Genet Resour Crop Evol (2018) 65:55–65
https://doi.org/10.1007/s10722-017-0508-2

RESEARCH ARTICLE

The impact of modern plant breeding on dominant Chinese wheat cultivars (*Triticum aestivum* L.) revealed by SSR and functional markers

Lingzhi Meng · Chao Xiang · Hongwei Liu · Li Yang · Chunyan Mai · Liqiang Yu · Yunliang Wei · Hongjie Li · Hongjun Zhang · Yang Zhou

Received: 17 October 2016 / Accepted: 7 March 2017 / Published online: 20 March 2017

Abstract The modern plant breeding is generally considered to be a practice that leads to a narrowing in genetic diversity of crops. The objective of the present study was to assess whether this practice has led to the reduction of genetic diversity in modern Chinese wheat cultivars. A set of 80 dominant Chinese wheat cultivars released from 1942 to 2011 was used to describe the genetic diversity based on 137 simple sequence repeat (SSR) and 52 functional markers. Several important properties about the genetic diversity were revealed. First, relative low genetic diversity level was detected on a genome-wide scale. A total of 752 alleles were detected with a range from 1 to 15, and the mean polymorphic information content value was 0.53 with a range from 0.00 to 0.87. Second, the genetic diversity significantly decreased from 2001 to 2011 at the genome-wide level. More importantly, significant differences of genetic diversity among the three different genomes were observed by the analysis of variance (ANOVA). The three genomes had clearly different changing trends over time: the A genome displayed a decreasing trend (regression coefficient (b) = −0.01); in contrast, the other two genomes, B and D, showed the increasing trends ($b = 0.01$ for the B genome, $P = 0.05$; $b = 0.01$ for the D genome, $P = 0.05$). Third, the analysis of qualitative variations in allelic composition over time indicated that, the more recent the cultivars were, the more similar they were to each other. Finally, the frequencies of favorable alleles related to important agronomic traits had been increasing over time or maintained high frequencies in all seven temporal groups. These findings indicate that modern wheat breeding results in not only a qualitative, but also a quantitative change in genetic diversity in the dominant Chinese wheat cultivars. A special attention should be paid to broaden the genetic base in the A genome.

Keywords Common wheat · Functional markers · Genetic diversity · Temporal trend · *Triticum aestivum*

Lingzhi Meng and Chao Xiang are Co-first authors.

Electronic supplementary material The online version of this article (doi:10.1007/s10722-017-0508-2) contains supplementary material, which is available to authorized users.

L. Meng · C. Xiang · H. Liu · L. Yang · H. Li · H. Zhang (✉) · Y. Zhou (✉)
Institute of Crop Sciences/National Key Facility for Crop Gene Resources and Genetic Improvement, Chinese Academy of Agricultural Sciences, Beijing 100081, China
e-mail: zhanghongjun01@caas.cn

Y. Zhou
e-mail: zhouyang@caas.cn

C. Mai · Y. Wei
National Center for Technological Innovation of Dwarf-male-sterile Wheat, Xinxiang 453731, China

L. Yu
Zhaoxian Institute of Agricultural Sciences, Zhaoxian 515300, China

Introduction

Genetic diversity is usually thought of as the amount of genetic variability among individuals of a variety, or population of a species (Brown 1983). Information on the genetic diversity in a crop is the basis for selection of superior crossing parents, establishing heterotic groups and has a significant impact on the improvement of crops (Landjeva et al. 2006; Zhang et al. 2011).

The practices of modern plant breeding accompanying strong selective pressure are generally considered to reduce the genetic diversity and increase the uniformity of crops (Vellvé 1993; Tanksley and McCouch 1997). Such a reduction can induce the modification in gene frequencies of crops and, consequently, changes in their resistances to biotic and abiotic stresses and their adaptability to climate changes (Donini et al. 2000; Roussel et al. 2004). The disadvantage of crop uniformity has been well documented by the history of disease epidemics, such as the Irish potato famine caused by late blight in the 1840s and the U.S. corn leaf blight in the 1970s (Fu and Somers 2009). It is essential, therefore, to explore the temporal changes in genetic diversity of crops at the molecular level. In wheat, the significant decreases in diversity were detected in Canadian hard red spring wheat using SSR markers (Fu and Somers 2009) and in 242 Chinese common wheat using five pairs of AFLP (amplified fragment length polymorphism) markers (Tian et al. 2005). In contrast, some reports show that the impact of modern breeding on genetic diversity has been negligible (Donini et al. 2000; Fu et al. 2003; Roussel et al. 2004; Huang et al. 2007). The meta-analysis showed that no substantial reduction in the diversity of crop cultivars has taken place using data from the 44 published papers related to the genetic diversity trends in crops (Wouw et al. 2010). These different conclusions could be resulted from the sample type of materials examined and the number and type of molecular markers (Roussel et al. 2005).

Chinese wheat breeding began in the 1920s (Zhuang 2003), has so far released over 2000 commercial cultivars, and has generated a significant impact on Chinese wheat production and food security. During the period 1942–2011, about 80 wheat cultivars were widely grown (Zhuang 2003; http://www.natesc.agri.cn/). Hence, these cultivars represent the bulk of dominant Chinese wheat cultivars in different breeding periods. Combining the SSR and functional markers (FMs), an attempt was done to clarify the impacts of modern wheat breeding on the genetic diversity and to provide evidence of reduction of diversity in Chinese wheat cultivars. This study was carried out: (1) to evaluate the genetic diversity at the genome-wide level; (2) to analyze temporal trends in genetic diversity in Chinese wheat cultivars over time, and (3) to compare the frequencies of favorable alleles associated with important agronomic traits in different breeding periods.

Materials and methods

Plant materials

According to the annual planting area, a total of 80 dominant Chinese wheat cultivars, consisting of 75 commercial cultivars, one landrace (Youzimai) and four foreign introduced cultivars (one from the USA and three from Italy), were evaluated in the present study. Among them, 71 had the annual planting area >333,500 hectares, and 61 had the largest annual planting area >667,000 hectares (Zhuang 2003; http://www.natesc.agri.cn/). Cultivars released or cultivated from seven different periods were grouped to facilitate the analysis of diversity change over time (Table 1). Detailed information on the code number, group, name, pedigree, released or cultivated year, maximum annual growing area and geographic origin for each cultivar is listed in the Online Resource 1.

Table 1 Seven temporal groups of 80 dominant Chinese wheat cultivars

Temporal group	Number of cultivars
1942–1960	9
1961–1980	11
1981–1989	12
1990–1995	11
1996–2000	13
2001–2005	13
2006–2011	11

DNA extraction and genotyping

Genomic DNA was extracted using the CTAB method (Murray and Thompson 1980). The total numbers of 137 clearly visible SSR markers distributed evenly on 21 chromosomes of wheat were selected (http://www.shigen.nig.ac.jp/wheat/komugi/maps/download.jsp) and polymorphism information previously reported by Hao et al. (2011) (Online Resource 2). A special consideration was given to the functional markers (FMs) associated with the processing quality, agronomic traits, resistance genes and introduced alien genes (Liu et al. 2012). A total of 97 FMs were used to detect the allelic variations, and finally 52 clearly amplified FMs identified 55 alleles and related to 23 genes (loci) were selected for data analyzing in this study (Online Resource 3).

The basic PCR amplifications were performed in a 20 μL reaction mixture containing 10 μL 2 × *Taq* PCR Master Mix (www.bibasic.com), 1 μL 50–100 ng μL^{-1} gDNA, 1 μL 10 p mol μL^{-1} of each primer and 7 μL sterilized ddH_2O (Guo et al. 2015). The thermo-cycling procedure was an initial denaturation at 94 °C for 5 min, followed by denaturing at 94 °C for 1 min, annealing at 52–60 °C for 30 s, and extension at 72 °C for 30 s to 2.0 min, and steps 2–4 were repeated 34 cycles with a final extension at 72 °C for 10 min. PCR amplification products were separated on 10% polyacrylamide gels and stained with GeneFinder™ (Bio-V, Xiamen, China), or on 2–5% agarose gels and stained with ethidium bromide.

Data analysis

The fragment size at each locus was scored using QuantityOne software version 4.62 (Bio-Rad Laboratories). The mean number of allele and polymorphism information content (PIC) were analyzed by PowerMarker software v3.25 (Liu and Muse 2005).

Analysis of variance (ANOVA) of genetic diversity in the 80 dominant Chinese wheat cultivars was performed according to the method described by Christiansen et al. (2002). The change trends in genetic diversity over time were analyzed by SAS software v9.2 (SAS institute inc.) based on the standard linear regression equation $y = a + bx$, where y is the dependent variable, a is the regression intercept, b is the regression coefficient and x is the independent variable.

In order to determine qualitative changes in allelic diversity over time, the four values, namely A, B, C and D, were calculated according to the method described by Roussel et al. (2005), where A is the percentage of alleles present in both successive temporal groups i and j, B is the percentage of alleles present in i and absent in j, C is the percentage of alleles present in j and absent in i, and D is the percentage of alleles absent in i and j.

Results

Global diversity at the genome-wide level

The evaluation on genetic diversity in the 80 wheat cultivars using 137 SSR loci and 52 FMs related to 23 genes (loci) is present in Table 2. A total of 752 alleles were detected at 160 loci. The number of alleles at each locus varied from 1 to 15 and the mean number of allele was 4.70. The mean PIC value across loci was 0.53 with a range from 0.00 to 0.87. Among the three genomes, the B genome had the highest mean number of allele (5.42, with a range of 1–15), followed by the A genome (4.68, with a range of 1–11) and the D genome (3.92, with a range of 1–11). The same trends were observed in the mean PIC values for the three genomes (0.58 for the B genome, 0.54 for the A genome and 0.47 for the D genome). ANOVA revealed that the B and A genomes had significantly higher genetic diversity than D genome. For the seven homoeologous groups, the mean number of alleles for groups 3 (5.15, 2–10), 5 (5.34, 2–12) and 7 (5.37, 2–15) were higher than other four groups of 1 (4.63, 1–11), 2 (3.96, 1–8), 4 (4.06, 2–8) and 6 (3.88, 2–7). The mean PIC values for groups 3 (0.56, 0.07–0.85), 5 (0.57, 0.09–0.87) and 7 (0.58, 0.16–0.87) were also higher than those of groups 1 (0.51, 0.00–0.86), 2 (0.50, 0.00–0.82), 4 (0.54, 0.21–0.81) and 6 (0.47, 0.11–0.78). The number of alleles ranged from 6 to 65 were detected for 21 chromosomes. The mean number of alleles ranged from 2.00 (4D) to 6.29 (7B), and the mean PIC values varied from 0.33 (6D) to 0.61 (4A, 1B and 7B).

Changes in genetic diversity over time

Variations in genetic diversity of the 80 dominant Chinese wheat cultivars during from 1941 to 2011

Table 2 Genetic diversities at genome/homeologous group/chromosome levels of 80 dominant Chinese wheat cultivars revealed by SSR and functional markers

Genome/group/chromosome	No. of loci	No. of alleles	Mean number of allele (range)	Mean PIC value (range)
Genome				
A	56	262	4.68 (1–11)	0.54 (0.00–0.86) A[a]
B	55	298	5.42 (1–15)	0.58 (0.00–0.87) A
D	49	192	3.92 (1–11)	0.47 (0.00–0.87) B
Homoeologous group				
1	30	139	4.63 (1–11)	0.51 (0.00–0.86) A
2	24	95	3.96 (1–8)	0.50 (0.00–0.82) A
3	26	134	5.15 (2–10)	0.56 (0.07–0.85) A
4	16	65	4.06 (2–8)	0.54 (0.21–0.81) A
5	29	155	5.34 (2–12)	0.57 (0.09–0.87) A
6	16	62	3.88 (2–7)	0.47 (0.11–0.78) A
7	19	102	5.37 (2–15)	0.58 (0.16–0.87) A
Chromosome				
1A	13	65	5.00 (2–10)	0.53 (0.02–0.86)
2A	7	23	3.29 (1–5)	0.46 (0.00–0.72)
3A	10	46	4.60 (2–7)	0.54 (0.07–0.77)
4A	7	33	4.71 (2–6)	0.61 (0.37–0.77)
5A	9	53	5.89 (2–11)	0.57 (0.09–0.84)
6A	6	26	4.33 (2–7)	0.53 (0.34–0.77)
7A	4	16	4.00 (2–6)	0.55 (0.18–0.77)
1B	8	46	5.75 (2–11)	0.61 (0.18–0.81)
2B	7	38	5.43 (1–8)	0.59 (0.00–0.82)
3B	11	63	5.73 (2–10)	0.59 (0.11–0.85)
4B	6	26	4.33 (2–8)	0.56 (0.21–0.80)
5B	11	60	5.45 (2–12)	0.58 (0.17–0.84)
6B	5	21	4.20 (3–6)	0.52 (0.27–0.66)
7B	7	44	6.29 (2–15)	0.61 (0.30–0.87)
1D	9	28	3.11 (1–7)	0.40 (0.00–0.81)
2D	10	34	3.40 (2–6)	0.46 (0.09–0.74)
3D	5	25	5.00 (2–7)	0.55 (0.22–0.77)
4D	3	6	2.00 (2-2)	0.34 (0.32–0.37)
5D	9	42	4.67 (2-11)	0.54 (0.09–0.87)
6D	5	15	3.00 (2–6)	0.33 (0.11–0.78)
7D	8	42	5.25 (2–8)	0.57 (0.16–0.81)
Total	160	752		
Overall mean			4.70 (1–15)	0.53 (0.00–0.87)

[a] The same letter indicates the difference is not significant as determined by the least significant difference test at $P < 0.05$

were further investigated with the aid of ANOVA (Table 3). The differences of genetic diversity were highly significant among the three different genomes ($P < 0.0001$) and among the seven homoeologous chromosome groups ($P = 0.0007$). There were no significant differences in genetic diversity among different temporal groups and interactions between temporal group × genome and temporal group × chromosome group.

The changes in diversity at the genome-wide and individual genome levels in the seven temporal groups are present in Fig. 1. On a genome-wide scale, there was erratic evolution of the genetic diversity over time. The mean PIC value increased from the period

Table 3 Analysis of variance of genetic diversity in 80 dominant Chinese wheat cultivars released from 1942 to 2011

Source of variation	df	Mean square	*F* value	*P* value
Temporal group	6	0.010	0.75	0.1186
Genome	2	0.119	21.02	<0.0001
Chromosome group	6	0.025	4.34	0.0007
Temporal group × Genome	12	0.005	0.93	0.5198
Temporal group × Chromosome group	36	0.003	0.50	0.9891
Error	84	0.006		
Total	146			

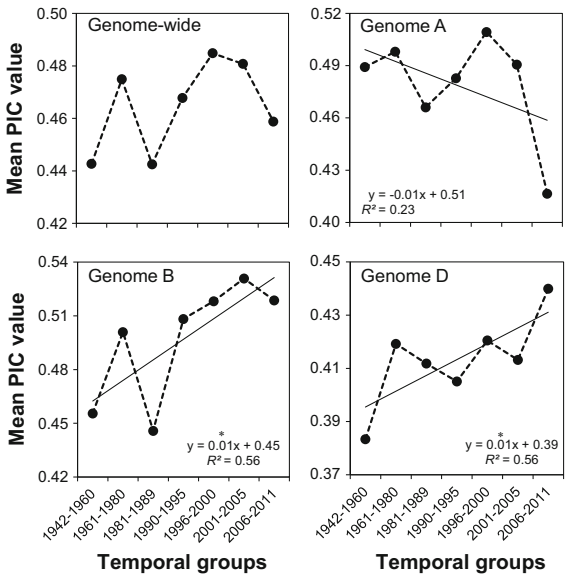

Fig. 1 Diversity changes of 80 Chinese wheat cultivars in the seven temporal groups on the genome-wide scale. *, the regression coefficient (*b*) is significant at $P \leq 0.05$

1942–1960 to the period 1960–1980, and fell to the lowest in the period 1981–1989; after that, this parameter began to rise in the 1990s and finally significantly decreased from 2001 to 2011. Different from the whole genome, the three genomes showed clearly different changing trends. The A genome displayed an decreasing trend from 0.49 in the period 1942–1960 to 0.42 in the period 2006–2011, and the regression coefficient (*b*) was −0.01. In contrast, the other two genomes showed the increasing trends over time. The B genome was from 0.46 in the period 1942–1960 to 0.52 in the period 2006–2011 ($b = 0.01$, $P = 0.05$), and the D genome was from 0.38 in the period 1942–1960 to 0.44 in the period 2006–2011 ($b = 0.01$, $P = 0.05$).

The variation of *A*, *B*, *C* and *D* in successive temporal group pairs is present in Fig. 2. The *A* values significantly increased over time (Fig. 2a), and the regression coefficient (*b*) was 1.89 ($P = 0.01$). In contrast, the negative slopes of the *B* and *C* curves were observed (Fig. 2b, c), especially for the *D* values ($b = -1.75$, $P = 0.01$). The *D* values were quite constant from the period 1942–1960/1961–1980 to the period 2001–2005/2006–2011 (Fig. 2d).

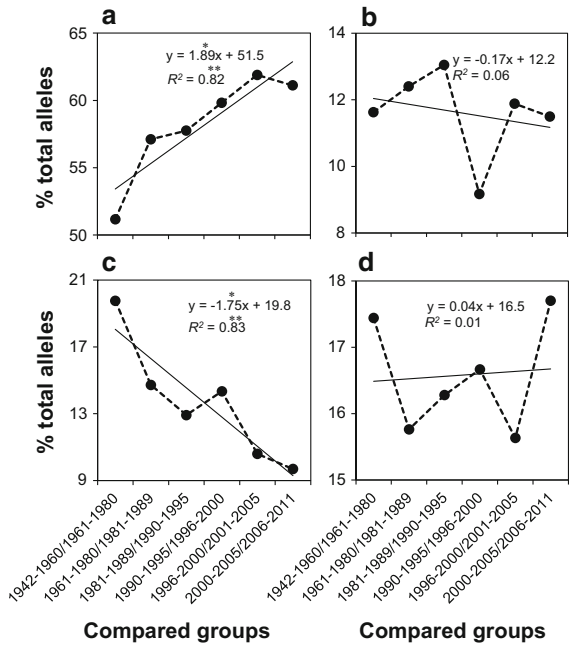

Fig. 2 Variation of percentage of total alleles in two successive temporal groups. **a**: Percentage of alleles simultaneously present in the groups *i* and *j*, **b**: percentage of alleles present only in *i* and absent in *j*, **c**: percentage of alleles absent in *i* and present only in *j*, and **d**: percentage of alleles simultaneously absent in both groups *i* and *j*. **, the regression coefficient (*b*) is significant at $P \leq 0.01$

Diversity changes in the loci associated with important agronomic traits over time

For the processing quality, ten loci *Pop-A1*, *Pop-D1*, *Talox-B1*, *Psy-B1*, *Glu-A1*, *Glu-B1*, *Glu-D1*, *Glu-A3*, *Glu-B3* and *Pin-b* were analyzed in the seven successive temporal groups (Table 4). At the *Pop-A1* locus, despite it had the similar mean allele frequency in the *Pop-A1a* (50.9%) and *Pop-A1b* (51.2%) alleles, the *Pop-A1b* allele associated with low polyphenol oxidase (PPO) activity had been increasing over time. *Pop-D1b*, another allele related to low PPO activity, had higher mean frequency (80.2%) than the *Pop-D1a* allele (30.3%). Compared to the *TaLox-B1a* allele (40.9%), the *TaLox-B1b* allele with lower lipoxygenase (LOX) activity had higher mean frequency (61.4%). Four allelic variations at the *Psy-B1* locus associated with yellow pigment (YP) content were identified. Among them, the mean frequencies of 29.5 and 59.8% for the *Psy-B1b* and *Psy-B1c* alleles associated with lower YP content respectively, whereas the corresponding values were 13.6 and 2.4% for the *Psy-B1a* and *Psy-B1d* alleles related to higher YP content. The significantly increasing trend was observed for the *Psy-B1b* allele by regression analysis ($b = 11.02$, $P \leq 0.05$) (Fig. 3a). At the *Glu-B1* locus, the allele frequency of *By8* varied from 0.0% in the period 1942–1960 to 27.3% in the period 2006–2011, and displayed a linear increasing trend over time ($b = 5.13$, $P \leq 0.01$) (Fig. 3b). The same changing trend was observed for the *Glu-A3b* allele at the *Glu-A3* locus ($b = 4.65$) (Fig. 3c). For the *Pinb-D1b* allele associated with superior milling and processing qualities, the frequency of this allele significantly increased with the time ($b = 3.69$, $P \leq 0.05$) (Fig. 3d).

Allelic variations at the loci *Rht-B1*, *Rht-D1*, *Ppd-A1*, *Ppd-B1*, *Ppd-D1*, *VRN-A1*, *VRN-B1*, *VRN-D1*, *TaCwi-A1* and *Dreb-B1* were analyzed in seven temporal groups (Table 4). The increasing trends were observed in alleles *Rht-B1b* ($b = 4.35$, $P \leq 0.05$) (Fig. 3e) and *Rht-D1b* ($b = 9.99$, $P \leq 0.01$) (Fig. 3f) associated with semidwarfing over time. The photoperiod-insensitive alleles *Ppd-B1a* and *Ppd-D1a* were predominated in the seven temporal groups, and the mean frequencies were 93.7 and 97.6% respectively. In contrast, the *Ppd-A1b* allele (97.6%) associated with photoperiod-sensitive had higher mean frequency than *Ppd-A1a* (0.0%). At the three vernalization loci, the consistent phenomenon was found that higher frequencies of recessive alleles *vrn-A1*, *vrn-B1* and *vrn-D1*present in the *VRN-A1*, *VRN-B1* and *VRN-D1* loci, respectively. Although the *TaCwi-A1b* allele had higher mean frequency, an increasing linear trend was displayed in *TaCwi-A1a* allele associated with higher thousand kernel weight ($b = 2.22$) (Fig. 3g). The Dehydration-responsive element binding (DREB) gene is used to improve drought tolerance in wheat. This gene predominated in all seven temporal groups with a mean frequency of 93.4%.

The 1B·1R chromosome translocation had an increasing trend over time ($b = 5.28$, $P \leq 0.05$) (Fig. 3h), and the mean frequency was 26.7%. As well, the increasing trend in frequency of *Gpc-B1* gene associated with high protein content is present in Fig. 3i ($b = 9.54$, $P \leq 0.01$).

Discussion

In the present study, the genetic diversity across 80 dominant Chinese wheat cultivars was evaluated using SSR markers and FMs at the whole genome level. The mean number of allele was 4.70, which is similar to previously reported estimates of 4.81 alleles among 95 U.S. elite wheat cultivars (Breseghello and Sorrells 2006) and 5.05 alleles among 90 Chinese elite winter wheat (Chen et al. 2012). However, this value was lower than those reported other studies. There were two possible reasons behind this phenomenon. One is that the wheat collections varied in different studies. Generally, the set of cultivars under evaluation that included many landraces had higher genetic diversity (Chao et al. 2007). Using 157 landraces and 97 modern cultivars, Hao et al. (2011) discovered 6727 alleles at 512 SSR loci with the mean of 13.1 alleles per locus. Likewise, evaluation on 559 French wheat accessions (landraces and commercial cultivars) based on 41 SSR markers resulted in the mean allele number of 14.5 (Roussel et al. 2004). In this study, only one landrace (Youzimai) was included, and the remaining 79 cultivars are modern cultivars. The domestication and breeding practices exerted on modern wheat cultivars by human, possibly resulted in the reduction of genetic diversity (Tanksley and McCouch 1997). Another reason is that the limited geographic sources of cultivars also decreased the genetic diversity. In the

Table 4 Allelic frequency at loci associated with imporant agronomic traits in dominant Chinese wheat cultivars from the seven temporal groups detected by functional markers

Trait	Locus	Allele	Frequency (%)							
			42–60	61–80	81–89	90–95	96–00	01–05	06–11	Mean
For processing quality genes										
Polyphenol oxidase activity	*Ppo-A1*	*Ppo-A1a*	71.4	50.0	33.3	77.8	41.7	44.4	37.5	50.9
		Ppo-A1b	42.9	50.0	66.7	22.2	58.3	55.6	62.5	51.2
	Ppo-D1	*Ppo-D1a*	87.5	88.9	50.0	100.0	81.8	83.3	70.0	80.2
		Ppo-D1b	12.5	55.6	50.0	0.0	27.3	16.7	50.0	30.3
Lipoxygenase activity	*TaLox-B1*	*TaLox-B1a*	0.0	57.1	11.1	66.7	58.3	42.9	50.0	40.9
		TaLox-B1b	100.0	57.1	90.9	33.3	41.7	57.1	50.0	61.4
Yellow pigment content	*Psy-B1*	*Psy-B1a*	0.0	0.0	0.0	33.3	0.0	33.3	28.6	13.6
		Psy-B1b	0.0	0.0	0.0	33.3	80.0	50.0	42.9	29.5
		Psy-B1c	100.0	100.0	100.0	33.3	40.0	16.7	28.6	59.8
		Psy-B1d	0.0	0.0	0.0	0.0	0.0	16.7	0.0	2.4
Bread- and noodle-making quality	*Glu-A1*	*Ax2**	55.6	36.4	58.3	18.2	15.4	7.7	18.2	30.0
	Glu-B1	*nonBx17*	11.1	0.0	8.3	45.5	76.9	38.5	81.8	37.4
		Bx17	0.0	27.3	8.3	18.2	0.0	46.2	18.2	16.9
		By8	0.0	0.0	0.0	9.1	15.4	23.1	27.3	10.7
	Glu-D1	*Dx5*	0.0	0.0	0.0	12.5	10.0	27.3	0.0	7.1
	Glu-A3	*GLuA3a*	0.0	0.0	0.0	10.0	10.0	9.1	9.1	5.5
		GluA3b	11.1	9.1	0	36.4	23.1	38.5	27.3	20.8
		GluA3d	33.3	20.0	0.0	40.0	30.0	45.5	27.3	28.0
		GluA3e	33.3	60.0	83.3	20.0	0.0	0.0	9.1	29.4
		GluA3 g	33.3	20.0	33.3	30.0	0.0	18.2	0.0	19.3
		GluA3ac	0.0	20.0	16.7	30.0	70.0	45.5	63.6	35.1
	Glu-B3	*Glu-B3a*	25.0	30.0	60.0	25.0	11.1	0.0	11.1	23.2
		Glu-B3b	0.0	10.0	0.0	0.0	0.0	0.0	0.0	1.4
		Glu-B3d	25.0	50.0	10.0	50.0	33.3	40.0	33.3	34.5
		GluB3 g	0.0	0.0	0.0	0.0	22.2	0.0	0.0	3.2
		GluB3 h	0.0	0.0	0.0	0.0	11.1	20.0	0.0	4.4
		GluB3i	50.0	10.0	40.0	0.0	0.0	20.0	0.0	17.1
		GluB3bef	0.0	0.0	10.0	0.0	0.0	20.0	44.4	10.6
		GluB3 fg	25.0	20.0	30.0	25.0	22.2	0.0	55.6	25.4
Grain hardness	*Pin-b*	*Pinb-D1a*	55.6	60.0	45.5	45.5	55.6	41.7	30.0	47.7
		Pinb-D1b	44.4	40.0	54.5	54.5	44.4	58.3	70.0	52.3
For agronomic traits										
Reduction in plant height	*Rht-B1*	*Rht-B1a*	100.0	90.9	100.0	100.0	81.8	76.9	66.7	88.0
		Rht-B1b	11.1	9.1	0.0	10.0	27.3	23.1	33.3	16.3
	Rht-D1	*Rht-D1a*	87.5	90.9	60.0	40.0	38.5	38.5	36.3	56.0
		Rht-D1b	12.5	9.1	40.0	60.0	61.5	61.5	72.7	45.3
Photoperiod response	*Ppd-A1*	*Ppd-A1a*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Ppd-A1b	100.0	90.9	100.0	100.0	100.0	92.3	100.0	97.6
	Ppd-B1	*Ppd-B1a*	88.9	90.9	91.7	100.0	92.3	92.3	100.0	93.7
		Ppd-B1b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Ppd-D1	*Ppd-D1a*	100.0	100.0	91.7	100.0	100.0	92.3	100.0	97.7
		Ppd-D1b	33.3	11.1	8.3	0.0	0.0	7.7	0.0	8.6

Table 4 continued

Trait	Locus	Allele	Frequency (%)							
			42–60	61–80	81–89	90–95	96–00	01–05	06–11	Mean
Vernalization response	*VRN-A1*	*Vrn-A1c*	11.1	0.0	16.7	0.0	0.0	7.7	0.0	5.1
		vrn-A1	90.9	100.0	83.3	100.0	100.0	92.3	100.0	95.2
	VRN-B1	*Vrn-B1*	11.1	18.2	0.0	25.0	7.7	8.3	0.0	10.0
		vrn-B1	90.9	81.8	100.0	75.0	92.3	91.7	100.0	90.2
	VRN-D1	*Vrn-D1a*	33.3	33.3	50.0	36.4	7.7	15.4	36.4	30.4
		Vrn-D1b	11.1	33.3	25.0	9.1	7.7	15.4	0.0	14.5
		vrn-D1	55.6	33.3	25.0	54.5	84.6	69.2	63.7	55.1
Kernel weight	*TaCwi-A1*	*TaCwi-A1a*	66.7	81.8	75.0	60.0	76.9	69.2	81.8	73.1
		TaCwi-A1b	100.0	100.0	100.0	90.0	100.0	92.3	90.9	96.2
Abiotic stress tolerance	*Dreb-B1*	*Dreb-B1*	88.9	100.0	100.0	81.8	92.3	100.0	90.9	93.4
For resistance genes										
Powdery mildew resistance	*Pm3*	*Pm3e*	0.0	0.0	0.0	9.1	0.0	0.0	0.0	1.3
For introduced alien genes										
Bread- and noodle - making quallity	*1BL·1RS*	ω-*secalin*	0.0	18.2	16.7	63.6	30.8	38.5	36.4	29.2
		1BL·1RS	0.0	18.2	25.0	45.5	23.1	38.5	36.4	26.7
Grain protein content and stipe rust	*Gpc-B1* and *Yr36*	*Gpc-B1*	0.0	18.2	25.0	54.5	33.3	38.5	72.7	34.6
		Yr36	100.0	100.0	100.0	100.0	92.3	100.0	100.0	98.9

evaluation of genetic diversity in 99 Chinese elite wheat cultivars from various geographic regions, the mean allele number was 11.7 based on 69 SSR markers (Guo et al. 2011). By contrast, 54 (67.5%) out of 80 cultivars were collected from the YHWR in this study. New cultivars are usually developed from the crosses among genetically related elite cultivars in the same wheat region, which will further narrow the genetic bases.

At the genome-wide level, the genetic diversity did not show successive reduction in seven temporal groups (Fig. 1). This phenomenon was also observed in Shandong cultivars of China during the 1950s to 2000s and European and French wheat varieties from 1840 to 2000 (Roussel et al. 2004, 2005; Peng et al. 2012). As suggested by Christiansen et al. (2002), the erratic evolution of genetic diversity could be explained by the history of wheat breeding. It was noteworthy that the obvious reduction in genetic diversity was observed during the last decade (2001–2011) in this study, which can be a consequence of both frequent use a small number of elite genotypes as parents and, simultaneously, lack of the innovation of germplasm resource. The three genomes showed clearly different changing trends: the A genome had decreasing trend in diversity over time, in contrast, the B and D genomes showed increasing trends, indicating that the stronger selection pressure was exerted on the A genome than on the other two genomes during wheat breeding. The A genome is central to wheat evolution, domestication and genetic improvement (Peng et al. 2011). A great number of genes associated with disease resistances and important agronomic traits were identified in the A genome (Ling et al. 2013). These genes might be selected and retained during the wheat breeding, which results in the reduction of diversity in the A genome. In this study, the analysis of qualitative changes in allelic composition over time showed an significantly increasing trend for the percentage of alleles present in both successive temporal groups ($b = 1.89$, $P = 0.01$), and the opposite trend ($b = -1.75$, $P = 0.01$) was observed for the percentage of alleles present in group i and absent in group j, indicating that the more recent the cultivars were, the more similar they were to each other. This result is consistent with that reported by Roussel et al. (2005) in the European bread wheat collected from 1840 to 2000. From these results, it is

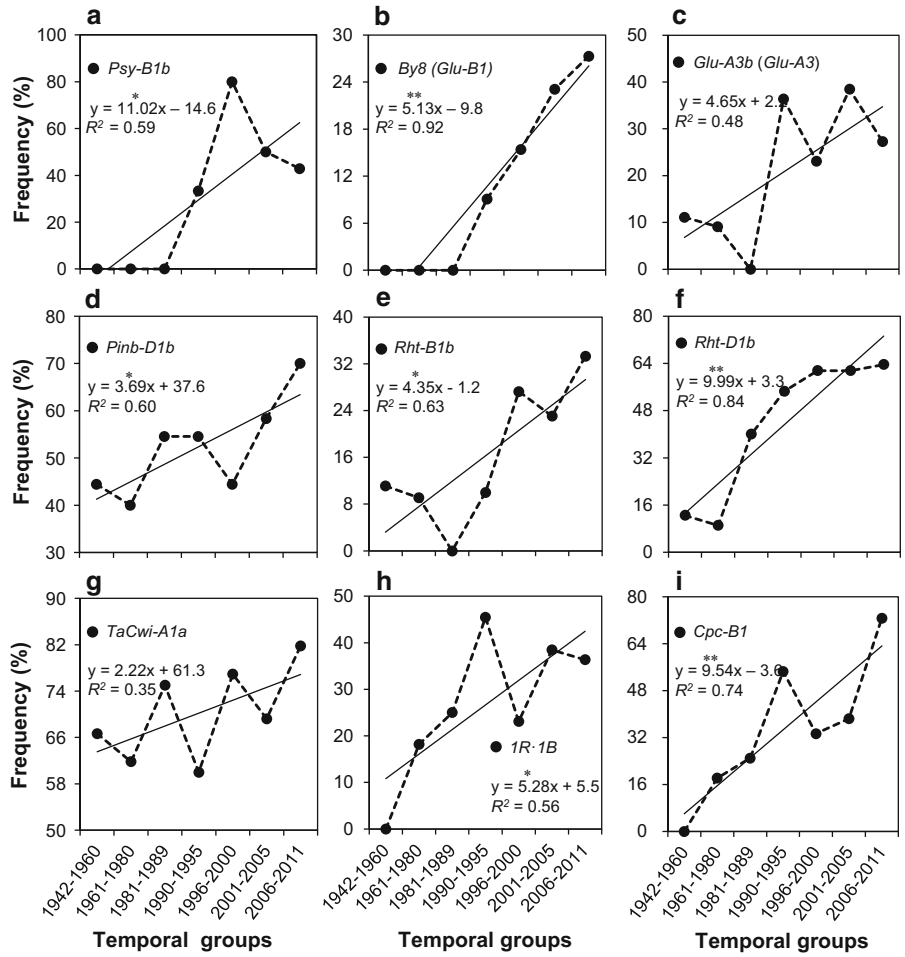

Fig. 3 Trends in changes of haplotype frequencies in successive temporal groups for the major functional markers associated with important agronomic traits. *, the regression coefficient (*b*) is significant at $P \leq 0.05$ and **, the *b* value is significant at $P \leq 0.01$

concluded that modern wheat breeding results in not only a qualitative, but also a quantitative change in diversity of dominant Chinese wheat cultivars.

In this study, a total of 52 FMs were used to examine the temporal changes in diversity. Overall, the frequencies of favorable alleles associated with important agronomic traits had the increasing trends or maintained high values in all the seven temporal groups (Table 4; Fig. 3). This is consistent with the result based on an analysis of qualitative variations in allelic composition (Fig. 2), indicating that the modern wheat breeding practices accompanying intensive selection pressure have always focused on economically important loci (Rasheed et al. 2015). Low PPO activity is mainly controlled by *Ppo-A1b* and *Ppo-D1a* alleles (Sun et al. 2005; He et al. 2007). In this study, the mean frequencies were over 50% for the both alleles in the seven temporal groups, and over 85% for the allelic combination *Ppo-A1b* + *Ppo-D1a* (data no shown), which is concordant with the breeder's selection because the low PPO activity is preferred for Chinese noodles (Liang et al. 2010). High- and low-molecular-weight glutenin subunits are largely controlled by the *Glu-1* and *Glu-3* loci (Luo et al. 2001). The frequencies of *By8* and *Dy5* alleles associated with strong gluten at the *Glu-B1* and *Glu-D1* loci had increasing trends (Fig. 3b and Table 4), which was consistent with the reports that the *Glu-D1* subunit *Dx5* + *Dy10* had a high frequency in most modern Pakistani wheat cultivars (Rasheed et al. 2015). The *Pinb-D1b,* one of the most important alleles associated with flour yield, lower ash, loaf

volume and crumb grain score (Hogg et al. 2005). A significantly increasing trend in frequency of the *Pinb-D1b* allele over time was found in the dominant Chinese wheat cultivars ($b = 3.69$, $P \leq 0.05$). Also, Rasheed et al. (2015) found that *Pinb-D1b* allele had a high frequency in modern cultivars, but was not present in the landraces, which concurs with our result. The semidwarfing genes *Rht-B1b* and *Rht-D1b* were widely used in modern commercial cultivars to reduce plant height and increase grain yield, and related FMs for these alleles were developed to differentiate the semi-dwarf and the wild-type alleles (Eills et al. 2002; Zhang et al. 2006). The increasing trends in the frequencies of the *Rht-B1b* and *Rht-D1b* allele were observed (Fig. 3e, f), and the frequency of *Rht-B1b* + *Rht-D1b* was over 80% in this study (data no shown). The high frequencies of *Rht-B1b* and *Rht-D1b* were also detected in modern cultivars from Pakistan (Rasheed et al. 2015), CIMMYT (Liang et al. 2010) and China (Zhang et al. 2006). It can be seen that modern wheat breeding practice has increased the frequencies of favorable alleles by direct or indirect selections in the dominant Chinese wheat cultivars over time, which results in the reduction of genetic diversity.

In conclusion, relative low genetic diversity level in the 80 dominant Chinese cultivars was detected on a genome-wide scale. The three genomes of wheat showed clearly different changing trends over time. Analysis of qualitative variations in allelic composition over time indicated that, the more recent the cultivars were, the more similar they were to each other. It was noteworthy that the frequencies of favorable alleles associated with important agronomic traits had been increasing over time or maintained high frequencies in the seven temporal groups. These findings indicated that modern wheat breeding practice results in not only a qualitative, but also a quantitative change in genetic diversity in the dominant Chinese wheat cultivars. The information on the extent and change trends obtained in this study will be helpful for developing appropriate science-based breeding strategies in future Chinese wheat breeding.

Acknowledgements The authors thank Dr. CY Hao and YG Xiao, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China, and Dr. GH Yin, Zhoukou Academy of Agricultural Sciences, Henan, China, for generously providing parts of wheat cultivars. This study was supported by the National Natural Science Foundation of China (31401468).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Breseghello F, Sorrells ME (2006) Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics 172:1165–1177

Brown WL (1983) Genetic diversity and genetic vulnerability-an appraisal. Econ Bot 37:4–12

Chao S, Zhang W, Dubcovsky J, Sorrells M (2007) Evaluation of genetic diversity and genome-wide linkage disequilibrium among U.S. wheat (*Triticum aestivum* L.) germplasm representing different market class. Crop Sci 47:1018–1030

Chen X, Min D, Yasir TA, Hu YG (2012) Genetic diversity, population structure and linkage disequilibrium in elite Chinese winter wheat investigated with SSR markers. PLoS ONE 7:e44510

Christiansen MJ, Andersen SB, Ortiz R (2002) Diversity changes in an intensively bred wheat germplasm during the 20th century. Mol Breed 9:1–11

Donini P, Law JR, Koebner RM, Reeves JC, Cooke RJ (2000) Temporal trends in the diversity of UK wheat. Theor Appl Genet 100:912–917

Eills MH, Spielmeyer W, Gale KR, Rebetzke GJ, Richards RA (2002) "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. Theor Appl Genet 105:1038–1042

Fu YB, Somers DJ (2009) Genome-wide reduction of genetic diversity in wheat breeding. Crop Sci 49:161–168

Fu YB, Peterson GW, Scoles G, Rossnagel B, Schoen DJ, Richards KW (2003) Allelic diversity changes in 96 Canadian oat cultivars released from 1886 to 2001. Crop Sci 43:1989–1995

Guo X, Gao A, Liu W, Yang X, Li X, Li L (2011) Evaluation of genetic diversity, population structure, and linkage disequilibrium among elite Chinese wheat (*Triticum aestivum* L.) cultivars. Aust J Crop Sci 5:1167–1172

Guo XR, Wang YY, Meng LZ, Liu HW, Yang L, Zhou Y, Zhang HJ (2015) Distribution of the *Vrn-D1b* allele associated with facultative growth habit in Chinese wheat accessions. Euphytica 206:1–10

Hao C, Wang L, Ge H, Dong Y, Zhang X (2011) Genetic diversity and linkage disequilibrium in Chinese bread wheat (*Triticum aestivum* L.) revealed by SSR markers. PLoS ONE 6:e17279

He XY, He ZH, Zhang LP, Sun DJ, Morris CF, Fuerst EP, Xia XC (2007) Allelic variation of polyhenol oxidase (PPO) genes located on chromosomes 2A and 2D and development of functional markers for the PPO genes in common wheat. Theor Appl Genet 115:47–58

Hogg AC, Beecher B, Martin JM, Meyer F, Talbert L, Lanning S, Giroux MJ (2005) Hard wheat milling and bread baking

traits affected by the seed-specific overexpression of puroindolines. Crop Sci 45:871–878

Huang XQ, Wolf M, Canal MW, Orford S, Koebner RMD, Röder MS (2007) Did modern plant breeding lead to genetic erosion in European winter wheat varieties? Crop Sci 47:343–349

Landjeva S, Korzun V, Ganeva G (2006) Evaluation of genetic diversity among Bulgarian winter wheat (*Triticum aestivum* L.) varieties during the period 1925–2003 using microsatellites. Genet Resour Crop Evol 53:1605–1614

Liang D, Tang JW, Pena RJ, Singh R, He XY, Shen XY, Yao DN, Xia XC, He ZH (2010) Characterization of CIMMYT bread wheats for high- and low-molecular weight glutenin subunits and other quality-related genes with SDS-PAGE, RP-HPLC and molecular markers. Euphytica 172:235–250

Ling HQ, Zhang S, Liu D, Wang J, Sun H, Zhang C, Fan H, Li D, Dong L, Tao Y, Gao C, Wu H, Li Y, Cui Y, Guo X, Xheng S, Wang B, Yu K, Liang Q, Yang W, Liu X, Cheng J, Feng M, Jian J, Zhang X, Luo G, Jiang Y, Liu J, Wang Z, Sha Y, Zhang B, Wu H, Tang D, Shen Q, Xue P, Zou S, Wang X, Liu X, Wang F, Yang Y, An X, Dong Z, Zhang K, Zhang X, Luo MC, Dvorak J, Tong Y, Wang J, Yang H, Li Z, Wang D, Zhang A, Wang J (2013) Draft genome of the wheat A-genome progenitor *Triticum urartu*. Nature 496:87–90

Liu K, Muse SV (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21:2128–2129

Liu Y, He Z, Appels R, Xia X (2012) Functional markers in wheat: current status and future prospects. Theor Appl Genet 125:1–10

Luo C, Griffin WB, Branlard G, McNeil DL (2001) Comparison of low- and high- molecular-weight wheat glutenin allele effects on flour quality. Theor Appl Genet 102:1088–1098

Murray M, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4326

Peng JH, Sun DF, Nevo E (2011) Domestication evolution, genetics and genomics in wheat. Mol Breed 28:281–301

Peng Q, Dai S, Guo Q, Cheng D, Li H, Liu A, Liu J, Zhao S, Song J (2012) The evolution of genetic diversity of wheat varieties released in Shandong province since 1950. Mol Plant Breed 10:228–237

Rasheed A, Xia X, Mahmood T, Quraishi UM, Aziz A, Bux H, Mahmood Z, Mirza JI, Mujeeb-Kazi A, He Z (2015) Comparison of economically important loci in landraces and improved wheat cultivars from Pakistan. Crop Sci 56:1–15

Roussel V, Koenig J, Beckert M, Balfourier F (2004) Molecular diversity in French bread wheat accessions related to temporal trends and breeding programmes. Theor Appl Genet 108:920–930

Roussel V, Leisova L, Exbrayat F, Stehno Z, Balfourier F (2005) SSR allelic diversity changes in 480 European bread wheat varieties released from 1840 to 2000. Theor Appl Genet 111:162–170

Sun DJ, He Zh, Xia CX, Zhang LP, Morris CF, Appels R, Ma WJ, Wang H (2005) A novel STS marker for polyphenol oxidase activity in bread wheat. Mol Breed 16:209–218

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Tian QZ, Zou RH, Jia JZ (2005) Gnetic diversity trend of common wheat (*Triticum aestivum* L.) in China revealed with AFLP merkers. Genet Resour Crop Evol 52:325–331

Vellvé R (1993) The decline of diversity in European agriculture. Ecologist 23:64–69

Wouw M, Hintum T, Kik C, Treuren R, Visser B (2010) Genetic diversity trends in twentieth century crop cultivars: a meta analysis. Theor Appl Genet 120:1241–1252

Zhang X, Yang S, Zhou Y, He ZH, Xia XC (2006) Distribution of the *Rht-B1b*, *Rht-D1b* and *Rht8* reduced height genes in autumn-sown Chinese wheats detected by molecular markers. Euphytica 152:109–116

Zhang LY, Liu DC, Guo XL, Yang WL, Sun JZ, Wang DW, Sourdille P, Zhang AM (2011) Investigation of genetic diversity and population structure of common wheat cultivars in northern China using DArT markers. BMC Genet 12:42

Zhuang QS (2003) Chinese wheat improvement and pedigree analysis. China Agriculture Press, Beijing