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Evaluation of genetic diversity, population structure, and linkage disequilibrium among elite Chinese wheat (*Triticum aestivum* L.) cultivars

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Abstract

Genetic diversity, population structure, and linkage disequilibrium (LD) were investigated among elite Chinese wheat (*Triticum aestivum* L.) cultivars originating from northern, Huanghuai and southwest wheat growing regions in China, using 69 diverse simple sequence repeat (SSR) markers. We observed an average of 11.72 (ranging from 3 to 50) alleles per SSR locus and an average gene diversity of 0.69 (ranging from 0.10 to 0.97). These results reveal that the diversity in elite Chinese cultivars was comparable to previously reported wheat germplasm SSRs in other countries. Cluster analysis of the SSR data, based on distance, divided all cultivars into six groups (A, B, C, D, E, and F) with a genetic similarity of 0.7, in agreement with their known pedigree data. The model-based clustering method divided these cultivars into four groups, partly corresponding to geographic regions of China, indicating that the genetic diversity of Chinese wheat cultivars was likely the result of regional adaptation. LD was investigated in these genotypes to determine the prospects for whole-genome association analyses. A low overall LD level (1.95%) was found with a mean r^2 of 0.14. LD decayed within a genetic distance of 33 centiMorgans ($r^2 > 0.2$) in all accessions, which was a slower decay than in previous studies of other crop populations. The suggested population structure and LD analysis in this study provide the basis for future association mapping for genes and quantitative trait loci in elite Chinese cultivars.

Keywords: Genetic relationship; SSR genotyping; STRUCTURE analysis; *Triticum aestivum* L.; UPGMA cluster analysis.
Abbreviations: HC1 - Huanghuai of China 1; HC2 - Huanghuai of China 2; HSWC - Huanghuai and Southwest China; LD - linkage disequilibrium; NC - northern China; QTL - quantitative trait loci; SDS - sodium dodecyl sulphate; SSR - simple sequence repeats.

Introduction

Studies on genetic diversity and the population structure of wheat germplasm are of great importance in improving wheat and developing strategies for optimal conservation of germplasm. DNA markers such as amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), and simple sequence repeats (SSR) can show the actual level of genetic diversity and therefore have been widely used for assessing genetic diversity among crop germplasm. Among these markers, SSRs or microsatellites are multiallelic, chromosome-specific, evenly distributed along chromosomes, and have been developed and widely used for studies of wheat genetic diversity and quantitative trait loci (QTL) mapping (Fahima et al., 1998, 2002; Huang et al., 2002; Khlestkina et al., 2004; Roussel et al., 2004, 2005; You et al., 2004; Peng and Lapitan, 2005; Teklu et al., 2006; Barakat et al., 2011). These studies have demonstrated a high level of wheat genetic diversity in different temporal and geographical areas. Linkage disequilibrium (LD) mapping is a valuable strategy for describing associations between markers and useful traits for crop improvement. This approach has a major advantage over conventional QTL mapping in that it does not require specific genetic populations and has been successfully used in maize, barley, rice, and common wheat to detect important markers or genes (Kraakman et al., 2004; Wilson et al., 2004; Breseghello and Sorrells, 2006; Agrama et al., 2007). LD, the nonrandom combination of alleles at two genetic loci, plays a key role in LD mapping and determines the resolution of an association study (Flint-Garcia et al., 2003). LD is affected by the population structure, mating system, recombination rate, and allele frequency (Flint-Garcia et al., 2003), with the population structure and mating system playing important roles. Using three barley groups (2-row, 6-row, and two-combination), Zhang et al. (2009) demonstrated that the population structure has an important impact on the extent of LD, especially interchromosomal LD. Therefore, knowledge of the population structure is a prerequisite in association mapping and can be used to avoid identifying false positive correlations between markers and traits (Pritchard et al., 2000; Pritchard and Donnelly, 2001; Maccaferri et al., 2005; Chao et al., 2007; Somers et al., 2007). On the other hand, the mating system can also affect the extent of LD. A higher LD level (up to 100 kb) has been detected in selfing species such as rice and sorghum, but a lower level (limited to a few hundred bp) was found in outbreeding species such as maize (Tenaillon et al., 2001; Garris et al., 2003; Hamblin et al., 2005). However, for both selfing and outbreeding species, LD has been reinforced in modern breeding by using a restricted number of parents in the hybridization schemes. Modern wheat breeding in China started in the 1910's when pure-line selection from landraces was first practiced. However, the improved wheat varieties were not widely used in farmers' fields until 1949. More than 2000 wheat cultivars were released for commercial production by the end of the 20th century. Fifty-nine outstanding varieties, each covering >667,000 hectares annually, have contributed significantly to the national wheat production (Zhuang et al.,

2003). To our knowledge, no information is available on the population structure and LD in elite Chinese wheat germplasm. In this study, we used 69 diverse SSR markers to evaluate the genetic diversity and population structure of 90 Chinese modern wheat cultivars and nine foreign-introduced lines using distance- and model-based methods. The Chinese modern wheat cultivars were representative of the outstanding varieties planted from 1949 to 2004. The major objectives of this study were to characterize the population structure within Chinese modern wheat cultivars and to determine the genomic distribution of LD between pairs of SSR loci.

Results

Genetic diversity in Chinese modern wheat cultivars

Sixty-nine polymorphic SSR markers dispersed throughout the genome (except 4DS, 5DL, 6DL, and 7DL) were used to test the genetic diversity of Chinese modern wheat cultivars. The allele number per marker and gene diversity are presented in Supplementary data 1B. In the 99 accessions, 809 alleles were detected. The number of alleles per locus ranged from three (*Xgwm624* and *Xgwm664*) to 50 (*Xgwm296*), with a mean value of 11.72 per locus. The gene diversity for 69 microsatellite loci varied from 0.10 to 0.97 for the least (*Xgwm154*) and the most (*Xgwm296*) informative markers, respectively (Supplementary data 1B).

Genetic relationships among Chinese modern wheat cultivars

UPGMA cluster analysis identified six groups (A, B, C, D, E, and F) by truncating the dendrogram at a value of 0.7 (Supplementary data 1C). The clusters of accessions within each group were in agreement with known pedigree data. Group A consisted of 15 cultivars, including the introduced Quality and CI12203 cultivars from America, and the Quality-derivative cultivars Bima 1, Bima 4, Beijing 8, Shijiazhuang 54, and Ji'nan 2. These Quality-derivative cultivars, as well as Jimai 30, Jimai 38, Bainong 3217, Ji'nan 9, and Yumai 13, were widely grown in >667,000 hectares annually over the last four decades. Group B included 76 cultivars, which can be subdivided into five subgroups (B1, B2, B3, B4, and B5). There were 39 cultivars in this group grown in >667,000 hectares annually. B1 was the largest subgroup, consisting of 38 Chinese modern cultivars and three genotypes from Italy (Funo, Mentana, and St1472/506). Five funo-derivatives, two Mentana-derivatives, and three St1472/506-derivative cultivars were included in this subgroup. Subgroup B2 cultivars included Abbondanza from Italy and its derivative cultivars such as Ganmai 8 and Ji'nan 13, one Quality-derivative cultivar (Wuyimai), and three other cultivars (Xinong 6028, Yumai 66, and Xuzhou 21). The cultivar Lovrin 10 from Romania and its four derivatives (Jingdong 8, Jimai 26, Shi 4185, and Een 1) as well as seven other cultivars mainly from the northern region of China (except Xuzhou 25) formed subgroup B3. Subgroup B4 included 13 cultivars mainly from the Huanghuai region of China, and also a northern region cultivar (Lunxuan 987). Subgroup B5 included Orofen from Chile and its derivative (Taishan 1) as well as a Huanghuai region cultivar (Fengchan 3). Groups C and D included two cultivars. The Pakistan cultivar (Mexipak 66) was well separated from the other cultivars and formed group E. The remaining three cultivars, including one landrace and two modern cultivars from Huanghuai and the southwest region, respectively, formed group F. The clusters divided by the distance-based method did not correspond well to the geographic regions of wheat production in China. This was due to the shared ancestral lines in breeding projects for different wheat growing areas. However, accessions from northern China were mostly included in group A (six accessions) and subgroup B3 (nine accessions). Accessions from the Huanghuai region were mostly included in group A (seven accessions) and subgroups B1 (28 accessions) and B4 (12 accessions). Accessions from southwestern China were mainly in subgroup B1 (six accessions). The genetic identity among the six groups was highest (0.985) between groups A and B and lowest (0.707) between groups C and F, indicating that the degree of diversity among the groups was very low (Table 1). In addition, the genetic similarity within each group varied from 0.716 to 0.845, indicating high identity among genotypes (Table 1).

Table 1. Mean genetic similarity within each group and genetic identity between pairs of six groups separated with the distance method.

Group	A	B	C	D	E	F
A	0.724*					
B	0.985**	0.719				
C	0.949	0.944	0.776			
D	0.876	0.8840	0.830	0.845		
E	0.857	0.855	0.796	0.811	-	
F	0.752	0.784	0.707	0.714	0.750	0.716

* Mean genetic similarity within the group (bold).
** Genetic identity between groups (Nei, 1978).

Population structure among Chinese modern wheat cultivars with the model-based clustering method

The model-based Bayesian clustering methodology was used to analyze 51 loosely linked SSR markers in all 99 accessions. The number of clusters did not peak in the range of two to ten subpopulations, and beyond four, the increase was not significant. In addition, α was relatively constant when $K \geq 4$, and hence $K = 4$ was selected as the optimal cluster number.

The four subpopulations [NC (northern China), HC1 (Huanghuai of China 1), HSWC (Huanghuai and Southwest China), and HC2 (Huanghuai of China 2)] are shown in Supplementary data 1A and are indicated by different colors in Figure 1. The NC subpopulation included 30 accessions, 15 of which originated from northern China. The remaining 15 were from the Huanghuai region, southwestern China, and foreign origins. Twenty-one out of 30 accessions in the HSWC subpopulation originated from the Huanghuai region. The HC1 (26 accessions) and HC2 (13 accessions) subpopulations had 69% and 92%, respectively, of their accessions originating from the Huanghuai region. These results indicated that the clusters divided by the model-based method partially corresponded to geographic regions. Supplementary data 1C indicates that the model-based clustering result was consistent with the genetic relationships based on their genetic distance measurement.

LD level in Chinese modern wheat cultivars

All 69 SSR markers were used to estimate the presence of LD in all accessions. There were 38,781 pairwise locus comparisons for all accessions, and the majority of locus pairs (94.9%) were independent loci. In all accessions, 755 locus pairs (1.95%) had a significant ($p < 0.01$) mean LD of 0.14, with an $r^2 > 0.2$ for 79 evaluated locus pairs (Table 2). The LDs for locus pairs within the same chromosomes and between chromosomes were calculated separately. There were 602 (1.64%) interchromosomal pairs of loci showing significant LD

Table 2. Presence and level of LD between SSR loci in the 99 wheat germplasm samples.

Classes	LD number (%) ($p < 0.01$)	Mean of r^2	Number of locus pairs with $r^2 > 0.2$
Intra Chromosome	153 (7.7)	0.21	45
Inter Chromosome	602 (1.64)	0.13	34
Genome wide	755 (1.95)	0.14	79

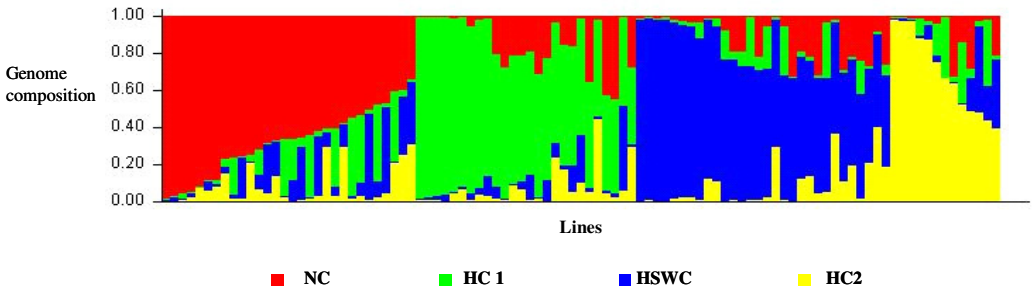

Fig 1. Population structure of the 99 wheat germplasms as estimated using the model-based Bayesian algorithm implemented in the program STRUCTURE. The subpopulations obtained with K = 4 are represented by different colors as indicated at the bottom.

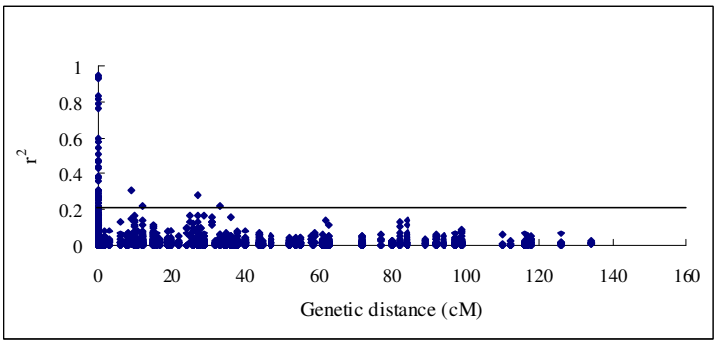

Fig 2. Scatterplot of the LD as a function of the intermarker distance within the same chromosome (cM) for 69 SSR loci on the entire wheat germplasm. Supplementary data 1C**.** UPGMA clustering of 99 wheat germplasms based on genetic similarity calculated from 68 SSR markers. The groups (capital letters, separated by vertical dashed lines) and subgroups (combined letters and numbers, separated by vertical dashed lines in bold) are indicated. The four model-based populations were depicted as ■ = NC (northern China), ▲ = HC1 (Huanghuai), ● = HSWC (Huanghuai and Southwest China), and △= HC2 (Huanghuai).

($p < 0.01$), 34 of which had $r^2 > 0.2$. Of the intrachromosomal locus pairs, 7.7% had a significant LD. In addition, intrachromosomal locus pairs had a higher mean r^2 value and more locus pairs with $r^2 > 0.2$ than interchromosomal locus pairs (Table 2). The scatter plots of LD (r^2) as a function of the intermarker distance (cM) within the same chromosome for all accessions indicated a clear LD decay with genetic distance (Fig. 2). LDs with $r^2 > 0.2$ extended to distances up to 33 cM, suggesting that the mapping resolution using these genotypes would generally be well below 33 cM.

Discussion

SSR diversity

In our current study, a total of 809 alleles were detected from 99 wheat accessions using 69 microsatellite markers. The mean number of alleles was 11.72, which is higher than that of European elite bread wheat germplasm (5.2–6.2) (Plaschke et al., 1995; Stachel et al., 2000), Argentine elite bread wheat cultivars (9.4) (Manifesto et al., 2001), Bulgarian winter wheat (6.8) (Landjeva et al., 2006), U.S. elite wheat cultivars (4.8–7.2) (Breseghello and Sorrells 2006; Chao et al., 2007), and elite durum wheat germplasm in Italy and other Mediterranean countries (Maccaferri, 2003) assessed with microsatellite markers. In contrast, 14.5 alleles were reported among 559 French wheat accessions (Roussel et al., 2004). Regarding the gene diversity of SSRs, elite Chinese wheat cultivars possessed a genetic diversity (0.69) comparable to that reported for elite U.S. wheat cultivars (Chao et al., 2007) and French wheat accessions (Roussel et al., 2004). The relatively higher genetic diversity present in the cultivars in our study may be because the cultivars were from various geographic regions of China. A genetic diversity study using only cultivated materials of the Shanxi province in China was reported by Hazen et al. (2002),

who used restriction fragment length polymorphisms and detected an average of 4.3 variants per probe. In addition, Shanxi wheat germplasm samples were characterized by a relatively low level of diversity (mean PIC value of 0.22).

Genetic relationships and population structure among elite Chinese wheat cultivars

The genetic distance-based clustering method separated the elite Chinese wheat germplasm into six clusters, corresponding to their pedigrees. The accessions originating from the same region did not form a single cluster, which was a result of repeated use of a few lines for wheat breeding across the country. This may also explain why accessions that originated in Huanghuai were clustered in three subpopulations based on the model clustering method. Regardless of the fact that the germplasm samples surveyed from different wheat production regions were almost identical, the model-based clustering method divided these germplasm samples into four subpopulations that were partially adapted to wheat production regions. The results indicate that the genetic diversity among the Chinese wheat germplasm samples was likely the result of regional adaptation, consistent with previous findings in wheat (Maccaferri et al., 2005; Chao et al., 2007). In addition, breeders in each wheat production region had different preferences— mostly high production in Huanghuai and efficient water use in the northern region. This partly resulted in genetic diversity of the wheat germplasm grown in different regions. The foreign introduced lines played an important role in Chinese wheat production. In our current study, nine foreign lines had been used as parents in wheat breeding programs. Some lines such as CI12203, Quality, Abbondanza, Funo, Mentana, and St1472/506 were grown directly in China, each covering >667,000 hectares annually. In addition, an introduced American line (Early Piemium) was used as an effective parent to produce Beijing 8, Shijiangzhuang 54, Xuzhou 14, Ji'nan 2, and Ji'nan 9, each grown in >667,000 hectares annually. Three Russian wheat lines (Аврора, Предгорная 2, and Скороспелка Л-1) were used as parents to breed Jingdong 8, Shan 7859, Yumai 7, and Taishan 1 (Zhuang et al., 2003). The introduced lines mentioned above, together with St2422/464, UP301, Triumph, Neuzucht, and Danmark 1, were used to create the Chinese modern varieties released before 2000, which were grown widely all over the country. However, these foreign lines did not appear in the varieties released after 2000, except for Neuzucht, a parent of Yumai 34. Our current study covers almost all elite wheat varieties in China used from 1949–2004, indicating that few foreign lines have been recently used in breeding strategies in China.

Linkage disequilibrium

In elite Chinese wheat cultivars, a fraction of marker pairs had only 1.95% significant LD, which was low compared to the reported values for 134 wheat varieties with 70% of locus pairs with significant LD (Maccaferri et al., 2005), 60% for 146 barley varieties (Kraakman et al., 2004), and 47.9% for 189 Canadian elite wheat varieties (Somers et al., 2007). However, Ecke et al. (2010) calculated LD among 845 amplified fragment length polymorphism loci distributed across the canola quality winter rapeseed genome and found a lower level of LD (0.78%) than that in our study. In addition, the percentage of significant LD in 43 elite U.S. wheat germplasm samples was also low (4.6%; Chao et al., 2007). Locus pairs on the same chromosome were previously shown to have a higher percentage of LD than those on independent chromosomes (Malysheva-Otto et al., 2006), consistent with what we observed in our study. We observed that 7.7% of locus pairs on the same chromosome showed significant LD, and only 1.64% of locus pairs on independent chromosomes showed significant LD. As a result of selection pressure, the LD on independent chromosomes was due to recombination or epistatic interactions between chromosomes (Gupta et al., 2005). The extent of LD relies heavily on the pollination behavior and population structure (Flint-Garcia et al., 2003). The data in our study indicated an LD decay within a 33 cM genetic distance with $r^2 > 0.2$ in all accessions. This represents a slower decay than that seen in previous studies of barley, maize, and elite U.S. wheat populations (Remington et al., 2001; Malysheva-Otto et al., 2006; Chao et al., 2007). This result could have important implications for association mapping of Chinese wheat cultivars, suggesting that fewer markers are required for genome-wide association studies using elite Chinese wheat accessions than the populations mentioned above.

Materials and methods

Plant materials

A total of 99 accessions, representing elite Chinese wheat cultivars, were included in our study (Supplementary data 1A). The 99 accessions included 90 Chinese cultivars: 49 were released before 2000, and each covered >667,000 hectares annually; most of the other 41 Chinese wheat cultivars were released recently; nine were introduced cultivars (two from America, one from Chile, four from Italy, one from Pakistan, and one from Romania). The Chinese wheat cultivars can be grouped into three main gene pools according to their origin: (1) 21 accessions were cultivated in the northern region of China; (2) 60 accessions were cultivated in the Huanghuai region of China; (3) nine accessions were cultivated in the southwest region of China. All these accessions were obtained from the National Crop Germplasm Resources Bank of China. The pedigree, origin, and year of release for each accession are reported in Supplementary data 1A. Pedigree information was obtained from Zhuang et al. (2003).

SSR genotyping

For each accession, genomic DNA was extracted from the fresh leaves of ten plants using the sodium dodecyl sulphate (SDS) method (Plaschke et al., 1995) and used as a template for PCR. A total of 69 polymorphic SSR primer pairs, at least one for each chromosome arm except 4DS, 5DL, 6DL, and 7DL, were selected for the genotyping analysis (Supplementary data 1B). These SSR primers included 61 GWMs, seven WMCs, and one GDM. Primer sequences are available at the GrainGenes web site:
http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker. PCR reactions were performed in a total volume of 20 μl containing 90 ng of template DNA, 200 μM each dNTP, 250 nM each primer, 1× PCR buffer, and 2.5 U Taq polymerase. PCR reactions were performed with an initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 50–60°C for 30 s, and 72°C for 60 s, and then a final 7 min at 72°C. The amplification products were separated on 6% (w/v) polyacrylamide denaturing gels (19:1 acrylamide: bis-acrylamide, 7 M urea) and stained with silver as described by Panaud et al. (1996). Fragments amplified were scored as present (1) or absent (0).

Data analysis

Molecular diversity within all accessions was estimated according to two parameters for each SSR locus, the total number of alleles and gene diversity (Weir, 1996) using PowerMarker software version 3.0 (Liu and Muse, 2005). To assess the relationship among all accessions, the SSR (0, 1) matrix was first transformed into a genetic similarity matrix. Cluster analysis using a distance-based method (SAHN method, UPGMA algorithm) of the 99 accessions was performed with NTSYS-pc software using the genetic similarity matrix (Gower, 1972). The genetic identity between groups suggested by the clusters was estimated with 1000 permutations using Popgen version 3.2. Analysis of the population structure based on a Bayesian Markov Chain Monte Carlo approach was performed with Structure version 2.1 software (http://pritch.bsd.uchicago.edu/structure.html) (Pritchard et al., 2000; Falush et al., 2003). Fifty-one loosely linked SSR markers (>40 centiMorgans (cM)) were chosen for structure analysis (Supplementary data 1B). The number of subpopulations (K) was set from two to ten, and each K value was repeated four times. For each run, the burn-in time and replication number were set to 100,000 with the admixture model and correlated to allele frequency according to Falush et al. (2003). The appropriate value of K was determined when the estimate of ln Pr(X/K) reached a minimum stable value. LD between polymorphic loci was evaluated using the TASSEL software package (http://www.maizegenetics.net/). SSRs were filtered for rare alleles (a percentage less than 5% in the whole population) to limit the inflation effect of low allele frequency on LD estimates. LD significance was determined with 100,000 permutations for each SSR pair. The squared correlation coefficients (r^2) between SSR markers were used for quantifying LD and were plotted against the genetic distance inferred from Somers et al. (2004). Markers that mapped to different chromosomes were used as unlinked markers. The LD decay was evaluated using Excel 2003.

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