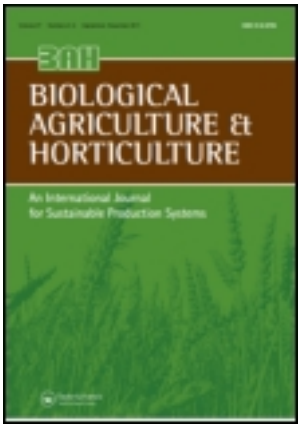

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Assessing genetic diversity and its changes of bread wheat in Qinghai Province, China, using agronomic traits and microsatellite markers

Hong-qin Li [a b], Huai-gang Zhang [a], Bao-long Liu [a], Deng-cai Liu [a] & Bo Zhang [a]

[a] Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, 810001, China

[b] Graduate University of Chinese Academy of Sciences, Beijing, 100049, China

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Assessing genetic diversity and its changes of bread wheat in Qinghai Province, China, using agronomic traits and microsatellite markers

Hong-qin Li[a,b], Huai-gang Zhang[a]*, Bao-long Liu[a], Deng-cai Liu[a] and Bo Zhang[a]

[a]*Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810001, China;*
[b]*Graduate University of Chinese Academy of Sciences, Beijing 100049, China*

Little is known about the diversity of wheat in Qinghai Province, China. Agronomic traits and microsatellite markers were used to survey genetic diversity and its change with time in 66 wheat cultivars registered from 1957 to 2009 in Qinghai Province. The average values of plant height, ear length, spikelets per ear, effective spikelets per ear, effective tillers per plant, internode length under spike, kernels per spike, grain weight per ear, 1,000-grain weight, and distance of spike base to auricle of flag leaf were studied. The mean Shannon-Weaver diversity index (*H'*) was 1.67 and increased with time. One hundred and eighty nine microsatellite markers also were used to examine genetic diversity at a molecular level which showed that the average number of alleles (*Na*), genetic diversity index (*He*), and allelic richness (*Rs*) were 3.69, 0.5, and 3.45, respectively, and also increased with time but did not lead to a significant differentiation among the decades. These results suggest that the modern wheat breeding practice did not cause a genetic reduction in Qinghai Province.

Keywords: agronomic trait; China; diversity; Qinghai Province: wheat

Introduction

Wheat is one of the most important food crops in the world. Breeders around the world are working to improve grain yield with better quality and disease resistance, and they have continuously introduced new varieties with those superior characteristics (Zhang et al. 2011). In this process, genetic diversity once played an important role because an accurate knowledge of genetic diversity among sources may increase the efficiency of plant breeding (Barrett and Kidwell 1998). However, researchers are concerned that modern breeding practices often introduce intensive selection with a narrow range of plant germplasm with limited allele introgressions over time, which might lead to crop uniformity and a reduction of genetic diversity on which breeding is based. In recent years, this perhaps led to a possible vulnerability of crop varieties to biotic and abiotic stresses (Roussel et al. 2004; Vellve 1993). Many breeders are very much aware of these risks of diversity reduction, but it is not clear to what extent scientific plant breeding has been instrumental in reducing crop diversity. However, there also is a hypothesis that plant breeding does not inevitably lead to a loss of genetic diversity. Reduction in diversity caused by intensive selection could be counterbalanced by introgression of novel germplasm.

So, in order to assess whether plant breeding has had an impact on genetic diversity, a variety of methods, including agronomic traits and molecular markers, were used

*Corresponding author. Email: hgzhang@nwipb.cas.cn

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to study the changes in genetic diversity in wheat. Among molecular markers, microsatellites are one of the most popular genetic markers due to their characteristic features of high polymorphism, abundant informativeness, convenience of assay by Polymerase Chain Reaction (PCR), and distribution throughout the genome (Gupta and Varshney 2000). Some reports demonstrated that a significant decline of genetic diversity has occurred in current crop cultivars. Roussel et al. (2005) analysed 480 European bread wheat (*Triticum aestivum* L.) cultivars released from 1840 to 2000 and found a clear reduction in genetic diversity after 1970. Similar results were reported by Fu et al. (2006), but there are some contrary results. By studying the 253 International Maize and Wheat Improvement Center (CIMMYT) or CIMMYT-related modern wheat cultivars, Reif et al. (2005) found that breeders averted the narrowing of the wheat germplasm base and subsequently increased genetic diversity through the introgression of novel materials. Landjeva et al. (2006) also revealed no declining trends in the diversity due to breeding activity.

Modern wheat breeding in Qinghai commenced in 1957 when the first cultivar, Abbondanza, was introduced from abroad and then a set of outstanding varieties were released for commercial production, especially after 1980. Gaoyuan338 once made a record production of 15.2 t ha^{-1} (Chen and Gong 2007). However, little information is available on the genetic diversity of wheat in Qinghai Province of China, which lies in the northeast of the Tibetan Plateau and has the greatest phenotypic diversity of spring wheat in the production regions of China (Dong et al. 2003). Compared with wheat in other areas, the spring wheats here have many special agronomic characteristics which could enable them to give stable and high yield to both irrigated and dry land farming regions (Chen 1994).

In this study, the 66 major wheat cultivars registered from 1957 to 2009 in Qinghai Province were analysed using agronomic traits and microsatellite markers. The main objectives were to assess (a) the phenotypic diversity of the collection covering 10 agronomic traits and (b) the genetic diversity of the collection using Simple Sequence Repeats (SSR) markers. Based on these results, valuable information could be provided to geneticists and breeders for future wheat improvement in Qinghai Province.

Materials and methods

A set of 66 bread wheat cultivars registered in Qinghai Province, China, during 1957–2009 for commercial cultivation were used in this study (Table 1). This set contained most of the varieties in this province.

Evaluation of morphological characters

The experiment was carried out in Ping'an Station of Ecological Agriculture (N36°30′, E101°59′, 2100 m), Northwest Institute of Plateau Biology, Chinese Academy of Sciences, based on a randomized complete block design with three replications. Ten plants selected randomly from each replication of each variety were used to score for the quantitative agronomic traits as follows: plant height (PH), ear length (EL), spikelets per ear (S/E), effective spikelets per ear (ES/E), effective tillers per plant (T/P), internode length under spike (IL), kernels per spike (K/S), grain weight per ear (GW), 1,000-grain weight (TW), and distance of spike base to auricle of flag leaf (DSL). The average value (X) and standard deviation (σ) for each quantitative trait were calculated and used to classify genotypes into 10 classes (i.e., the first group $Xi < (X - 2\sigma)$; the tenth group $Xi > (X + 2\sigma)$) with every

Table 1. Wheat cultivars and their registration year.

Cultivar	Registered year	Cultivar	Registered year	Cultivar	Registered year
Abbondanza	1957	Qingchun891	1994	Gaoyuan314	2001
Gaoyuan182	1969	Chaichun901	1994	Minhe665	2001
Xiangnong3	1970	Gaoyuan356	1994	Gaoyuan115	2001
Mobo	1976	Gaoyuan158	1994	Lantian3	2001
Gaoyuan506	1978	Zhangchun811	1994	Gaoyuan142	2002
Huzhuhong	1979	Qingchun570	1996	Lemai6	2003
Gaoyuan338	1981	Qingchun254	1996	Qingchun144	2003
Qingnong524	1984	GaoyuanV028	1997	Ningchun26	2003
Qingnong469	1984	Minhe853	1998	Moyin1	2003
Hanhai304	1986	Lemai5	1998	Moyin2	2003
Gaoyuan602	1987	Gaoyuan205	1998	Humai14	2004
Humai11	1988	Gaoyuan175	1998	Ganchun20	2004
Humai12	1988	Gaoyuan913	1998	Shanhan901	2005
Chaichun044	1988	Gaoyuan584	1999	Yuanzhuo3	2005
Qingchun533	1988	Gaoyuan932	1999	Humai15	2005
Chaichun236	1988	Minhe588	1999	Tongmai1	2005
Xinzhe9	1988	Gaoyuan363	1999	Qingchun37	2005
Chaichun018	1988	Gaoyuan448	1999	Qingchun 38	2005
Gaoyuan466	1990	Humai13	2000	Qingchun 39	2005
Gaoyuan465	1990	Qingchun587	2000	Caoxuan5	2007
Qingchun415	1993	Gaoyuan671	2000	Gaoyuan437	2008
Dongchun1	1994	Qingchun952	2001	Gaoyuan412	2009

0.5σ for one group. The Shannon-Weaver diversity index (H') (Shannon and Weaver 1949), was used to determine phenotypic diversity in this collection.

$$H' = -\sum Pi \ln Pi$$

Where Pi is the proportion of the total number of entries in the ith class.

DNA extraction and SSR analysis

DNA was extracted from two-week-old fresh leaves from five plants of each cultivar using a modified Hexadecyltrimethylammonium Bromide (CTAB) method (Colosi and Schaal 1993). A set of 200 SSR markers distributed throughout the 21 wheat chromosomes were initially tested. One hundred and eighty nine primers that showed consistently good amplification were used for the final study with all 66 wheat genotypes. These primers were selected based on Röder et al. (1998) and Somers et al. (2004). Amplification reactions were carried out in a 20 μl reaction mixture. The basic PCR procedure was 94 for 5 min, 94 for 1 min, 55 for 30 s, and 72 for 1 min, and then a return to step 2 for 35 cycles. Different primers may have different annealing temperatures (Röder et al. 1998). The amplified products were incubated at 72 for 10 min and stored at 4. The amplifications were separated by 6% polyacrylamide gel electrophoresis (PAGE) and stained with silver as described by Panaud et al. (1996).

The presence of the fragment was coded by 1, 2, 3, and so forth, according to its molecular weight, and the absence of the fragment was coded by 0. The reason of using this method was that it could reflect the superiority of SSR in displaying the allele characteristic, and the raw data could easily be transformed to the input format of the

software. The genetic diversity was assessed by using various statistical parameters including the number of alleles (*Na*), allelic richness (*Rs*) (Petit and El Mousadik 1998), and genetic diversity index (*He*) of Nei (1973) using the software package FSTAT (Goudet 2002). The significance of difference in *Na*, *He,* and *Rs* of the cultivars between each pair of the decade was calculated using a nonparametric Wilcoxon-matched pairs test (Sokal and Rohlf 1995). Pairwise *Fst* values of the difference in number of different alleles between decades and Analysis of Molecular Variance (AMOVA) were calculated in the software package Arlequin ver. 3.0 (Excoffier et al. 2005).

Results

Analysis of agronomic traits in 66 wheat cultivars

Mean values for agronomic traits measured for 66 cultivars are presented in Table 2. A large variability among these cultivars was noted and the coefficient of variation of the 10 agronomic traits was between 8.81% (effective spikelets per ear) and 27.5% (distance of spike base to auricle of flag leaf). Generally, the wheat in Qinghai Province was tall and there were 18 cultivars taller than 100 cm. Thousand grain weight also was large with 39 cultivars, 59% of the text materials, having 1,000-grain weight exceeding 45 g.

Results of phenotypic diversity (*H'*) for each trait are presented in Table 3. The Shannon-Weaver index differed slightly between the 10 traits in the range of 1.51 for effective tillers/plant to 1.73 for distance of spike base to auricle of flag leaf with an average of 1.67. In order to further understand the diversity change with time, the 66 cultivars were classified into four groups according to their registered year. Because the cultivars registered in 1950s and 1960s were few, they were included in the group of the 1970s. Among the 10 traits, the *H'* of six traits (spikelets per ear, effective spikelets per ear, kernels per ear, grain weight per ear, 1,000-grain weight, and distance of spike base to auricle of flag leaf) showed an upward trend, while the *H'* of the other four traits (plant height, spike length, effective tillers per plant, and internode length under spike) increased before 1990 and dropped after 1990 in the range of 0.01 and 0.24. The mean value of *H'* for the 10 agronomic traits was treated as the *H'* for the cultivar. The *H'* of the cultivar showed a trend of increase from 1.33 in the 1970s to 1.88 in the 2000s. The *H'* of the varieties before 1990 was lower, but it improved greatly in the 1990s. It also increased in the 2000s but only by 0.02 over the 1990s.

Table 2. Analysis of agronomic traits in 66 wheat cultivars.

Trait	Maximum	Minimum	Average	Range	CV (%)
Plant height (cm)	148.78	67.19	92.82	81.59	14.49
Spike length (cm)	17.37	7.73	10.94	9.64	15.36
Spikelets per ear	24.43	14.69	20.62	9.74	9.65
Effective spikelets per ear	23.23	14.38	19.76	8.85	8.81
Effective tillers per plant	11.20	4.63	7.19	6.57	17.94
Internode length under spike (cm)	55.23	26.04	38.88	29.19	15.33
Kernels per ear	78.83	39.51	62.70	39.32	11.64
Grain weight per ear (g)	3.75	1.69	2.81	2.06	16.73
1,000-grain weight (g)	56.23	31.61	45.91	24.62	12.57
Distance of spike base to auricle of flag leaf (cm)	32.89	6.18	18.11	26.71	27.50

Table 3. Genetic diversity index (H') of agricultural traits in different periods.

Trait	1970s	1980s	1990s	2000s	Average
Plant height	1.33	1.56	1.89	1.65	1.61
Spike length	1.24	1.55	1.91	1.79	1.62
Spikelets per ear	1.33	1.63	1.90	1.95	1.70
Effective spikelets per ear	1.33	1.68	1.85	1.95	1.70
Effective tillers per plant	1.01	1.42	1.82	1.78	1.51
Internode length under spike	1.24	1.70	1.91	1.90	1.69
Kernels per ear	1.33	1.79	1.77	1.98	1.72
Grain weigh per ear	1.56	1.58	1.73	1.94	1.70
1,000-grain weight	1.33	1.55	1.95	1.99	1.70
Distance of spike base to auricle of flag leaf	1.56	1.70	1.82	1.85	1.73
Average	1.33	1.62	1.86	1.88	1.67

SSR analysis

At the molecular level, the awnless cultivars were separated from the awned cultivars to show the differences between the two groups. One hundred and eighty nine of the 200 SSR primer pairs throughout all the chromosomes showed polymorphisms and were used to characterize and estimate the genetic diversity of the 66 wheat genotypes. A total of 714 alleles were detected from the 189 amplified loci. The total number of alleles per locus ranged from two for many loci to ten for locus *Xgwm*294 on chromosome 2A, with an average number of 3.78. The highest values of *He, Na,* and *Rs* all occurred in the B genome with 0.50, 3.83, and 3.59, respectively (Table 4). The A genome was the second with 0.58, 3.57, and 3.31; the last was the D genome with 0.43, 3.01, and 2.82. If the seven homologous groups are compared, it can be seen that the highest value of *He, Na,* and *Rs* occurred with chromosome 4 while the lowest *He* occurred with chromosome 5 and the lowest *Na* and *Rs* occurred with chromosome 3. In awnless and awned subsets, the *Na* and *Rs* of awnless were a little higher than those of awned, while the *He* value of the two subsets was the same.

Table 4. Mean values of number of alleles (*Na*), Nei's genetic diversity index (*He*), and allelic richness (*Rs*) for each homologous group of chromosome and genome.

Homologous Group	*He*			*Na*			*Rs*		
	Awnless	Awned	Total	Awnless	Awned	Total	Awnless	Awned	Total
1	0.46	0.44	0.46	3.01	3.00	3.27	2.94	2.95	3.07
2	0.51	0.54	0.53	3.65	3.48	4.01	3.52	3.42	3.70
3	0.49	0.51	0.50	3.08	3.10	3.25	3.01	3.04	3.07
4	0.55	0.57	0.57	4.30	4.11	4.69	4.15	4.04	4.38
5	0.44	0.42	0.44	3.17	3.18	3.55	3.08	3.11	3.26
6	0.53	0.53	0.53	3.57	3.39	3.73	3.46	3.34	3.50
7	0.55	0.52	0.54	3.76	3.46	3.99	3.67	3.38	3.74
A	0.47	0.46	0.48	3.30	3.11	3.57	3.20	3.05	3.31
B	0.49	0.50	0.50	3.50	3.45	3.83	3.40	3.39	3.59
D	0.42	0.43	0.43	2.84	2.76	3.01	2.76	2.71	2.82
Average	0.49	0.49	0.50	3.42	3.30	3.69	3.32	3.24	3.45

Genetic variation within decadal periods

The changes of *Na, He,* and *Rs* during the four periods are shown in Table 5. The *Na* and *Rs* increased consistently from the 1970s to the 2000s, and the increasing range was 43.10% and 10.65%. The Wilcoxon-matched pairs test showed that the increase of *Na* and *Rs* was significant at $p < 0.05$. The differences between the 1990s and 2000s for the two parameters were not significant. The *He* between the four periods did not show a significant difference. The difference between the change of *He* and the other two indices might be caused by the existence of rare alleles because *He* is an indicator of both allele numbers and frequencies.

Table 5. The average *Na, He,* and *Rs* in the four decades.

Decade	*Na* ± SD	*He* ± SD	*Rs* ± SD
1970s	2.39 ± 1.01 a	0.49 ± 0.29 a	2.16 ± 0.81 a
1980s	2.91 ± 1.27 b	0.50 ± 0.24 a	2.30 ± 0.76 ab
1990s	3.27 ± 1.40 c	0.49 ± 0.21 a	2.31 ± 0.70 b
2000s	3.42 ± 1.52 c	0.51 ± 0.22 a	2.39 ± 0.74 b

Note: Estimates followed by the same letter are not significantly different at $p < 0.05$.

Genetic differentiation among decadal periods

The results of AMOVA for the effect of decades for the whole set are shown in Table 6. The genetic differentiation (*Fst*) among the four decades was 0.014 and not significant, indicating little temporal drift among decadal periods. The pairwise comparison of *Fst* showed that significant genetic differentiation existed in two pairs of decades (1970s–2000s and 1990s–2000s), while no significant differentiation was found between other periods.

AMOVA also indicated that the variance component within subsets (96.94%) was far higher than that among subsets (3.06%). For the awnless subset, none of the differences between decades were significant. For the awned subset, significant differentiation was observed between the 1970s and the 2000s. The *Fst* value was 0.1807, indicating that the genetic difference between the two periods was greater.

Table 6. Pairwise Fst values between periods for awnless, awned, and the whole set.

Decade	Awnless	Awned	Whole
1970s–1980s	0.1018 NS	0.1417 NS	0.0711 NS
1970s–1990s	0.0931 NS	0.1211 NS	0.0577 NS
1970s–2000s	0.1181 NS	0.1807*	0.0903*
1980s–1990s	0.0658 NS	0.0684 NS	0.0393 NS
1980s–2000s	0.0321 NS	0.0869 NS	0.0368 NS
1990s–2000s	0.0476 NS	0.0851 NS	0.0358**
Among all decades	− 0.007 NS	0.027 NS	0.014 NS

Note: $*p < 0.05$; $**p < 0.01$; Nonsignificant (NS) $p > 0.05$.

Discussion

Agronomic traits and diversity survey

Plant height is a major agronomic metric in wheat breeding because of its association with lodging, seedling growth capacity, and weed control (Donald and Hamblin 1976). Appropriate plant height is a prerequisite for attaining the desired yield level in wheat

breeding programmes. Compared to the plant height in other places (Liu and Sun 2006), the wheat in Qinghai Province was taller with a mean value 92.82 cm. This might reflect the special ecology here with strong sunshine and lack of rain so the wheat is less subject to lodging with taller height. The average 1,000-kernel weight was 45.91 g in the wheat tested, whereas in some of the varieties it was as high as 56 g. This germplasm can be used as donor parents to improve seed weight, which ultimately increases grain yield.

Phenotypic diversity (*H'*) ranged from 1.51 for effective tillers/plant to 1.73 for distance of spike base to auricle of flag leaf with an average of 1.67. This estimate was slightly lower than the one reported for accessions of Hebei wheat with $H' = 1.76$, which was based on eight agro-morphological traits (Li et al. 2009). The increase of *H'* with time also suggested that modern breeding did not narrow the genetic diversity of wheat in Qinghai Province.

Diversity analysis based on microsatellites

The major aim of this model experiment was to assess the genetic diversity in wheat breeding programmes in Qinghai Province in China. There were an average number of 3.78 alleles per locus, which was lower than that of U.S. elite wheat cultivars (4.8–7.2) (Breseghello and Sorrells 2006), Russian wheat (6.7) (Peng et al. 2009), and Western European elite wheat varieties (7.49) (Le Couviour et al. 2011). However, it was higher than that of Sichuan (2.4) (Zhang et al. 2002) and Shandong (3.65) (Pu et al. 2011) Provinces in China. The reason may be that the U.S., Russian, and European studies included more varieties, from a country or even a continent, but the Chinese studies included varieties from a single Province with similar geographic regions.

Nei's (1973) gene diversity or expected heterozygosity (*He*) is another common diversity index in population genetics. Huang et al. (2002) obtained a *He* value of 0.77 in an analysis of a set of common wheat germplasm from across all wheat-producing regions. Khlestkina et al. (2004) showed a value of *He* equal to 0.70 (0.46–0.82) in evaluating Siberian common spring wheat. In this study, a mean *He* value of 0.50 was calculated for the 66 varieties in Qinghai Province. The correlation between gene diversity and allele number is positive, so it was lower due to fewer alleles in these varieties.

The different contribution of the three genomes to genetic variation also was confirmed in this study: B genome was the most variable, A genome followed, and D genome was the least variable. This is consistent with the findings of several wheat research groups who utilized microsatellite markers.

The results revealed that the estimates of the average number of alleles/locus (*Na*), gene diversity (*He*) and allelic richness (*Rs*) did not show any significant reduction, which meant that the wheat breeding in Qinghai Province did not lead to any significant loss of genetic diversity in Qinghai registered wheat cultivars. This could be explained by the fact that in other places, some materials were used as parents in many breeding programmes, primarily owing to their resistance to disease, but in Qinghai, the disease was not as serious as elsewhere, so there was no material used repeatedly as parents and, thus, did not lead to crop uniformity.

In Qinghai Province, breeders particularly select the awnless varieties because the farmers prefer awnless wheat. AMOVA showed that the difference of SSR allelic diversity between awnless and awned subsets was small and that just 3.06% of the total genetic variance was variation between the two subsets. This indicated that breeders' selection emphasis on awnless varieties did not cause its genetic diversity difference from the awned varieties. Though it was generally thought that modern breeding reduced the genetic diversity especially after the

"green revolution," there was disagreement about it. (van de Wouw et al. 2010). In the present study, both type of analysis agronomic and microsatellites-based provided that there was not genetic reduction in the breeding practices in Qinghai Province, but it was imperative to introduce new materials in the future programmes because the diversity here was low.

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