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

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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
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 (Roussell 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, codominant nature and high degree of polymorphism (Singh et al., 2007; Royo et al., 2010; Ruiz et al., 2012; Laide 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-Gene 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 (H2CO) 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.

<table>
<thead>
<tr>
<th>Cultivar designation</th>
<th>Name of cultivar</th>
<th>Pedigree information*</th>
<th>Origin</th>
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<td>93R177 / 912-2-1-2</td>
<td>Gansu</td>
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<tr>
<td>4</td>
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<td>Zhongliang22+ gDNA of oil sunflower</td>
<td>Gansu</td>
</tr>
<tr>
<td>5</td>
<td>Chancellor</td>
<td>Carina/Mediterranean//Dietz / Carina/3/P-1068/3×Purplestraw</td>
<td>-</td>
</tr>
<tr>
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</tr>
<tr>
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<td>SXAF-7/87-121</td>
<td>Gansu</td>
</tr>
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<td>Lantian23/Zhou92031</td>
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<td>Shan167/ Guinong22/T. Spelta album</td>
<td>Beijing</td>
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<td>Lin87-4535/81168-4-3//Longyuan932</td>
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<td>Shan167/C591</td>
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<td>Kenya Kongoni</td>
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<td>Xinyumai836</td>
<td>-</td>
<td>Henan</td>
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<td>36</td>
<td>Yumai368</td>
<td>-</td>
<td>Henan</td>
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<td>37</td>
<td>Zhoumai19</td>
<td>Neixiang185 / Zhoumai9</td>
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<tr>
<td>38</td>
<td>Guan368</td>
<td>-</td>
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<td>39</td>
<td>Zhoumai32</td>
<td>Zhoumai12/ Wenmai6 // Zhoumai13</td>
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<td>41</td>
<td>Zhou99233</td>
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<td>Henan</td>
</tr>
<tr>
<td>42</td>
<td>Punong1</td>
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</tr>
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<td>43</td>
<td>Pu02056</td>
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<td>48</td>
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<th>No.</th>
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<td>Benyumai21/Yumaia2//Yumaia7</td>
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<tr>
<td>54</td>
<td>Zhengmai9023</td>
<td>[Xiaoyan6/Xinong65/83(2)/33/84(1)(4)3] F3/3Shan213</td>
<td>Henan</td>
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<td>Yumai47/PH82-2-2</td>
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<tr>
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<td>Zhou mai16</td>
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<tr>
<td>57</td>
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<td>Duokang893/Wenmai6//Bainong64/ Wenmai6</td>
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<td>59</td>
<td>Lantian15</td>
<td>Lantian10/Ibis</td>
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<tr>
<td>60</td>
<td>Yujiao0338</td>
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<td>Henan</td>
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</table>

*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.

<table>
<thead>
<tr>
<th>Marker (locus)</th>
<th>Marker sequence</th>
<th>Chromosomal location</th>
<th>Annealing temperature (°C)</th>
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<td>Xgwm273 F</td>
<td>ATGGGACGGACGAGATGCTTT</td>
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<tr>
<td>Xgwm273 R</td>
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<tr>
<td>Xbarc229 F</td>
<td>GGGCGCTGGGATTTGATGAT</td>
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<tr>
<td>Xbarc229 R</td>
<td>TCGGGATAGCGAGACCAT</td>
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<tr>
<td>Xgwm294 F</td>
<td>GGAATGGGATTAAGAGAGACG</td>
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<tr>
<td>Xgwm294 R</td>
<td>GCAGATGTCAATGCGAGA</td>
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<tr>
<td>Xwmc382 F</td>
<td>cATgAAtggAggcAcTgAAcA</td>
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<tr>
<td>Xwmc382 R</td>
<td>ccTTccggTcAgcAaAc</td>
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<td>Xgwm319 F</td>
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<tr>
<td>Xgwm319 R</td>
<td>CGGTGTCGTGTGTATGAC</td>
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<tr>
<td>Xgwm210 F</td>
<td>TGCATCAAGAATAGTGGAAG</td>
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<tr>
<td>Xgwm210 R</td>
<td>TGAGAAGAAGGCTCACACCT</td>
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<td>Xgwm257 F</td>
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<td>Xwmc317 R</td>
<td>TcAgcAaAcTTTcTccTcCcc</td>
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<td>Xwmc41 F</td>
<td>TcCctcTcCccAcgcgcgtATAg</td>
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<td>Xgwmc174 F</td>
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<td>Xgwm311</td>
<td>R</td>
<td>CTACGTGCACCACCATTTCCTT</td>
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</table>

The 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:

\[ PIC = 1 - \sum_{i=1}^{n} (f_i)^2 \] for n alleles

Where \( f_i \) = frequency of \( i \) 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-Wiener 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:

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

Where \( P_i \) = number of \( i \) 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.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Number of alleles</th>
<th>Expected allele size (bp)</th>
<th>Range of allele sizes (bp)</th>
<th>Polymorphic information content</th>
<th>Shannon-Weaver diversity index</th>
</tr>
</thead>
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<tr>
<td>Xwmc382-2A</td>
<td>10</td>
<td>270</td>
<td>250-450</td>
<td>0.882</td>
<td>3.290</td>
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<tr>
<td>Xgwm294-2A</td>
<td>7</td>
<td>96</td>
<td>90-160</td>
<td>0.761</td>
<td>2.185</td>
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<td>Xgwm126-5A</td>
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<td>196</td>
<td>190-225</td>
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<td>1.358</td>
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<td>280</td>
<td>220-300</td>
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<td>1.474</td>
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<td>Xcfa2019-7A</td>
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<td>217</td>
<td>190-260</td>
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<tr>
<td>Xwmc346-7A</td>
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<td>203</td>
<td>180-270</td>
<td>0.729</td>
<td>1.775</td>
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<tr>
<td>Xwmc525-7A</td>
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<td>206</td>
<td>195-280</td>
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<td>Total</td>
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<td>90-550</td>
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<tr>
<td>Mean</td>
<td>6.1</td>
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<td>-</td>
<td>0.724</td>
<td>1.864</td>
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</table>

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...
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.
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 alleles per 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 this genome benefiting genomes A and B (Perugini, 2001; Prasad et al., 2000). It is believed that the low genetic diversity of genome D emanated from evolution of hexaploid wheat. During evolution of hexaploid wheat, genomes A and B produced more tetraploid wheat species. 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 tauschi, 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).

### 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).

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

<table>
<thead>
<tr>
<th>Locus</th>
<th>Expected allele size (bp)</th>
<th>Range of allele sizes (bp)</th>
<th>Polymorphic information content</th>
<th>Shannon-weaver diversity index</th>
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<tbody>
<tr>
<td>Xbarc142-5B</td>
<td>10</td>
<td>208</td>
<td>175-350</td>
<td>0.834</td>
</tr>
<tr>
<td>Xwmc810-5B</td>
<td>9</td>
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<td>150-300</td>
<td>0.868</td>
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<td>Xgwm273-1B</td>
<td>8</td>
<td>171</td>
<td>170-490</td>
<td>0.875</td>
</tr>
<tr>
<td>Xwmc397-6B</td>
<td>8</td>
<td>160</td>
<td>155-300</td>
<td>0.822</td>
</tr>
<tr>
<td>Xgwm210-2B</td>
<td>6</td>
<td>303</td>
<td>160-550</td>
<td>0.760</td>
</tr>
<tr>
<td>Xgwm257-2B</td>
<td>7</td>
<td>190</td>
<td>180-350</td>
<td>0.794</td>
</tr>
<tr>
<td>Xwmc317-2B</td>
<td>8</td>
<td>139</td>
<td>115-290</td>
<td>0.804</td>
</tr>
<tr>
<td>Xgwm319-2B</td>
<td>3</td>
<td>170</td>
<td>110-495</td>
<td>0.461</td>
</tr>
<tr>
<td>Xwmc291-3B</td>
<td>6</td>
<td>233</td>
<td>210-350</td>
<td>0.806</td>
</tr>
<tr>
<td>Xgwm108-3B</td>
<td>3</td>
<td>135</td>
<td>110-150</td>
<td>0.374</td>
</tr>
<tr>
<td>Xbarc232-5B</td>
<td>4</td>
<td>368</td>
<td>160-390</td>
<td>0.689</td>
</tr>
<tr>
<td>Xgwm408-5B</td>
<td>4</td>
<td>182</td>
<td>175-210</td>
<td>0.636</td>
</tr>
<tr>
<td>Xwmc75-5B</td>
<td>6</td>
<td>206</td>
<td>195-500</td>
<td>0.787</td>
</tr>
<tr>
<td>Xwmc276-7B</td>
<td>2</td>
<td>292</td>
<td>250-390</td>
<td>0.132</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>-</td>
<td>110-550</td>
<td>-</td>
</tr>
<tr>
<td>Mean</td>
<td>6.2</td>
<td>-</td>
<td>1.608</td>
<td>-</td>
</tr>
</tbody>
</table>
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.

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

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.

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

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.

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

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
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

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

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REFERENCES


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