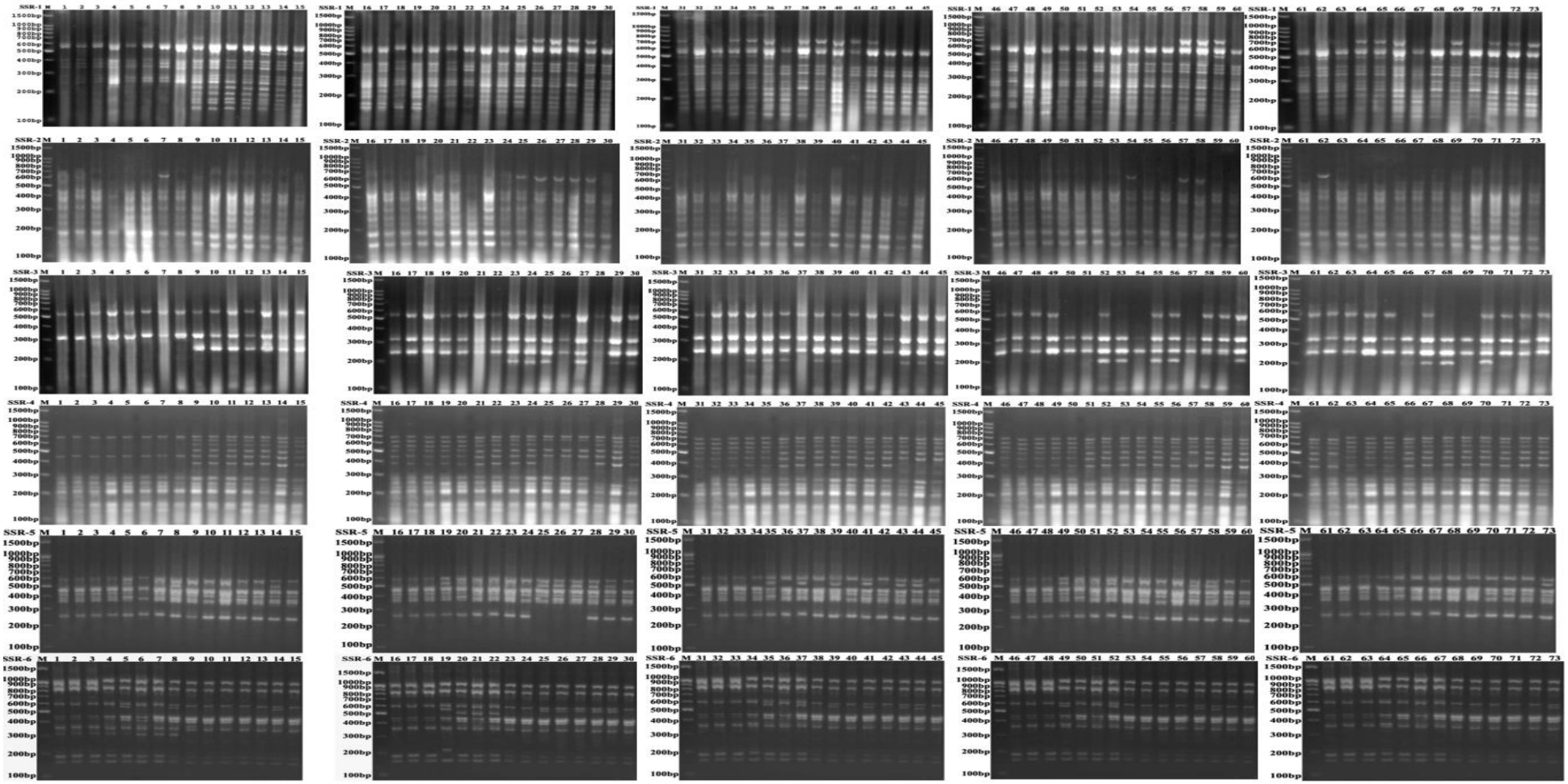

Fig. 1: ISSR banding profile of 8 durum (lanes 1 to 8) and 65 bread (lanes 9 to 73) wheat genotypes amplified with the primers SSR1 to SSR 6. Lane M represented the DNA ladder.

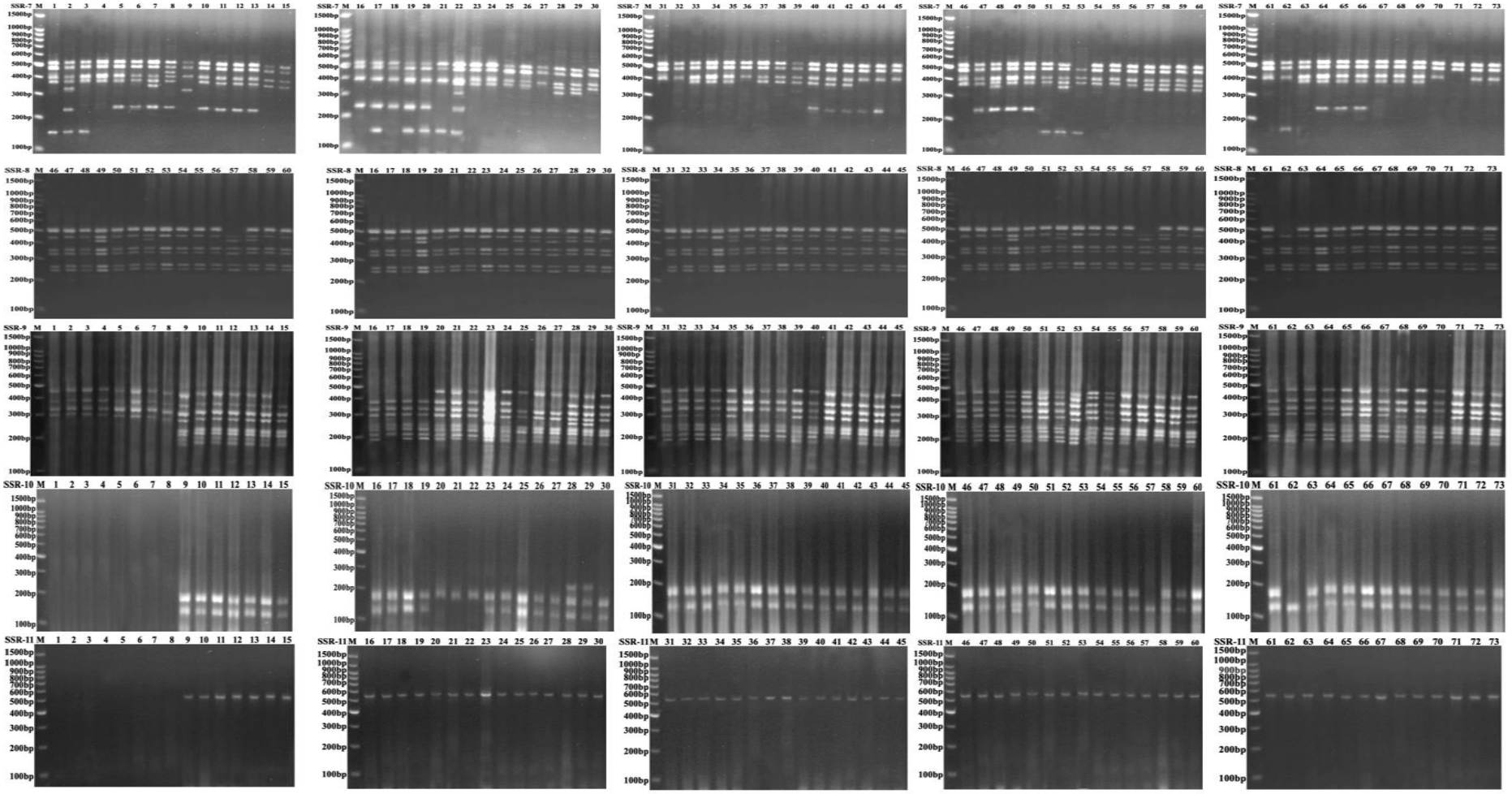

Fig. 1: ISSR banding profile of 8 durum (lanes 1 to 8) and 65 bread (lanes 9 to 73) wheat genotypes amplified with the primers SSR7 to SSR 11. Lane M represented the DNA ladder.

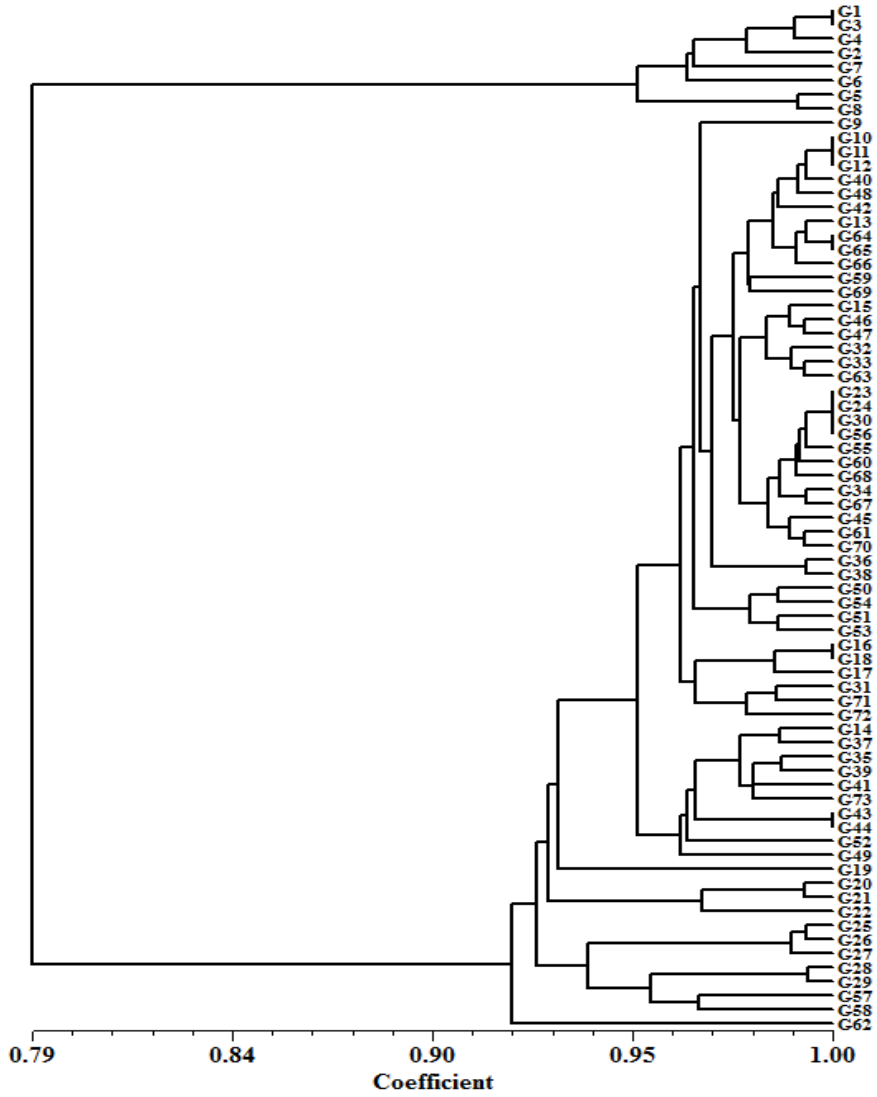

Fig. (3): Dendrogram of 73 wheat genotypes developed from SSR data using UPGMA analysis. The scale is based on Dice coefficients of similarity.

number of bands and the number of polymorphic bands amplified by the eleven SSR primers. The high level of polymorphism is in accordance with the previously published results **(Ro¨der *et al.,* 1995; Bryan *et al.,* 1997; Islam *et al.,* 2012; Akfirat and Uncuoglu, 2013; Wang *et al.,* 2013; Salem and Mattar, 2014** and **Khaled *et al.,* 2015)**

The high level of polymorphism in the present study indicative of greater genetic diversity between the tested wheat genotypes, which include 65 bread wheat and 8 durum wheat genotypes, and

confirmed the efficiency of SSR molecular markers in detecting polymorphism among wheat genotypes. It is generally reported that polymorphism between cultivars can arise through nucleotide changes that prevent amplification by introducing a mismatch at one priming site; deletion of a priming site; insertions that render priming site too distant to support amplification and insertions or deletions that change the size of the amplified product **(Powell *et al.,* 1996).** Polymorphism also considered as a useful selection tool in monitoring alien genome introgression in wheat breeding programs.

Primers differentiating bread wheat and durum wheat genotypes

Eleven alleles at molecular size [219 bp and 188 bp (SSR-1), 239 bp (SSR-3), 635 bp and 503 bp (SSR-4), 358 bp and 331 bp (SSR-8), 250 bp, 226 bp and 210 bp (SSR-9) and 532 bp (SSR-11)], were present only in bread wheat genotypes, while one allele at molecular size 312 bp generated with primer (SSR-6) was unique to durum wheat genotypes. These alleles could be used as marker to distinguish the durum wheat from the bread wheat genotypes.

Genotype Specific Primers

Eleven SSR primers produced a total of 9 unique bands. There were 9 unique fragments (10.11%) that could distinguish genotypes. Maximum number of unique bands (3) was scored with the primer SSR-6. Out of the 73 wheat genotypes, only seven varieties (G22, G6, G9, G19, G8, G72 and G49) possessed unique bands. These unique alleles were; two in G19 [one positive at 215 bp and one negative at 189 bp (SSR-6)], two negative alleles at 439 bp and 389 bp (SSR-2) in G22, one negative allele at 361 bp (SSR-5) in G6, one positive marker at 363 bp (SSR-6) in G9, one positive and one negative alleles with 435 bp and 403 bp (SSR-7) in G8 and G72, respectively, and one positive allele at 93 bp (SSR-10) in G49. Unique SSR alleles have also been reported by **Akfirat and Uncuoglu (2013).**

Polymorphism information content (PIC)

The PIC values obtained here are typical of dominant markers in which only two alleles (i.e. states) – presence (1) and absence (0) – were assumed for a particular locus (i.e. band or row) and thus by definition the highest frequency for a locus is 0.5. The PIC value, a parameter indicative of the degree of informativeness of a marker, in this study, the PIC ranged from 0.06 (primer SSR-6) to 0.2 (primer SSR-11) with an average value of 0.13 **(Table 2).** These results reflect the wide range between the frequencies of alleles at loci of SSR studied in the tested wheat varieties.

Achtara *et al.* (2010) reported that the PIC values in 49 wheat varieties (37 durum and 12 bread wheat) ranged between 0.0-0.88. However, **Bryan *et al.* (1997)** found that PIC value with an average 0.51 from 49 SSR primer pairs isolated from hexaploid wheat genome. The genetic differentiation of 60 wheat cultivars selected for adaptation and end-use from Hungary, Austria, and German using 42 microsatellite showed, on average, PIC value of 0.57 **(Stachel *et al.,* 2000).**

There was significant correlation between PIC and the number of alleles ($r = 0.52$, $p<0.01$). Therefore, the number of alleles can be used for the evaluation of genetic diversity. The results obtained in the present investigation were in agreement with those of **(Huang *et al.,* 2002; Achtara *et al.,* 2010; Wang *et al.,* 2013; Khavarinejad, 2014** and **Reza *et al.,* 2015)** who reported that the PIC value was correlated with the number of alleles.

Resolving power (RP)

The resolving power (Rp) is another method used to measure the ability of primers or techniques to distinguish between genotypes **(Prevost and Wilkinson, 1999).** The estimates of RP ranged from 0.22 in SSR-11 to 2.79 in SSR-1 with an average of 1.17 per primer **(Table 2). Prevost and Wilkinson (1999)** reported that the RP index provides a moderately accurate estimate of the number of genotypes identified by a primer. RP was significantly associated with the other molecular indices e.g. PPB and PIC ($r = 0.23$ and $r = 0.19$, respectively) in this study. The resolving power provides information on the ability of a primer, i.e. SSR-1 (PR = 2.79), to reflect the genetic or taxonomic relationships of a group of genotypes under study.

Genetic similarity (GS)

The genetic similarity (GS) for pairs of the genotypes was calculated using the software NTSYS program according of Dice coefficients. The bivariate (1/0) data and Dice similarity coefficient matrix of the 73 wheat genotypes, based on the data of eleven SSR primers, were used to construct the dendrogram **(Fig. 3).**

The similarity indices based on all possible pairs of genotypes ranged from 0.73 to 1.00 with an average 0.87. Close similarity was observed between G1/G3, G10/G11/G12, G64/G65, G23/G24/G30/G56, G16/G18 and G43/G44 indicating that these genotypes were

similar to each other at the DNA loci tested by the eleven SSR primers.

The other genotypes displayed moderate to high genetic similarity between them (GS ≤ 0.73) which reflects low degree of genetic variability among these genotypes at the tested loci. Several authors have reported a large genetic similarity in wheat **(Sehgal *et al.,* 2012 and Wang *et al.,* 2013)**

The dendrogram **(Fig. 3)** grouped the 73 wheat genotypes according to their ploidy levels into two main clusters within a close-off 0.79 GS. Cluster 1 included the durum wheat genotypes, while cluster 2 contained all bread wheat genotypes.

The dendrogram also reflected the differences in genetic diversity within the two ploidy levels and discriminated all genotypes. In cluster 1, the durum wheat genotypes were divided at 0.95 GS into two sub-clusters, the first contains the genotypes (G1, G2, G3, G4, G6 and G7) and the second included G5 and G8.

The bread wheat genotypes were clustered together in several sub-clusters within high similarity levels (GS ≤ 0.92) reflecting low variability between them at the tested SSR loci.

Powell *et al.* (1996) reported that several factors might affect the estimates of genetic relationships between individuals i.e., number of markers used, distribution of markers in the genome (genome coverage) and the nature of evolutionary mechanisms underlying the variation measured.

The above discussion amply demonstrates the utility of microsatellites, which can be profitably utilized in wheat not only for detecting polymorphism and tagging genes **(Prasad *et al.,* 1999** and **Salem and Mattar, 2014)** but also for genotype identifica-tion and for estimation of genetic diversity.

These results are in agreement with those obtained by **(Ro¨der *et al.,* 1998; Pestsova *et al.,* 2000; Achtara *et al.,* 2010; Islam *et al.,* 2012; Spanic *et al.,* 2012; Bousba *et al.,* 2013; Wang *et al.,* 2013; Amir *et al.,* 2014** and **Khavarinejad, 2014).**

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