academicJournals

Vol. 15(21), pp. 960-973, 25 May, 2016
DOI: 10.5897/AJB2015.15185
Article Number: 5F2E9F858604
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Assessment of genetic diversity among sixty bread wheat (*Triticum aestivum*) cultivars using microsatellite markers

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Received 19 December, 2015; Accepted 20 April, 2016.

Assessment of genetic diversity among wheat cultivars is important to ensure that a continuous pool of cultivars with varying desirable traits is maintained. In view of this, a molecular study was conducted to assess the genetic diversity of sixty wheat cultivars using sixty microsatellite markers. Amplified alleles from each cultivar were scored after running in 6% poly acrylamide gel electrophoresis (PAGE). A dendrogram was constructed based on the genetic similarity coefficient of un-weighted pair-wise group method with arithmetic average (UPGMA). The results showed that 276 alleles were amplified by 48 polymorphic microsatellite markers averaging 5.7 alleles per locus. A total of 12 markers did not amplify any alleles from the 60 cultivars. Polymorphism of alleles and genetic diversity measured by polymorphic information content (PIC) and Shannon index (SI) respectively, found that genome A had the highest genetic diversity followed by genome B while genome D was the lowest diverse. Cluster analysis resulted in formation of four clusters comprising of 3, 7, 9 and 41 cultivars. Genetic distance between the clusters ranged from 0.56 to 0.87 and most cultivars showed high diversity between genetic distances of 0.65 and 0.75. The four clusters and their similarities will help breeders to breed new disease resistant cultivars and make rational deployment of cultivars in production based on the established relationships.

Key words: Genetic diversity, molecular marker, microsatellite (SSR marker), *Triticum aestivum.*

INTRODUCTION

Common wheat (*Triticum aestivum* L.) is among the most important cereals currently grown in most parts of the world. The crop is among the three world's major cereal export earners with others including maize and rice (Tong et al., 2003; Abdellatif and Abouzeid, 2011). It forms more than 40% of the world's commonly consumed food and 95% of people in the developing countries eat wheat or maize in form of flour as a main food source (Akhtar et

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al., 2011; Coventry et al., 2011). The crop provides one fifth of the global required calories (Reynolds et al., 2011; Friedrich et al., 2014). Currently, wheat is grown on approximately 216 million hectares of land worldwide with an estimated production of 605 million tons (Abdellatif and Abouzeid, 2011). China is the largest wheat producer and consumer in the world (FAO, 2014). As at 2013, the crop was produced on approximately 24 million hectares of land yielding 121 million tons nationally, representing 11.2 and 17.6% of the world's total harvest area and production tonnage, respectively (FAO, 2014; Li et al., 2014). The crop is mostly produced in 30 provinces across China with 1.9 million hectares (8%) covered by spring wheat and 22.3 million hectares (92%) grown with winter wheat. Spring wheat is mainly grown in the northeastern, central northern and northwestern China including parts of Gansu, Xinjiang and Qinghai provinces, while winter wheat is mainly grown in eastern China including parts of Henan, Shandong, Anhui and Hebei provinces among others (Liu et al., 2014).

In order to sustain high levels of wheat production in China, one of the most important requirements is the maintenance of a diverse pool of wheat cultivars where 'superior' gene/alleles can be obtained for genetic improvement programs. Intensive activities aimed at improving wheat crop such as selection of cultivars with desirable attributes have led to a reduced genetic diversity over time, increased disease incidences, a decline in crop yield and compromised drought tolerance among many other biotic and abiotic challenges (Roussel et al., 2004; Fu et al., 2005; Mir et al., 2012).

Presently, it is extremely difficult to increase the land area for wheat production in China due to pressure from human population growth, urbanization and competition from other crops (Fu et al., 2001; Lu et al., 2007; Lu and Fan, 2013). By preserving the genetic diversity, growers could achieve a high improvement rate of desired attributes such as pest resistance and high yields in the available wheat cultivars while maintaining land size.

Microsatellite markers also called simple sequence repeats (SSR) or short tandem repeats (STR) (Tautz, 1989; Edwards et al., 1991; Jacob et al., 1991; Kalia et al., 2011) are among the most popular molecular markers used in genetic diversity studies. This type of markers is characterized by its high efficiency, reproducibility, co-dominant nature and high degree of polymorphism (Singh et al., 2007; Royo et al., 2010; Ruiz et al., 2012; Laido et al., 2013; Meti et al., 2013). Microsatellites are vital in cultivar identification and also offer an advantage during pedigree analysis as they are genus specific (Romero et al., 2009; Abdullah et al., 2012). Several studies conducted to identify the genetic diversity of wheat cultivars using SSRs, had shown consistent results with the polymorphism expressed being significantly more reliable than that reported using other types of markers (Corbellini et al., 2002; Ahmed et al., 2010; Khodadadi et al., 2011; Shakeel and Azam, 2012; Spanic et al., 2012).

The aim of the present study was to utilize microsatellite markers in order to assess the genetic diversity of sixty wheat cultivars collected from several parts of main wheat growing regions in China. The outcome of this research could assist breeders to set up the appropriate guidelines for proper management of the wheat cultivars, as a precursor towards the implementation of future programs.

MATERIALS AND METHODS

Selection of cultivars, DNA extraction and PCR protocol

A total of 60 wheat cultivars comprising 57 wheat cultivars collected from parts of main wheat growing regions of China and 3 cultivars collected from USA and Italy were evaluated for genetic diversity. Detailed information of cultivars is shown in Table 1.

Ten seeds of each wheat cultivar were sown on trays in greenhouse located at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China. About 15 days after sowing, when three to four leaves had been developed, seedling leaves were detached and their DNA was extracted following Zheng (2010) CTAB extraction method.

To test DNA purity, all extracted DNA samples were run on 2% Agarose gel of 1% TBE buffer solution and the image was captured using Gel Documentation and Image Analysis System after staining in Ethidium bromide solution for 5 min. For PCR reaction, the DNA was diluted in the range between 50 and 80 ng/µl and the mixture comprised 5 µl PCR master mix, 2 µl double distilled water, 1 µl of 10 mM Forward primer, 1 µl of 10 mM Reverse primer and 1 µl of DNA template, with a final volume of 10 µl. PCR protocol was applied using Bio-Gener Technology, Gene explorer PCR machine with the following conditions: 94°C for 3 min, 35 cycles of 94°C for 1 min, 50 to 60°C (depending on SSR primer annealing temperature) for 30 s, 72°C for 30 s and a final extension of 72°C for 10 min before soaking at 4°C. A total of sixty wheat microsatellite markers were used to estimate the genetic diversity among the sixty cultivars used herein (Table 2). SSR markers that had linkage to designated and temporarily designated wheat powdery mildew resistance genes were selected for the study. This preference was due to the fact that a subsequent study that followed the present one required the utilization of the same cultivars and markers for molecular disease resistance assessment. Marker sequences, chromosomal locations and corresponding annealing temperatures were retrieved from the graingenes website (http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker).

Simple sequence repeat protocol

SSR protocol for 6% poly-acrylamide gel electrophoresis (PAGE) was used. The gel glass was stained in 1500 ml of water containing 3 g silver nitrate solution. Thereafter, the alleles were enhanced in 2000 ml of water solution containing 3 ml of 37% formaldehyde (H_2CO) and 30 g sodium hydroxide.

Data analysis

All clearly amplified alleles on the cultivars were treated as a single locus. Scoring was based on presence and absence of the alleles. Bivariate 1 and 0 data matrices obtained from the stained gel were used to construct a dendrogram based on the genetic similarity coefficient. Sahn-clustering of un-weighted pair-group method with

Table 1. Names of wheat cultivars, pedigree information and origin.

Cultivar designation	Name of cultivar	Pedigree information*	Origin
1	Lantian095	-	Gansu
2	Tian0015	-	Gansu
3	Tian01-104	93R177 / 912-2-1-2	Gansu
4	05bao1-1	Zhongliang22+ gDNA of oil sunflower	Gansu
5	Chancellor	Carina/Mediterranean//Dietz/ Carina/3/P-1068/3×Purplestraw	-
6	Tian00127	(Baidatou/C184-3-4-1)F2//85-173-4	Gansu
7	Lantian23	SXAF4-7/87-121	Gansu
8	Lantian20	CappelleDesprez/Lantian10	Gansu
9	Tian03-142	9589-8-1-2-1/Qing 95-111	Gansu
10	Tian00296	9362-13-3-4/8748-0-2-1	Gansu
11	TianTian9681	863-13/87148-1-1-2-2-2	Gansu
12	Lantian093	Lantian23/Zhou92031	Gansu
13	AvocetYrA	Avocet	USA
14	Longchun26	Yong3263/Gaoyuan448	Gansu
15	TianTian96-86	863-13/8560-2-2-1	Gansu
16	Tian02-195	Wenmai8/Tian96-1c1	Gansu
17	Tian03-160	0037-1-2/9938-2-2-1	Gansu
18	Tian02-204-1	Wenmai8/9157-3-2-2-1	Gansu
19	Longjian101	8487/85-173-12-2	Gansu
20	Longjian127	7402/Lv419//7415	Gansu
21	Tian989	9362-13-4-4/lantian1	Gansu
22	Zhongzhi2	Shan167/ Guinong22/*T. Spelta* album	Beijing
23	Longjian102	Lin87-4535/81168-4-3//Longyuan932	Gansu
24	Tian98101	9362-13-4-4/Tian94-3	Gansu
25	03bao1-1	Lantian10+ DNA of oil sunflower	Gansu
26	Zhongliang27	90293///Zhongliang12/Zhongsi// Bulgaria10/Xiannong4	Gansu
27	N. Strampelli	LIBERO//S.Pastou/C.Jrometh.lig	Italy
28	Zhongzhi4	Mianyou2/Zhongzhi1	Beijing
29	Zhongzhi1	Shan167/C591	Beijing
30	Lantian097	92R137/87-121 //Shan167	Gansu
31	Taikong06	Space-flight mutation from Yumai49	Henan
32	Kenya Kongoni	C18154/2×Fr/2/Romm/3/WIS.245-II-50-7/C8154/2/2×Fr	USA
33	Keyuan5	-	Henan
34	Xinmai19	(C5/xinxiang3577) F3d1s/Xinmai9	Henan
35	Xinyumai836	-	Henan
36	Yumai368	-	Henan
37	Zhoumai19	Neixiang185 / Zhoumai9	Henan
38	Guoan368	-	Henan
39	Zhoumai32	Zhoumai12/ Wenmai6 // Zhoumai13	Henan
40	Yangao03710	-	Henan
41	Zhou99233	-	Henan
42	Punong1	-	Henan
43	Pu02056	Zhoumai16/ Yumai24	Henan
44	Xinxuan2039	-	Henan
45	Zhengnong01059	-	Henan
46	Guomai301	G883/ Pumai9	Henan
47	Zhongxin01	-	Henan
48	Zhongyu885	-	Henan
49	04zhong70	-	Henan
50	Zheng366	-	Henan

Table 1, Contd.

51	Lankao008	-	Henan
52	Tianmin198	R81/Bainong64//Yanzhan4110	Henan
53	Zhengyumai9989	Benyumai21/Yumai2//Yumai57	Henan
54	Zhengmai9023	[Xiaoyan6/Xinong65//83(2)33/84(14)43] F3/3Shan213	Henan
55	Zhengmai366	Yumai47/PH82-2-2	Henan
56	Zhou mai16	Zhoumai9/Zhou8425B	Henan
57	Yanzhan4110	[(C39/Xibei78(6)9-2)/(FR81-3/ Aizao781-4)] /Aizao781-4	Henan
58	Bainong160	Duokang893/Wenmai6//Bainong64/ Wenmai6	Henan
59	Lantian15	Lantian10/Ibis	Gansu
60	Yujiao0338	-	Henan

*Cultivars with a dash (-) indicate that their pedigree information could not be traced.

Table 2. SSR markers (loci), sequence, location on the wheat chromosome and their annealing temperatures.

Marker (locus)	Marker sequence	Chromosomal location	Annealing temperature (°C)
Xgwm273 F	ATTGGACGGACAGATGCTTT	1B	55
Xgwm273 R	AGCAGTGAGGAAGGGGATC		
Xbarc229 F	GGCCGCTGGGGATTGCTATGAT	1D	58
Xbarc229 R	TCGGGATAAGGCAGACCACAT		
Xgwm294 F	GGATTGGAGTTAAGAGAGAACCG	2A	55
Xgwm294 R	GCAGAGTGATCAATGCCAGA		
Xwmc382 F	cATgAATggAggcAcTgAAAcA	2A	61
Xwmc382 R	ccTTccggTcgAcgcAAc		
Xgwm319 F	GGTTGCTGTACAAGTGTTCACG	2B	55
Xgwm319 R	CGGGTGCTGTGTGTAATGAC		
Xgwm210 F	TGCATCAAGAATAGTGTGGAAG	2B	60
Xgwm210 R	TGAGAGGAAGGCTCACACCT		
Xgwm257 F	AGAGTGCATGGTGGGACG	2B	61
Xgwm257 R	CCAAGACGATGCTGAAGTCA		
Xwmc356 F	gccgTTgcccAATgTAgAAg	2B	61
Xwmc356 R	ccAgAgAAAcTcgccgTgTc		
Xwmc317 F	TgcTAgcAATgcTccgggTAAc	2B	61
Xwmc317 R	TcAcgAAAccTTTTccTccTcc		
Xwmc41 F	TcccTcTTccAAgcgcggATAg	2D	61
Xwmc41 R	ggAggAAgATcTcccggAgcAg		
Xwmc445 F	AgAATAggTTcTTgggccAgTc	2D	51
Xwmc445 R	gAgATgATcTccTccATcAgcA		
Xwmc291 F	TAccAcgggAAAggAAAcATcT	3B	61
Xwmc291 R	cAcgTTgAAAcAcggTgAcTAT		
Xgwm108 F	CGACAATGGGGTCTTAGCAT	3B	60
Xgwm108 R	TGCACACTTAAATTACATCCGC		
Xgwm415 F	GATCTCCCATGTCCGCC	5A	55
Xgwm415 R	CGACAGTCGTCACTTGCCTA		
Xgwm126 F	CACACGCTCCACCATGAC	5A	60
Xgwm126 R	GTTGAGTTGATGCGGGAGG		
Xwmc75 F	gTccgccgcAcAcATcTTAcTA	5B	61
Xwmc75 R	gTTTgATccTgcgAcTcccTTg		
Xgwm408 F	TCGATTTATTTGGGCCACTG	5B	55
Xgwm408 R	GTATAATTCGTTCACAGCACGC		
Xwmc810 F	GGCACCGATGCTTCCA	5B	61

Table 2, Contd.

Xwmc810 R	GCCCCAACCACCTCCC		
Xbarc232 F	CGCATCCAACCATCCCCACCCAACA	5B	65
Xbarc232 R	CGCAGTAGATCCACCACCCCGCCAGA		
Xbarc142 F	CCGGTGAGAGGACTAAAA	5B	52
Xbarc142 R	GGCCTGTCAATTATGAGC		
Xgwm67 F	ACCACACAAACAAGGTAAGCG	5B	60
Xgwm67 R	CAACCCTCTTAATTTTGTTGGG		
Xgwm174 F	GGGTTCCTATCTGGTAAATCCC	5D	55
Xgwm174 R	GACACACATGTTCCTGCCAC		
Cfd57 F	ATCGCCGTTAACATAGGCAG	5D	60
Cfd57 R	TCACTGCTGTATTTGCTCCG		
Xgwm583 F	TTCACACCCAACCAATAGCA	5D	60
Xgwm583 R	TCTAGGCAGACACATGCCTG		
Xgwm205 F	CGACCCGGTTCACTTCAG	5D	60
Xgwm205 R	AGTCGCCGTTGTATAGTGCC		
Xgwm583 F	TTCACACCCAACCAATAGCA	5D	60
Xgwm583 R	TCTAGGCAGACACATGCCTG		
Xgwm292 F	TCACCGTGGTCACCGAC	5D	60
Xgwm292 R	CCACCGAGCCGATAATGTAC		
Xwmc553 F	cggAgcATgcAgcTAgTAA	6A	60
Xwmc553 R	cgccTgcAgAATTcAAcAc		
Xwmc684 F	CGAATCCAACGAGGCCATAGA	6A	61
Xwmc684 R	GCAATCAGGAGGCATCCACC		
Xpsp3131 F	GCTAGTCCCGACGCCCTATC	6B	61
Xpsp3131 R	GAGGAAGGAGCTTTGGTTTCTCC		
Xwmc397 F	AgTcgTgcAccTccATTTTg	6B	61
Xwmc397 R	cATTggAcATcggAgAccTg		
Xgwm325 F	TTTCTTCTGTCGTTCTCTTCCC	6D	60
Xgwm325 R	TTTTTACGCGTCAACGACG		
Xbarc183 F	CCCGGGACCACCAGTAAGT	6D	58
Xbarc183 R	GGATGGGGAATTGGAGATACAGAG		
Xcfa2240 F	TGCAGCATGCATTTTAGCTT	7A	60
Xcfa2240 R	TGCCGCACTTATTTGTTCAC		
Xcfa2019 F	GACGAGCTAACTGCAGACCC	7A	60
Xcfa2019 R	CTCAATCCTGATGCGGAGAT		
Xcfa2257 F	GATACAATAGGTGCCTCCGC	7A	60
Xcfa2257 R	CCATTATGTAAATGCTTCTGTTTGA		
Xwmc346 F	cTgAAgTTccAgccAAcAcA	7A	61
Xwmc346 R	ATTcccTcATcccgTTgc		
Xwmc525 F	gTTTgAcgTgTTTgcTgcTTAc	7A	61
Xwmc525 R	cTAcggATAATgATTgcTggcT		
Xbarc1073 F	GCGGGCACAATATTCTAATGGACAAAG	7B	55
Xbarc1073 R	GCGCAGATGCAGAGGCCAGGGGTC		
Xwmc276 F	gAcATgTgcAccAgAATAgc	7B	51
Xwmc276 R	AgAAgAAcTATTcgAcTccT		
Xcfa2040 F	TCAAATGATTTCAGGTAACCACTA	7B	60
Xcfa2040 R	TTCCTGATCCCACCAAACAT		
Xgwm611 F	CATGGAAACACCTACCGAAA	7B	55
Xgwm611 R	CGTGCAAATCATGTGGTAGG		
Xpsp3033 F	GTTGGCAGTGTAAATCGGTG	7B	61
Xpsp3033 R	GAGCCACGTATGCAATGGACG		
Xgwm46 F	GCACGTGAATGGATTGGAC	7B	60

Table 2. Contd.

Xgwm46 R	TGACCCAATAGTGGTGGTCA		
Xgwm297 F	ATCGTCACGTATTTTGCAATG	7B	55
Xgwm297 R	TGCGTAAGTCTAGCATTTTCTG		
Xpsp3029 F	CCATCGATGAGGATCTCCTCGGGCA	2A, 6A	58/61
Xpsp3029 R	GCAACAGGACCATGGTCG		
Xwmc289 F	cATATgcATgcTATgcTggcTA	5B,5D	61
Xwmc289 R	AgccTTTcAAATccATccAcTg		
Xgwm296 F	AATTCAACCTACCAATCTCTG	2D,7D	55
Xgwm296 R	GCCTAATAAACTGAAAACGAG		
Xgwm265 F	TGTTGCGGATGGTCACTATT	2A,4A	55
Xgwm265 R	GAGTACACATTTGGCCTCTGC		
Xgwm111 F	TCTGTAGGCTCTCTCCGACTG	7B, 7D	55
Xgwm111 R	ACCTGATCAGATCCCACTCG		
Xgwm344 F	CAAGGAAATAGGCGGTAACT	7A, 7B	55
Xgwm344 R	ATTTGAGTCTGAAGTTTGCA		
Xgwm159 F	GGGCCAACACTGGAACAC	5B, 5D	60
Xgwm159 R	GCAGAAGCTTGTTGGTAGGC		
Xgwm382 F	GTCAGATAACGCCGTCCAAT	2A,2B,2D	60
Xgwm382 R	CTACGTGCACCACCATTTTG		
Xpsp3003 F	GATCGACAAGGCTCTAATGC	1A,5A,7D,	63
Xpsp3003 R	CAGGAGGAGAGCCTCTTGG		
Xcfd81 F	TATCCCCAATCCCCTCTTTC	7D,5D,4D	60
Xcfd81 R	GTCAATTGTGGCTTGTCCCT		
Xcfd39 F	CCACAGCTACATCATCTTTCCTT	4B,4D,5A	60
Xcfd39 R	CAAAGTTTGAACAGCAGCCA		
Xgwm356 F	AGCGTTCTTGGGAATTAGAGA	2A,6A,7A	55
Xgwm356 R	CCAATCAGCCTGCAACAAC		
Xgdm93 F	AAAAGCTGCTGGAGCATACA	2A,2D,4B	55
Xgdm93 R	GGAGCATGGCTACATCCTTC		
Xwmc273 F	AgTTATgTATTcTcTcgAgccTg	7A,7B,7D	51
Xwmc273 R	ggTAAccAcTAgAgTATgTccTT		
Xgwm526 F	CAATAGTTCTGTGAGAGCTGCG	2A, 2B, 7A, 7B	55
Xgwm526 R	CCAACCCAAATACACATTCTCA		
Xgwm311 F	TCACGTGGAAGACGCTCC	2A, 2B, 2D, 6B	60
Xgwm311 R	CTACGTGCACCACCATTTTG		

arithmetic average (UPGMA) were applied using the software NTSYSpc 2.1 (Numerical Taxonomy and Multivariate Analysis System), version 2.1 (Rohlf, 2000). Polymorphism information content (PIC) was calculated using the following formula:

$PIC = 1 - \sum_{i=1}^{n}(fi)^2$ for *n* alleles

Where *fi* = frequency of *i*th allele for *n* alleles at a locus (Powell et al., 1996).

PIC measures the informativeness of the DNA markers over a set of genotypes during gene mapping, molecular breeding and germplasm evaluation (Peng and Lapitan, 2005; Varshney et al., 2007; Wang et al., 2007).

A molecular marker with lower PIC indicates less informativeness in expressing the polymorphism of its alleles at a locus while higher PIC value indicates the high ability of the marker to express polymorphism of alleles at a locus. Shannon-weaver index (SI) was calculated as described by Chen and Li (2007). The index estimates species diversity in a community at a particular time. The diversity index, also known as the Shannon-Wiener species diversity index or simply the Shannon index, calculates the number of different species in a community (species richness) and the proportion of individuals from a single species as compared to the number of individuals of other species in the same community. A Shannon-Weaver diversity index of zero indicates that only one species is present in the community; as diversity increases, so does the index number.

The most diverse communities have an index of seven or higher. The formula used for index calculation was:

$SI = -\sum_{i=1}^{n}(Pi \ln[Pi])$ for *n* species

Where *Pi* = number of *i*th individuals in a particular *n* species divided by the total number of individuals of all species in the community.

Table 3. Number of alleles, range of allele sizes, polymorphic information content (PIC) and Shannon-Weaver diversity index (SI) for genome A of wheat loci.

Locus	Number of alleles	Expected allele size (bp)	Range of allele sizes (bp)	Polymorphic information content	Shannon-Weaver diversity index
Xwmc382-2A	10	270	250- 450	0.882	3.290
Xgwm294-2A	7	96	90- 160	0.761	2.185
Xgwm126-5A	4	196	190- 225	0.674	1.358
Xwmc553-6A	8	395	375- 550	0.817	2.364
Xwmc684-6A	7	190	150- 290	0.833	2.234
Xcfa2240-7A	6	280	220- 300	0.675	1.474
Xcfa2257-7A	4	167	150- 220	0.503	1.006
Xcfa2019-7A	5	217	190- 260	0.728	1.638
Xwmc346-7A	6	203	180- 270	0.729	1.775
Xwmc525-7A	4	206	195- 280	0.640	1.316
Total	61		90-550	-	-
Mean	6.1		-	0.724	1.864

RESULTS

Polymorphism of SSR markers and genetic diversity

Number of amplified alleles per locus, PIC and SI values varied among wheat genomes A, B and D in the 60 cultivars analyzed. In genome A, locus Xwmc382-2A had the highest number of alleles (10) followed by Xwmc553-6A, which had 8 alleles (Table 3). Locus Xwmc382-2A also had the highest PIC value of 0.882 as well as the highest SI value of 3.290. Locus Xwmc553-6A was second with PIC of 0.817 and SI value of 2.364. The lowest number of alleles per locus (4) in genome A was recorded in the loci Xgwm126-5A, Xcfa2257-7A and Xwmc525-7A. Locus Xcfa2257-7A showed the lowest PIC and SI values of 0.503 and 1.006, respectively (Table 3). For a total of 10 polymorphic loci in the A genome, 61 alleles were recorded and their molecular sizes ranged from 90 to 550 bp. Alleles in locus Xwmc382-2A ranged from 250 to 450 bp and significant polymorphism was observed between 250 and 320 bp (Figure 1).

In genome B, locus Xbarc142-5B amplified 10 alleles, being the highest number of all polymorphic loci in the genome, with a range from 175 to 350 bp. Significant polymorphism was observed between 150 and 290 bp (Figure 2). The locus had the highest PIC and SI values of 0.834 and 2.787, respectively (Table 4). This was followed by loci Xwmc810-5B and Xgwm46-7B, having 9 alleles each. Locus Xwmc810-5B showed allele sizes ranging from 150 to 300 bp while the PIC and SI values were 0.868 and 2.533, respectively (Table 4).

The lowest number of alleles (2) in B genome was recorded in locus Xwmc276-7B with a range between 250 and 390 bp. The locus also exhibited the lowest PIC (0.132) and SI (0.378) value. Loci Xgwm108-3B and Xgwm319-2B amplified 3 alleles each and their molecular sizes ranged from 110 to 495 bp (Table 4).

In D genome, the highest number of alleles (9) was recorded in locus Xwmc445-2D (Figure 3) followed by locus Xgwm325-6D with 7 alleles. Xwmc445-2D also had the highest SI and PIC values of 2.543 and 0.815, respectively (Table 5). This was followed by locus Xgwm325-6D, which had an SI value of 1.992 and PIC of 0.776. A total of 40 alleles were recorded in the 8 polymorphic loci of the genome D, with an average of 5 alleles per locus (Table 5). Size of alleles ranged from 150 to 380 bp while average PIC and SI values were 0.613 and 1.294, respectively.

Fifteen markers amplified alleles from multiple loci of the A, B and D wheat genomes. For instance, marker Xgwm344 (Figure 4) amplified alleles from loci in genome A and B for chromosome 7 (Table 6). The marker amplified 7 alleles, as markers Xwmc273 and Xcfd39. It also had the highest PIC and SI values of the group, 0.860 and 1.810 respectively. The lowest number of alleles (4) was found in markers Xcfd81, Xgwm382, Xgwm356 and Xgwm311. These four markers had their SI values below 1.0 (Table 6).

Out of 60 markers studied herein, 48 amplified a total of 276 alleles with an average of 5.7 alleles per locus. 61 alleles were amplified in genome A, 93 in genome B and 40 in genome D. A total of 82 alleles were amplified from markers that detected multiple loci in the wheat genome. Genome A had the highest PIC mean value of 0.724, while the lowest one was recorded in genome D (0.676). Genome A also had the highest SI value of 1.864 while the lowest one of 1.312 was recorded in those markers that detected multiple loci. Sizes of the alleles ranged from 90 to 550 bp with an overall PIC and SI values of 0.703 and 1.543 respectively (Table 7).

Cluster analysis

Cluster analysis represented by a dendrogram plotted

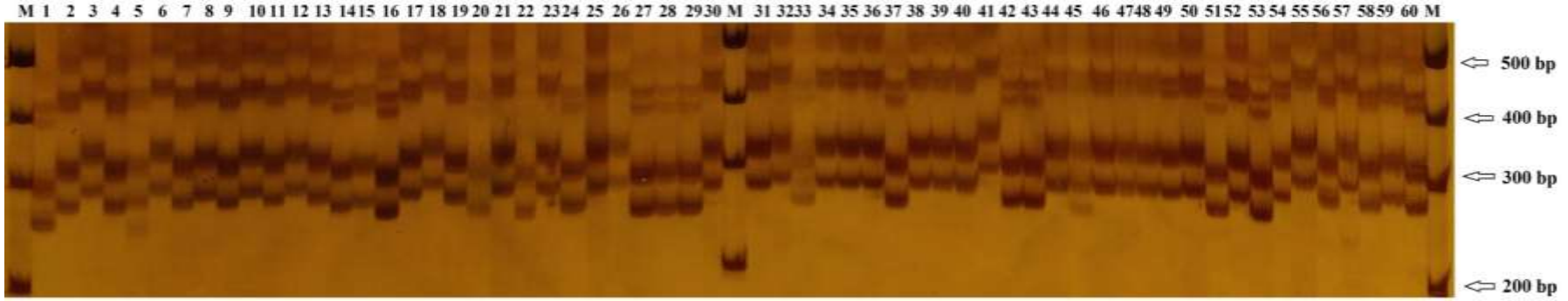

Figure 1. Amplified alleles on locus Xwmc382-2A for 60 wheat genotypes, M is 100 bp Marker.

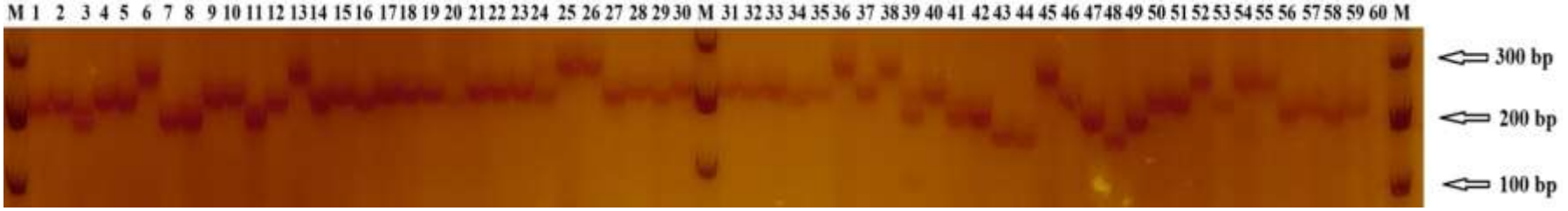

Figure 2. Amplified alleles on locus Xbarc142-5B for 60 wheat genotypes, M is 100 bp marker.

using the UPGMA method, revealed four major clusters. The genetic distance between clusters ranged from 0.56 to 0.87 and most cultivars showed a high degree of diversity within a range of 0.65 to 0.75 (Figure 5). Cluster 1 was made up of three cultivars namely, Lantian095, Tian00127 and Zhongliang27. The cultivars were spread within a distance range of 0.647 to 0.687. Cluster 2 comprised of seven cultivars, included Tian0015, 05bao1-1, Chancellor, Tian03-142, Tian96-86, Tian01-104 and Longchun26 spread on a distance range of 0.642 to 0.752. Cluster 3 was the largest and most diverse cluster consisting of 41 cultivars. It contained several sub-clusters within a genetic distance range of 0.642 to 0.87. The fourth cluster was composed of 9 cultivars namely Lantian23, Lantian093, Tian9681, Longjian101, Tian989, Lantian20, Tian00296, Zhongxin01 and Zheng366. Its genetic distance ranged between 0.643 and 0.832 (Figure 5).

DISCUSSION

Polymorphism of SSR markers in wheat genomes

SSR markers have been used widely in genetic studies due to their high polymorphism in the genomes (Gupta and Varshney, 2000; Kalia et al., 2011; Jamalirad et al., 2012). In this study, a total of 276 alleles were identified by 48 polymorphic markers with an average of 5.7 alleles per locus. The results are comparable to findings reported elsewhere. In assessing genetic diversity of 62 Sichuan wheat landraces using 114 SSR markers, Li et al. (2013) reported an average of 4.76 alleles per locus, which is slightly lower as compared to the findings herein. Wang et al. (2007) also reported a mean of 3.3 alleles per locus when 60 durum wheat accessions were analyzed using 26 SSR markers. Hazen et al. (2002) found 4.7 and 6.8 alleles per locus in two assays using 24 wheat accessions obtained from Shaanxi province.

Table 4. Number of alleles, range of allele size, polymorphic information content and Shannon-Weaver diversity index for genome B of wheat loci.

Locus	Number of alleles	Expected allele size (bp)	Range of allele sizes (bp)	Polymorphic information content	Shannon-weaver diversity index
Xbarc142-5B	10	208	175- 350	0.834	2.787
Xwmc810-5B	9	196	150- 300	0.868	2.533
Xgwm46-7B	9	187	160- 490	0.853	2.363
Xgwm273-1B	8	171	170- 490	0.875	2.179
Xwmc397-6B	8	160	155- 300	0.822	1.877
Xgwm210-2B	6	303	160- 550	0.760	1.645
Xgwm257-2B	7	190	180- 350	0.794	1.999
Xwmc317-2B	8	139	115- 290	0.804	2.059
Xgwm319-2B	3	170	110- 495	0.461	0.383
Xwmc291-3B	6	233	210- 350	0.806	1.480
Xgwm108-3B	3	135	110- 150	0.374	0.521
Xbarc232-5B	4	368	160- 390	0.689	1.056
Xgwm408-5B	4	182	175- 210	0.636	1.025
Xwmc75-5B	6	206	195- 500	0.787	1.830
Xwmc276-7B	2	292	250- 390	0.132	0.378
Total	93		110- 550	-	-
Mean	6.2		-	0.700	1.608

However, Spanic et al. (2012) reported a higher mean value of 8.44 alleles per locus following an assessment of 30 wheat genotypes using 24 SSR markers, while Jamalirad et al. (2012) found a mean value of 9.26 allelesper locus when 70 wheat genotypes were evaluated with 50 SSR markers. In some cases, the average number of alleles per locus as 12.06 (Abdellatif and Abouzeid, 2011) and 16.8 (Laido et al., 2013).

Genome A had the highest PIC value followed by genome B while the lowest PIC value was recorded in genome D. The highest PIC value in genome A was recorded for locus Xwmc382-2A (0.882). Kitavi et al. (2014) found a highest PIC of 0.86 in marker Xtxp 265 and a mean PIC value of 0.54, when 30 sorghum accessions were analyzed using 22 markers. Fu et al. (2006) reported a highest PIC value of 0.98 when 37 eSSR markers were tested on 75 Canadian hard red spring wheat. Salem et al. (2008) also obtained a highest PIC value of 0.816 for locus Xgwm437 when 15 SSR markers were analyzed in 9 wheat varieties. In another study, the highest PIC value of 0.93 was found with 45 markers on sixteen bread wheat samples (Cifci and Yagdi, 2012). The results herein are, therefore, consistent with previously reported findings.

Genetic diversity of wheat cultivars

Genetic diversity as measured by Shannon Weaver Index revealed that genome A was the most diverse followed by genome B and then genome D was the least (genome A>genome B>genome D). Similar results were reported by Li et al. (2013). Schuster et al. (2009) also found that genome A had the higher genetic diversity followed by genomes B and D, when analyzing 23 SSRs in 36 Brazilian cultivars. Furthermore, Zhang et al. (2011) reported a low level of polymorphism in D genome when testing DarT markers in 111 common wheat cultivars from northern China. Studies on molecular markers and many other agronomic traits have shown the genetic base of cultivated wheat (Parker et al., 2002; Prasad et al., 2000). The low genetic diversity of genome D has caused a delicate genetic basis for modern cultivated wheat (Jia et al., 2001; Zhang et al., 2002; Chen and Li, 2007).

However, the donor of genome D, *Aegilops squarrosa* was more diverse than cultivated wheat (Dudnikov, 2000; Pestsova et al., 2000; Gianibelli et al., 2001). It is believed that the low diversity of genome D emanated from evolution of hexaploid wheat. During evolution of hexaploid wheat, genomes A and B produced more tetraploid wheat including *Triticum turgidum*, *Triticum turani-cum*, *Triticum dicoccoides*, *Triticum dicoccum*, *Triticum polonicum*, *Triticum carthlicum* and *Triticum durum*. These tetraploid wheat species were able to cross with hexaploid wheat thereby enriching the genetic diversity of A and B genome species. The crossing was carried out with *Aegilops tauschii*, resulting in production of more hexaploid wheat. On the other hand, D genome species did not produce any tetraploid wheat species. This resulted in minor genes exchange in genome D and, consequently, led to the reduction of genetic diversity in this genome benefiting genomes A and B (Perugini, 2007; Wang et al., 2007).

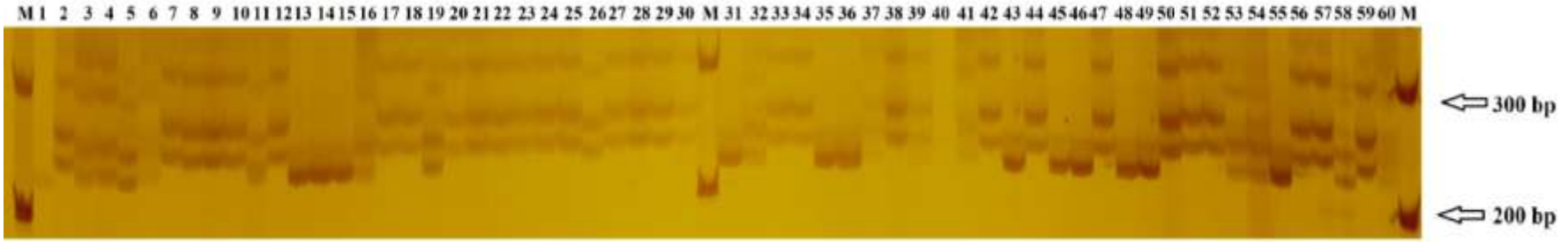

Figure 3. Amplified alleles of locus Xwmc445-2D for 60 wheat genotypes, M is 100 bp marker.

Table 5. Number of alleles, range of allele size, polymorphic information content (PIC) and Shannon-weaver diversity index (SI) for genome D of wheat loci.

Locus	Number of alleles	Expected allele size (bp)	Range of allele sizes (bp)	Polymorphic information content	Shannon-weaver diversity index
Xwmc445-2D	9	229	220- 380	0.815	2.543
Xwmc41-2D	3	163	150- 310	0.571	0.798
Xgwm174-5D	5	233	195- 300	0.746	1.241
Xgwm292-5D	6	214	205- 300	0.753	1.688
Xgwm583-5D	3	265	210- 380	0.317	0.744
Cfd57-5D	3	291	280- 300	0.228	0.429
Xbarc183-6D	4	179	175- 310	0.701	0.917
Xgwm325-6D	7	183	150- 250	0.776	1.992
Total	40		150- 380	-	
Mean	5.0		-	0.613	1.294

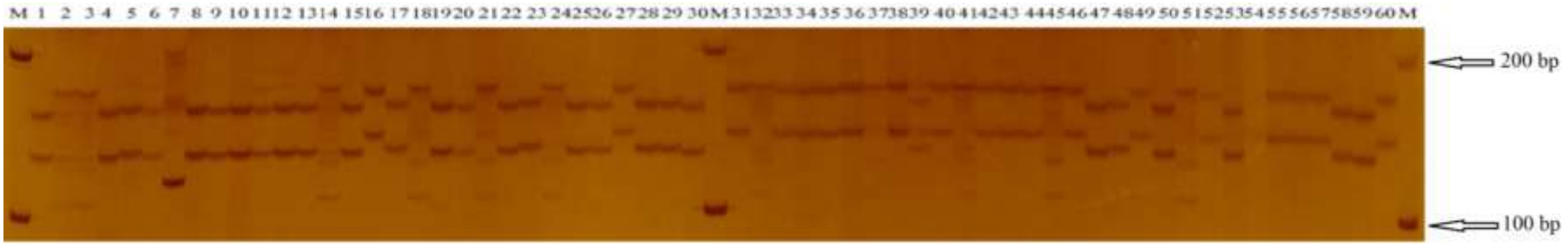

Figure 4. Amplified alleles on locus Xgwm344 for 60 wheat genotypes, M is 100 bp marker.

Table 6. Number of alleles, expected allele size, range of allele size, polymorphic information content (PIC) and Shannon-Weaver Diversity index (SI) for multiple loci in wheat genomes.

Marker	Chromosome	Number of alleles	Expected allele size (bp)	Range of allele sizes (bp)	Polymorphic Information Content	Shannon-Weaver Diversity Index
Xgwm344	7A, 7B	7	141	120-190	0.860	1.810
Xgwm526	2A, 2B, 7A, 7B	6	184	150-250	0.622	1.774
Xpsp3029	2A, 6A	6	180	160-450	0.638	1.319
Xgdm93	2A,2D,4B	6	135	125-175	0.653	1.548
Xwmc273	7A,7B,7D	7	279	190-400	0.787	1.795
Xpsp3003	1A,5A,7D	6	210	195-450	0.760	1.260
Xgwm111	7B, 7D	6	206	150-290	0.752	1.729
Xcfd39	4B,4D,5A	7	175	150-210	0.771	1.798
Xwmc289	5B,5D	5	200	175-490	0.767	1.217
Xcfd81	7D,5D,4D	4	283	170-310	0.710	0.742
Xgwm265	2A,4A	5	179	125-295	0.676	1.414
Xgwm296	2D,7D	5	182	150-220	0.681	1.129
Xgwm311	2A, 2B, 2D, 6B	4	120	120-225	0.629	0.416
Xgwm382	2A,2B,2D	4	86	80-190	0.645	0.726
Xgwm356	2A,6A,7A	4	216	195-290	0.588	0.999
Total		82		120-490	-	-
Mean		5.5		-	0.703	1.312

Table 7. Total number of alleles, range of allele size, polymorphic information content and Shannon-Weaver Diversity index for A, B and D genomes of wheat loci.

Genome	Number of alleles	Mean of alleles per genome	Range of allele sizes (bp)	Average polymorphic information content	Shannon-Weaver diversity index
A	61	6.1	90- 550	0.724	1.864
B	93	6.2	110- 550	0.700	1.608
D	40	5.0	150- 380	0.613	1.294
Multiple A, B, D	82	5.5	120- 490	0.703	1.312
Grand total	276	-	90- 550	-	-
Grand mean	5.7	5.7	-	0.685	1.520

Clustering of wheat cultivars

Cluster analysis using UPGMA method delineated the 60 cultivars into four clusters comprising of 3, 7, 9 and 41 cultivars. Within the major cluster consisting of 41 cultivars, several sub-clusters were formed, showing the effectiveness of microsatellite markers in genetic diversity assays. Several studies using SSR have resulted in successful clustering of wheat cultivars. This type of markers is very effective in delineating diversity based on parental source by grouping cultivars with similar pedigree information (Plaschke et al., 1995; Kitavi et al., 2014) as well as grouping based on agronomic characteristics and geographical origin (Naceur et al., 2012). Depending on the degree of diversity, two (Tahir, 2008; El-Bakatoushi, 2010) or three clusters (Hazen et al., 2002; Wang et al., 2007) can be formed following the UPGMA analysis. In addition, as high as 9 (Naceur et al., 2012) and 13 clusters (Schuster et al., 2009) have been reported in genetic diversity studies. Grouping into four clusters herein is, therefore, within the expected ranges as compared to previously reported results. The 41 cultivars grouped in cluster 3 should be of significant attention to breeders as this may offer a useful guide when doing rational deployment in the field. Most of the cultivars studied herein have not been fully utilized in breeding programs. As such, by belonging to one cluster, it shows that these 41 cultivars share genetic similarities from their parental source, which could make them easily compatible when transferring desirable traits.

Conclusion

The present study contributes further to developing suitable science-based approaches for molecular

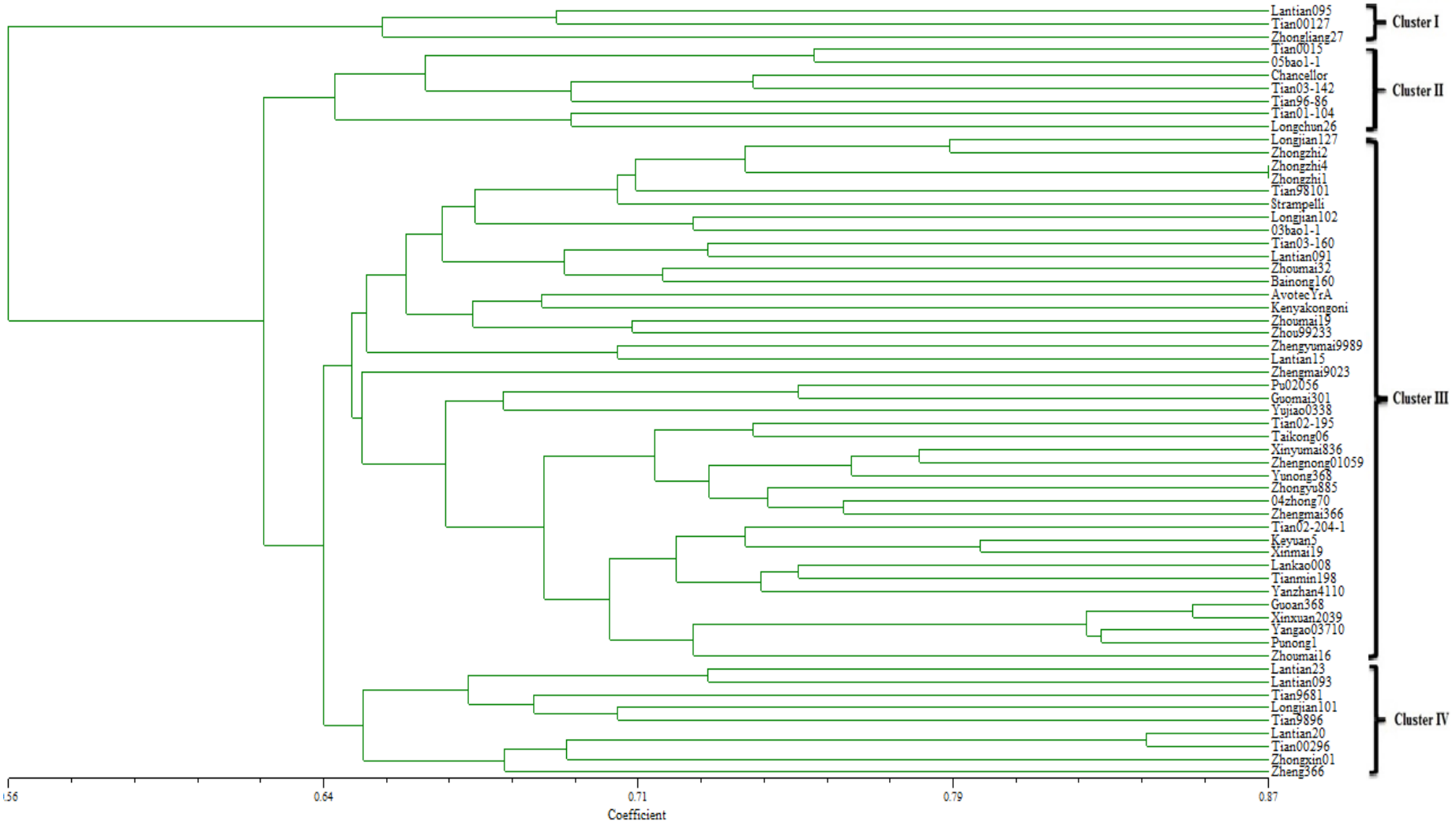

Figure 5. A dendrogram indicating genetic diversity of 60 wheat cultivars.

techniques in wheat. It offers an effective and reliable monitoring of wheat genetic diversity, which should be the starting point for future selection programs. Genome A was the most diverse and having most polymorphic loci as shown by SI and PIC values. Genome B was second, followed by genome D. Cluster analysis using UPGMA method delineated the 60 cultivars into four main clusters and several sub-clusters. Furthermore, it

was verified that microsatellite markers are effective in conducting genetic diversity studies as a total of 276 alleles were identified by using 48 wheat SSR markers with an average of 5.7 alleles per locus.

The present molecular genetic assay managed to shed more light on the genetic relatedness of wheat cultivars. This might assist breeders to set up the appropriate guidelines for successful breeding of wheat cultivars based on the established relationships.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors thank the Chinese Government through the Chinese Scholarship Council (CSC) for provision of funds to carry out this work.

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